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MICRODISSECTION STUDIES. I.

The Visible Structure of Cell Protoplasm and Death Changes

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A study of the physical properties of protoplasm requires for its foundation a knowledge of the consistency of protoplasmic structures in the living cell. Barber's pipette holder, a mechanical device for manipulating microscopically fine pipettes and glass needles in a hanging drop has proved most valuable for this purpose as with it one may dissect living cells under direct microscopic observation. The technique used will be described in a paper which will shortly appear. One must be fully alive to the difficulty of discriminating between protoplasmic structures and artifacts frequently produced during dissection. Considerable experience was, therefore, found to be necessary for a proper interpretation of the results obtained.

Marine invertebrate and plant ova lend themselves well to microvivisection as their post-operative development is easily followed. The bulk of this paper, therefore, deals with results obtained from studies on the protoplasm of Asterias and Arbacia ova at Woods Hole and that of Echinarachnius, Cerebratulus and Fucus ova at South Harpswell.

It is remarkable how much tearing and pulling with a needle living protoplasm will undergo without showing injury. One may puncture a cell with a needle and drag the needle through the cytoplasm back

[1] This paper is based on the work of two summers at Woods Hole Marine Biological Laboratory, and one summer at the South Harpswell Laboratory. The writer wishes to express his indebtedness to Prof. F. R. Lillie for accommodation at Woods Hole and to Prof. H. V. Neal for facilities accorded at South Harpswell. An abstract was read before the American Physiological Society, December, 1916.

and forth cutting through the sides of the cell and if the procedure be slow and gradual the tear closes up behind as the needle proceeds and the process may be continued almost ad libitum without producing an ill effect. If, on the other hand, the needle be carried rapidly through the cytoplasm, a few thrusts only are necessary to induce rapid disorganization. The effects of injury are probably cumulative. If injurious effects be made to follow one another without giving the cell time for recovery, the additive effects of the injury soon manifest themselves in disorganization of the protoplasm resulting in the death of the cell.

The cytoplasm. In the marine eggs studied, the protoplasm consists of a hyaline fluid matrix in which are imbedded granules of various sizes. The fluid offers no perceptible resistance to the needle and an indication of its very slight viscosity lies in the fact that when the needle is moved through the fluid, the only granules displaced are those in the immediate vicinity of the needle. The fluid is water miscible. If, for example, the cell surface be torn, the interior cytoplasm pours out and mixes completely with the surrounding water. Also, if a drop of water be injected slowly and gradually into the egg by means of the mercury injection method, the water diffuses throughout the cytoplasm diluting it. A dilution can be produced in this way sufficient to produce Brownian movement of the imbedded granules. The surface film of such an egg is so much weakened that a mere touch suffices to burst it open when everything except the smallest granules disappears in solution. This water soluble hyaline protoplasm coagulates with ease on mechanical injury. Mere compression will often cause an egg to coagulate into a solid mass. It can then be cut into pieces which hold their shape. This is apt to lead one to the erroneous conclusion that the substance of a cell is usually a solid protoplasmic gel.

When a concentrated aqueous solution of neutral red or brilliant cresyl blue[2] is injected into the interior of an egg, the dye spreads from the tip of the pipette diffusely staining the hyaline cytoplasm. Within a few seconds a granule here and a granule there begins to stain. In a short time all the dye is taken up by the scattered granules leaving the cytoplasm colorless. At the tip of the needle where the concentration of the stain is at its greatest all the granules finally stain. Elsewhere colorless granules lie scattered among the colored ones. The stain is

[2] For the use of the brilliant cresyl blue and for noting the effect of acetic acid on the macrosomes, I am indebted to Mrs. M. R. Lewis.

never permanent. Within a few minutes to an hour, depending on the amount injected, the stain completely fades away. A continuously stained condition can be maintained only by the presence of a superfluity of the dye.

The granules in the cytoplasm of the eggs studied vary considerably within certain limits in size (fig. 1). They have been described by E. B. Wilson (2) and Kite (6). One may classify them into two groups. The smallest granules or microsomes, as they may be called, are minute specks considerably below 1 μ in diameter but plainly visible with the high powers of the microscope, their refractive indices differing considerably from that of the surrounding cytoplasm. The larger granules or macrosomes (Wilson's alveolar spheres), vary from 2 to 4 μ in

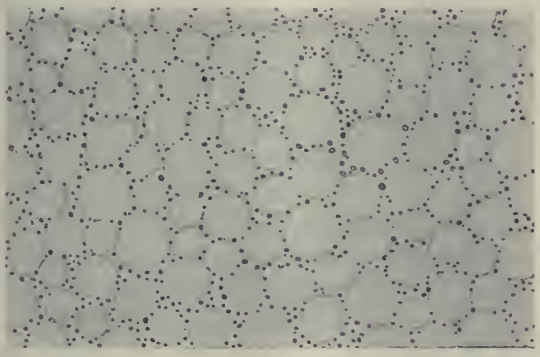

Fig. 1. Illustrating the protoplasm of a living Echinarachnius egg with microsomes and translucent macrosomes (the latter 2 to 4 μ in diameter) crowded together in a hyaline fluid.

diameter and may be circular, oval or irregularly polygonal in shape, exhibiting simple outlines with no diffraction rings. They are closely crowded throughout the egg and among them are scattered the microsomes. The macrosomes, constantly but in almost imperceptible degrees, change in shape and position and it is probable that they are as constantly disappearing and reappearing in the hyaline cytoplasmic matrix. They are very translucent in Echinarachnius and so contribute to the remarkable transparency of the egg. In the Cerebratulus egg they are comparatively opaque.

The difference between the micro and macrosomes is brought out most prominently on injury of the cell by acids or by the needle. If a hanging drop of sea water containing eggs be subjected to the fumes of

acetic acid for a few seconds, the macrosomes quickly disappear leaving the microsomes in a hyaline coagulated mass of cytoplasm. The dissolution of the macrosomes occurs also when disorganization of the protoplasm is induced by rapidly repeated tearing of the cytoplasm with the dissecting needle. The macrosomes swell and gradually but rapidly fade into the surrounding cytoplasm which now becomes very liquid and in which the microsomes exhibit a dancing Brownian movement. The disorganized area then spreads on all sides, and, if the initial injury be extensive and no protective membrane intervenes (see farther on), soon involves the entire cell. If the injurious tear be such as to destroy the surface film in one spot, the disorganized liquefied cytoplasm flows out and mixes completely with the sea water and, in a few seconds the entire cell, except for the dancing microsomes, disappears from view. The microsomes appear to be the most resistant structures of the cell.

Wilson's conclusions (2) that there exists "a complete gradation in size from the largest alveoli" (macrosomes, as I call them) "down to the microsomes" is based, I believe, on a misconception derived from crushing protoplasm. On crushing protoplasm, the macrosomes go into solution and the disintegrating protoplasm as it flows out, tends to break up into spherules of varying sizes, by the formation of surface films. These spherules vary extremely in size and may fuse with one another on touching. They swell readily in water or may coagulate and are quite different from the macrosomes of the uninjured egg. Kite (3) describes the formation of more or less rounded masses in dying Asterias eggs and rightly distinguishes them from structures normally present in protoplasm.

Frequently before all the disorganized cytoplasm has poured out and mixed with the surrounding water, the outflow ceases and the whole mass changes into a solid coagulum which may or may not show a network structure. The complexity in constitution of the protoplasm must account for the fact that a very slight difference in the manner of injury, or in the state of the protoplasm, at the time of injury may produce such widely differing end results, viz., a coagulation into a solid jelly or a complete dissolution into the surrounding medium. The coagulum consists of a hyaline gel with granules arranged in a delicate meshwork. Occasionally upon disintegration the cytoplasm begins to set before the macrosomes have quite gone into solution. Such a mass often resumes dissolution with a succession of peculiar spasmodic jerks

apparently due to the macrosomes which, here and there, swell and carry into solution regions surrounding them.

In cells killed by fixing reagents, the death changes take place without destruction of the cell wall. If the fixing agent act rapidly, the macrosomes tend to swell simultaneously and coagulation sets in before the microsomes are irregularly dispersed. The precipitation network incident to coagulation is produced regularly and the result is a fairly symmetrical alveolar structure with microsomes imbedded in the alveolar walls. Flowing may occur in the disintegrated cytoplasm if the fixing agent be slow in producing coagulation and a variety of distorted figures may result. The prolonged action of acetic or hydrochloric acid tends to dissolve the microsomes and, with various fixing

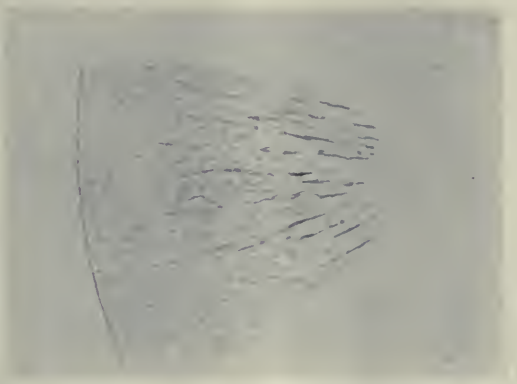

Fig. 2. Drawing of a sector of an Echinarachnius egg in the astral stage fixed in Bouin's picroformol and stained with iron hematoxylin. The effect of fixation is seen in the fibrous appearance of the astral rays and in the network structure of the coagulated cytoplasm.

reagents, a variation in coagulation effects occurs so that one obtains granular and network precipitates which may simulate protoplasmic structure but in reality completely mask it and often lead to erroneous conclusions regarding cell inclusions.

The cell aster is a structure whose nature is entirely masked on fixation. In the living cell the rays are probably paths due to a centripetal flow of a hyaline fluid in an otherwise temporarily gelatinized cytoplasm. When a cell containing an aster is fixed, the reagent used disorganizes the cytoplasm in the way described above. The rays are then compressed between artificially produced alveoli, and their substance be-

ing precipitated, gives the appearance, familiar in fixed material, of fibers or rows of granules which seem to merge peripherally into an alveolar network (Fig. 2).

An acid reaction of protoplasm on mechanical injury can be demonstrated in the Arbacia egg where many of the macrosomes are deep reddish brown chromatophores. On injury to the cell, the color diffuses into the liquid cytoplasm, changing from brown to an orange pink hue,[3] denoting a distinct increase in acid reaction. Also in cells whose granules are stained wth neutral red, injury causes the dye to diffuse out of the macrosomes as they dissolve and give to the liquid cytoplasm a rose color. Acid fuchsin also, which is avidly taken up by acid regions, stains the injured disorganized cytoplasm while the normal cytoplasm remains colorless.

The peripheral layer of the cell. The surface layer of the egg cells studied is very dense in consistency as compared with the cell interior into which it merges insensibly. In the unfertilized egg, the cell granules are imbedded in it up to the very line of division between the egg and surrounding medium. With the needle the surface may be pulled out into long strands without otherwise disturbing the contour of the cell. On being released the strands tend to curl and retract slowly till they disappear. If a more rapid tear be made, and if the cell be under compression, the spot torn bulges out as the internal cytoplasm presses on the weakened surface. The surface layer of the swelling protuberance is very easily broken, upon which the interior may pour out. The cytoplasm then either disintegrates entirely in the surrounding water or, if remaining normal, reëstablishes a film on its surface. When left undisturbed the new surface film gradually strengthens into a definite ectoplasmic layer and the protuberance slowly retracts until the original contour of the egg is reëstablished. If the point of attachment of the protuberance be small, the protuberance may be pinched off to form a spherule of cytoplasm which to all appearances is normal.

If a tear of the surface layer or of any part of the cytoplasm be so injurious that disorganization sets in, a film may form around the disorganized area separating it from the sound cytoplasm. The recovery of an injured cell is only brought about by the formation of a membrane-like film which prevents extension of the injury. A suc-

[3] This color reaction can be demonstrated in an aqueous suspension of the echinochrome made by drawing off a quantity of the colored body fluid into distilled water.

cession of films may form, as one after the other, they succumb to the steady advance of the destroying process and the film which finally holds out may enclose only a fraction of the original cell but what it encloses will be normal protoplasm. The retention of the forming surface film is aided by the presence of solid structures in the cytoplasm. These act as bases which shorten the span to be bridged over by the new film. In the mature fertilized egg, the developing sperm aster which is a gelatinized ball of cytoplasm frequently acts in this way.

When the surface layer is injuriously torn and the disorganized interior cytoplasm flows out, the torn edges of the surface layer curl out and rapidly dissolve, upon which the entire cell disappears in the surrounding water. If the disorganization of the interior has been produced without destruction of the surface layer, the surface often becomes transformed into a rigid coagulum enclosing the fluid products of the disintegrated protoplasm.

The readiness with which the surface film can be reformed is seen in the following experiment on the unfertilized mature Arbacia egg. If an egg be compressed in a hanging drop and then pushed along with a blunt needle, peculiar currents can be produced in the egg substance. The currents pass directly from the pushing object in a straight line through the egg to the anterior end where they curve outward and flow back along the surface to be caught again in the flow from the pushing object. The egg surface is thus being continually reformed by an outflow of its interior, much as the falling sides of a fountain of water are formed by the jet that is streaming up at the centre. The readiness, however, with which the internal cytoplasm may be transformed into the substance of the surface layer is limited. If the egg be pushed beyond this limit no surface layer forms and the cytoplasm at once disorganizes and dissolves in the surrounding water. If one stops before reaching this limit, the egg can be made to round up and will continue a normal existence.

In protozoa, the surface layer of protoplasm, the ectoplasm, is very pronounced. If the ectoplasm of Paramoecium bursaria be torn, internal pressure causes the endoplasm to flow out through the tear which is at first a gaping hole in the ectoplasm. The contractility of the ectoplasm, however, is such that the torn rim curves in. In this way the free edges of the tear tend to approach and if the tear be slight they meet and further outflow of the endoplasm is stopped. In time the concavity on the surface produced by such an injury fills out and the cell resumes its normal shape. If the gap in the tear be too wide for

this method of repair, a surface film bridging the gap forms which may break repeatedly until the outflow lessens the internal pressure sufficiently to allow the newly formed film to persist. Within a few hours, evidence of the tear is no longer visible, either the film stiffens into a definite ectoplasmic layer or the entire body of the cell contracts to bring the original edges of the gap together making the ectoplasm again a continuous layer. A paramoecium may be readily cut into pieces by squeezing it between the coverslip and a fine glass rod, or a knife which is not sharp enough to cut through the ectoplasm. The knife edge bears down upon the surface of the cell until the floor of the groove formed touches the ectoplasm on the other side. The surfaces of contact fuse and further pressure of the knife separates the cell into two portions possessing no gap through which the fluid endoplasm may escape. The cautions usually given in the technique of cutting up protozoa is significant when we bear the above in mind. The knife must be as sharp as possible (which will still be blunt from the point of view of the paramoecium), the cutting edges must be free from nicks and, in cutting, the experimenter must bear down upon the knife without giving it a drawing movement. Either of the two latter precautions, if unheeded, prevents a continuous surface of contact for the ectoplasmic layer of opposite sides and when the knife is removed the endoplasm will flow out through the gap thus destroying the cell. In the marine ova studied, the ectoplasmic layer is very thin but the same condition holds true, viz., that cut pieces will persist only when the cut surface can be bridged over by a morphologically definite film.

In summary, we may say that the surface layer is a highly extensile, contractile and viscous gel capable of constant repair. Its establishment and maintenance is a property essential to protoplasm. With the film intact the mass of protoplasm maintains itself and the life of the cell is assured. When the film is destroyed the cytoplasm flows out, the macrosomes swell and disappear, the whole mass completely disorganizes and disappears in solution in the surrounding water.[4]

In regard to the difference in permeability of the surface layer of a cell and its interior, I have so far only tested the diffusion of the three

[4] Kite describes the cytoplasm of the Asterias eggs as "a quiet translucent gel which can be cut into small pieces with comparative ease." His paper is a pioneer one in microdissection research. The observations recorded were necessarily fragmentary and the differences between the surface layer of the egg and its interior as also the ease with which protoplasm forms gel membranes escaped his notice.

basic dyes, neutral red, cresyl blue and janus green (6). A droplet was injected into the cell simultaneously with the application of a similar droplet to its external surface. For all three dyes diffusion into the cytoplasm took place equally rapidly whether applied to the egg surface or injected into the interior.

Insect germ cells. The germ cells of certain insects, Periplaneta, Disosteira and Anasa, were studied both in modified Ringer's fluid and in the serous fluid of the insects used (3, 4). By pricking the walls of the cysts in the testes, spermatocytes in different stages of development flow out as isolated cells. In the serous fluid they may be kept alive two or three days, during which all stages of cell division may be observed. Except for the nucleus and the mitochondrial network which surrounds it, the resting cell consists of a hyaline fluid cytoplasm. By very slow action with the needle, strands of protoplasm may be pulled out which retract on being released. Injury, however, very easily manifests itself upon which the cell outline fades and the cytoplasm disappears in solution in the surrounding medium.

Adult somatic cells. These vary greatly in consistency owing probably to the amount of metaplasmic material into which their protoplasm has been transformed. Of the various types, only a few possess the fluid consistency of embryonic and germ cell protoplasm. With the exception of the leucocytes, they are comparatively resistant to mechanical injury and are tough and fairly rigid bodies. The nerve cell is probably a rigid gel. The muscle fiber is also a gel. Its substance can be easily pulled out into strands which retract completely when released. Continued injury causes the muscle substance to pass into a very rigid hyaline gel which may be cut into discrete non-glutinous pieces. Gland cells swell readily when punctured and torn with the needle. They seem to exist both in sol and gel states. Mucosa cells consist of a soft, very extensile gel. They tend to round up when isolated. The leucocyte possesses a protoplasm which is very like the undifferentiated protoplasm of the germ cells. If a leucocyte with pseudopodia be touched with the needle, the pseudopodia are retracted and the cell becomes spherical. If the tip of a very fine needle be inserted gradually into the leucocyte a puncture may be made without apparent injury. The diminutive size of the cell, however, renders necessary very little accummulation of injurious effects to disorganize the cell. This usually occurs with almost explosive rapidity, the entire cell going into solution. Dead leucocytes are coagulated bodies and may be cut into non-glutinous pieces.

The cell nucleus. Observations on the cell nucleus of all the ova studied agree with those of Kite (5) that the resting nucleus is hyaline and exists in the sol state. In the germinal vesicle of the immature egg a definite membrane seems to bound the nuclear substance. It is extensile but easily destroyed. Evidence that this membrane is a morphological structure is shown on withdrawing some of the nuclear contents with a micropipette. The nucleus then partially collapses throwing the nuclear surface into irregular folds. Suspended in the nuclear fluid in which it may be pushed about with ease is the germinal spot or nucleolus distinctly visible on account of the difference in its refractive index from that of the nuclear fluid. It frequently contains one or more vacuoles and does not appear to be solid for it may be cut into two, each part rounding up like a droplet.

A rapid tearing of the germinal vesicle with the needle point produces an injury which is accompanied by some remarkable changes. The nucleolus at once swells and fades from view, the nuclear membrane entirely dissolves and, as the nuclear fluid comes into contact with the surrounding cytoplasm, immediate disintegration takes place. The destructive action spreads and may involve the entire egg unless a protective film forms to enclose the area of destruction as in a vacuole. A vacuole of this kind gradually works to the surface of the egg, where it is eventually extruded and expelled. When this has occurred the egg resumes its normal appearance although smaller than before and minus its nucleus. The disintegrative action of the nuclear substance very quickly disappears on extraction from the cell. It is, however, possible, by acting rapidly, to produce the destruction of one cell by injecting into it the substance of the germinal vesicle of another cell. If the nuclear substance remains more than five or ten seconds in the micropipette it is found to be innocuous on injection. With the normal breakdown of the germinal vesicle in the maturing egg, the cytoplasm acquires an increased sensitiveness to injury by the needle. This sensitiveness gradually passes off during the polar body formation.

In the mature egg both nucleus and cytoplasm are comparatively resistant to injury. The nucleus is a hyaline sphere which behaves like an immiscible fluid drop in the cytoplasm. One may divide it into two and each part rounds up into a droplet. On coming into contact the droplets run together. Such a process is no hindrance to normal nuclear activity, for in one case an egg so treated was subsequently fertilized and segmented normally. Rapid thrusts of the needle into the nucleus cause injury, which manifests itself either in

a swelling followed by complete dissolution or a rapid coagulation with the production of a granular meshwork precipitate simulating the nuclear network of fixed cells.

SUMMARY

1. Protoplasm is a hydrophilic colloid which, in early germ cells, egg cells and Protozoa, usually exists in the sol state with a surface layer in the gel state. Adult somatic cells generally are gels in which one cannot demonstrate a cell membrane possessing a consistency different from that of the cytoplasm within.

2. The microscopically visible granules in the cytoplasm of the egg of Arbacia may be classified into two groups: (a) The microsomes, which are considerably less than one micron in diameter and constitute the most resistant parts of the cell maintaining themselves after complete disorganization of the cell; (b) the macrosomes, which range from 2–4 micra in diameter and are very sensitive to injury.

3. The external surface of the egg cell is a gel which passes gradually into the sol in the interior. The surface gel is very extensile and contractile and is readily regenerated on injury. Tearing of this surface, if unrepaired, results in the pouring out of the internal cytoplasm and dissolution.

4. A remarkable property of protoplasm is its ability to form a protective gel film not only on its external surface but also around an injured area which is in the process of disorganization. The disorganized mass thus insulated is eventually expelled from the cell.

5. A continuous but gradual application of mechanical injury can be sustained by a cell for some time without evidence of harm done. A short but rapid application produces instant local destruction, the spread of which may involve the entire cell.

6. Disorganization of the cytoplasm of the egg cells studied takes place in the following way: First, the macrosomes swell and go into solution, and second, the liquid hyaline cytoplasm may flow out and disappear in the surrounding water or it may suddenly set forming a rigid coagulated mass. The coagulation structure gradually coarsens with the production of a network or granular precipitate.

7. Injury is accompanied by a swelling and an apparent increase in the acid reaction of the part involved.

8. The comparatively rigid ectoplasm and the fluid endoplasm of Protozoa are directly comparable with the surface layer and the internal cytoplasm respectively of the marine ova studied.

9. The surface layer and the internal cytoplasm appear to be equally permeable to the basic vital dyes used.

10. The germinal vesicle of an immature egg consists of a hyaline liquid enclosed in a gel like membrane. The nucleolus is an immiscible droplet floating in the vesicle and is very sensitive to mechanical injury.

11. The contents of the germinal vesicle of an immature egg, if brought into contact with egg cytoplasm, either by mechanical rupture of the vesicle or by injection, produce instant destruction of the cytoplasm. This is not true for the mature nucleus nor for the segmentation nucleus.

12. The cytoplasm of an immature egg is comparatively impervious to mechanical tearing, that of a mature egg is very much more sensitive.

13. In the mature egg the nucleus behaves as a fluid droplet whose substance is immiscible with the cytoplasm. It may be divided into two droplets which unite on touching. It coagulates with ease on mechanical injury.

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ADDENDUM

If the injection of an aqueous solution into an egg be not very carefully and gradually done (see page 2) the mechanical compression caused by the force of the injection will produce a coagulation film about the injected droplet to form a vacuole. This film exhibits diosmotic properties similar to that of the gelled surface of the egg for, as Kite observed (6), such a vacuole filled with hypertonic sea water increases in size while one containing distilled water loses its water and decreases in size.

THE OXYGEN PRESSURE NECESSARY FOR TISSUE ACTIVITY

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The ordinary plasma culture is sealed into a very small chamber of a hollow ground slide. The cultures are made by placing small fragments of tissue into layers of liquid coagulable plasma on the surface of a cover glass. The cover is placed over the hollow ground slide so that the tissue and the plasma hang in the hollow chamber. It is sealed to the slide with vaseline and paraffin (1). From the air in the hollow chamber the cells obtain the oxygen necessary for their activities and it has been of interest that in such a small chamber the cells may continue to grow actively for several days. This observation had already led to the belief that these cells may grow in an atmosphere of a low concentration of oxygen. This belief was further substantiated by the fact that the renewal of the air had little or no noticeable effect on the growth or activity of the cells.

That the oxygen essential for activity in the culture is derived from the air chamber was determined by placing two small glass tubes under the cover and replacing the air in the chamber by hydrogen and sealing. No growth took place in these cultures.

A considerable amount of work has recently been done to determine at ordinary atmospheric pressure the per cent of oxygen necessary to maintain a flame as well as to maintain the life of organisms. Clowes (2) and Loevenhart (3) noted that alcohol ceases to burn in an atmosphere containing 15 per cent of oxygen. The latter author noted that ether and Madison illuminating gas cease to burn at 13 per cent of oxygen, while hydrogen continues to burn until the atmosphere contains 6.6 per cent of oxygen.

With the animal conditions are different. Loevenhart found rabbits would live in an atmosphere containing 3.5 per cent of oxygen, but that they died when this was reduced to 3 per cent.

In the present series of experiments an attempt has been made to

determine the effect of pure oxygen and various partial pressures of oxygen on the growth of the cells *in vitro*. Although at the present time the apparatus is still not perfect and a few of the results given here have a slight error, it seems that in their present form they warrant publication.

MATERIALS AND METHODS

The gas and mixture of gases tested have been kept at atmospheric pressure. The partial pressure of oxygen has been lowered by diluting it with nitrogen. The culture chambers used have a large air space, forty to sixty times as large as that of the ordinary culture chamber.

To make these determinations it was necessary to prepare pure oxygen and nitrogen and to devise a culture from which gases could not diffuse. The cultures, therefore, were made in sealed glass chambers.

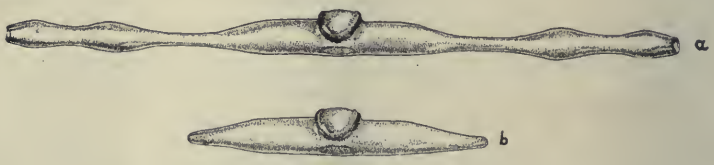

Fig. 1. *a*, The complete culture chamber; *b*, the sealed culture chamber.

The culture chamber. The culture chamber as shown in figure 1, a, is blown from soft glass tubing $\frac{1}{4}$ inch in diameter. In place of the cover glass used in the ordinary cultures, one small part of one side of the tube is blown out, made very thin and flattened. Opposite this place the tube is again blown out and flattened. This latter flat surface forms a base for the culture and allows free transmission of light which is essential for examining the cultures under the microscope. On each side of the central portion the tube is constricted so that it can be easily sealed with a small flame. Beyond this point, it is left full size but corrugated to allow a rubber tube to be tightly fitted. These culture chambers are easy of construction and are made in the laboratory as they are needed.

The culture chambers thus prepared are boiled in soap and water, left to soak several hours in sulphuric acid, rinsed in tap and distilled water. They are then steamed for several minutes to remove soluble substances in the glass and sterilized by dry heat.

Oxygen. The oxygen is prepared by dissociating a 1 per cent aqueous solution of sulphuric acid with an electric current. This method of preparing oxygen is well known. The particular apparatus

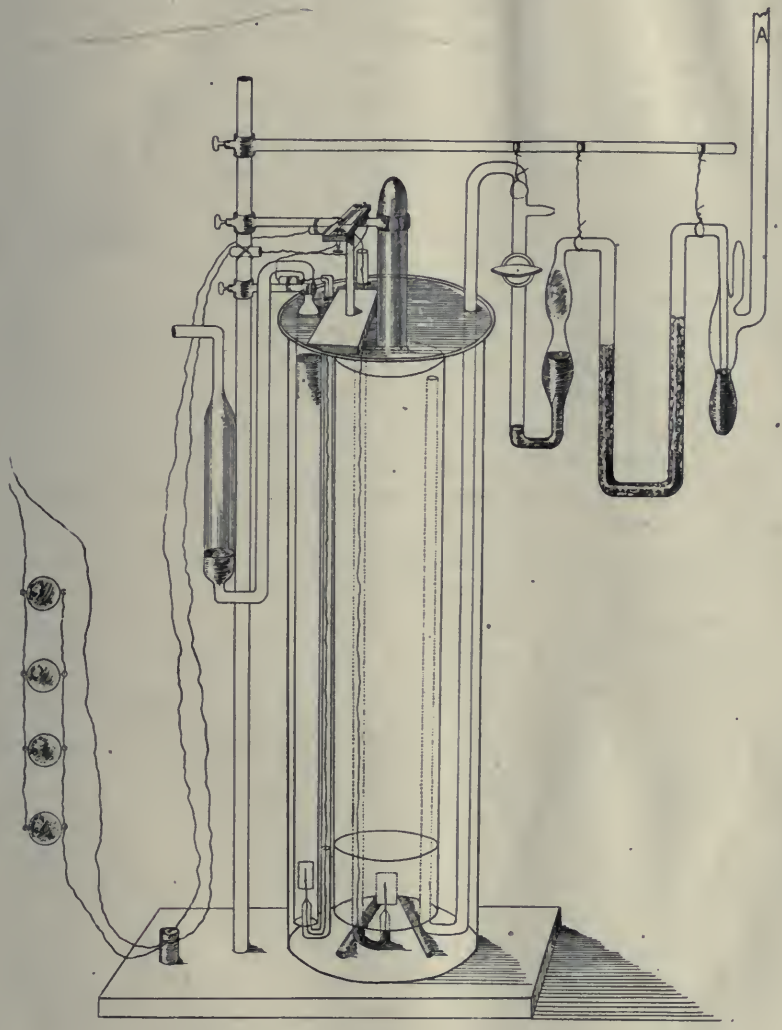

Fig. 2. An automatic oxygen-hydrogen generator.

used works automatically and is shown in figure 2. The current used is D.C. 110 volts, which has been passed through a resistance of one to four 16-candle power carbon lights. The apparatus is made

automatic by interrupting one of the wires with a platinum pointed switch, which is operated by a float.

Oxygen prepared in this manner is something over 99 per cent pure. It contains, however, a small amount of ozone, hydrogen and water vapor. Since the ozone attacks organic matter and may become injurious especially if rubber tubing is used at any point, the gas, before using, is bubbled through olive oil. To remove any traces of acid, it is further passed through a tube containing soda lime and bubbled through an aqueous solution of potassium hydrate 40 per cent. No rubber connections are used in any part of the apparatus. The apparatus is continuous except for one joint which is ground glass.

Nitrogen. The nitrogen is commercial. It contains about 2 per cent of oxygen which is removed by bubbling the gas through several long cylinders containing pyrogallol and potassium hydrate. These are mixed according to the proportions given by Hempel (page 149) (4). One hundred and twenty grams of potassium hydrate are dissolved in 80 cc. of water. To this are added 5 grams of pyrogallol dissolved in 15 cc. of water. Hempel states that no carbon monoxide is formed when a solution of these proportions is used. To insure against this possibility the gas is bubbled through two cylinders containing Sandmeyer's solution (Hempel, page 203) (4). It is then passed through a tube containing soda lime and bubbled through a solution of 40 per cent potassium hydrate.

Preparation of cultures and tissues. For these experiments fragments of heart muscle, and skin of chick-embryos from five to sixteen days of age have been used. The medium is plasma prepared from the blood of adult chickens. When thin layers of plasma are placed in these large culture chambers some slight evaporation may take place. To prevent this from causing an injurious hypertonicity, the plasma, previous to being used, is diluted 0.1 with sterile distilled water. Fragments of both heart muscle and skin are planted in each culture.

It is impossible to prepare these cultures in the ordinary manner. The method used has been to mix together quickly outside four or five pieces of tissue with a drop of liquid plasma. The plasma and tissue are then sucked, before the plasma clots, into the end of a long slender pipette which may be passed into the culture chamber, and the drop of plasma containing the tissue fragments deposited on the thin, flat surface which has been prepared for it (fig. 1, a). The culture chamber is then gently shaken so that the drop of plasma spreads in a layer no greater than 0.5 mm. in thickness, and the tissue fragments

become scattered in this layer over the surface of the glass. The fragments of tissue are 1 mm. or less in diameter.

As soon as the plasma has clotted, the chambers are turned over so that the culture hangs into it. The side walls of the culture chamber are moistened with sterile water so as to further prevent as far as possible any evaporation of the culture medium. In each experiment, one, two or three cultures are used. Besides these in most instances, three control cultures are also prepared. The air in one of the controls is replaced by nitrogen gas. In the other it is replaced with pure oxygen, the third is sealed at once and acts as an air control. This last culture, which contains air, controls the tissue and plasma as well as the activity observed in the cultures where pure oxygen gas is being tested, but it does not control any possible toxic substances in the nitrogen. An attempt to partially control this was made by substituting air for oxygen in several experiments. It was possible by this means to use for the very low dilutions of oxygen a quantity of nitrogen, which has been proven not to be toxic in those cultures where a higher partial pressure of oxygen has been tested and an active growth of cells has been observed. The cultures prepared have shown no evidence of toxic substances in the nitrogen gas.

Since it is essential for all these determinations that the gases used be saturated with water vapor at the temperature at which the cells are grown, the measuring of gases and the filling of the culture chambers with the gas to be tested are always made in the incubator. The arrangement of the apparatus within the incubator is illustrated in figure 3. The oxygen and nitrogen are passed through slender glass tubes, *A* and *B* respectively, across to the middle of the incubator where they are each bubbled through cylinders containing distilled water, *c-A* and *c-B* respectively. From the water cylinders the gas passes into T-tubes, one arm of each of which is open and fitted with a stopcock. To this open arm the cultures in which the pure gases are to be tested are attached. The other arm passes to the measuring cylinder. The measuring cylinder is arranged so that not only these but other gases may be tested. The gases are measured over mercury. In order to keep them saturated a thin layer of freshly boiled distilled water is run over the mercury surface before they are admitted. The measurements are made at the temperature of the incubator. The measuring cylinder is fitted at the top with a 3-way cock. One arm of the cock is open and to this the culture chambers (*b*) are attached, by means of a rubber tube.

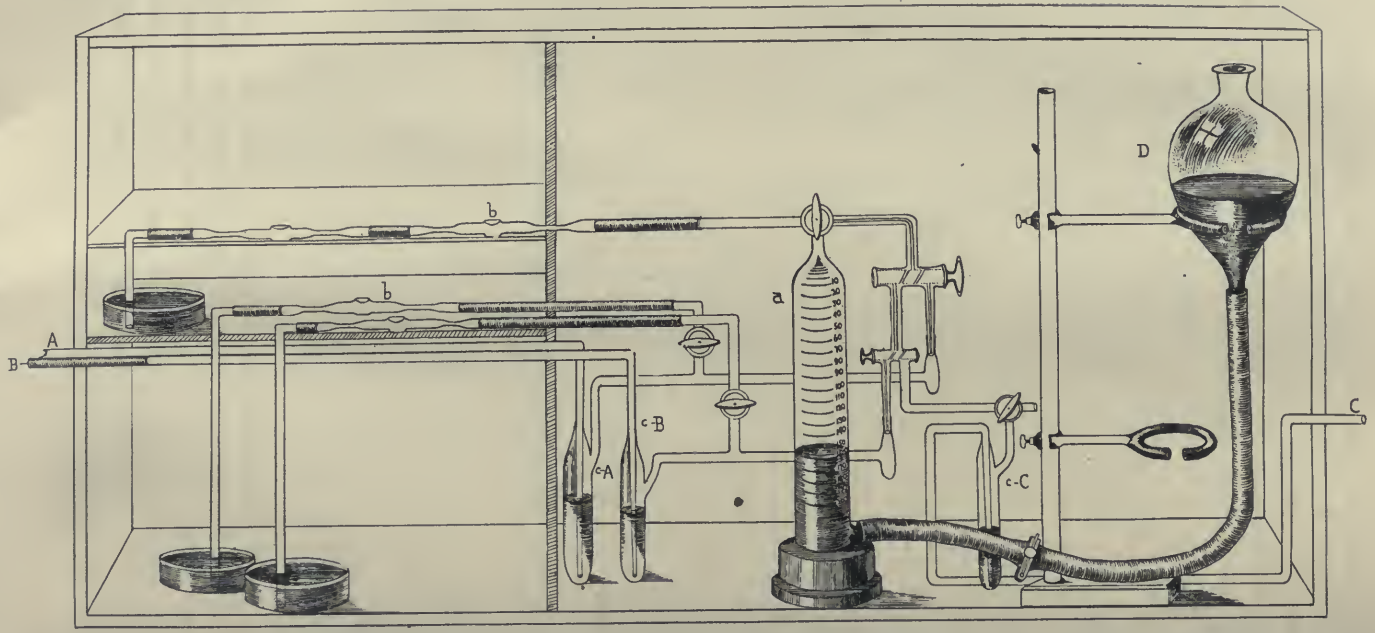

Fig. 3. Gas conducts, washing cylinders, measuring cylinder and cultures within the incubator. *A*, tube carrying oxygen; *B*, tube carrying nitrogen; *c-A*, oxygen washing cylinder; *c-B*, nitrogen washing cylinder; *b*, culture chambers.

The air in the culture chambers is replaced by pure gases or a mixture of gases in the measuring cylinder by allowing these gases to pass rapidly through the culture chambers at regular intervals during a period of one or two hours. The free open end of the culture chamber is fitted with a rubber tube and a piece of glass tubing. The free end

TABLE 1.

DATE	PARTIAL PRESSURE OF O AND N		CORRECTED PERCENTAGE OF O BY HALDANE METHOD	NUMBER OF EXPERIMENTS	AVERAGE GROWTH OF EXPERIMENTS	AVERAGE GROWTH OF CONTROL (AIR)	BAROMETER	PARTIAL PRESSURE OF O AT 39°C.
	Number of cubic centimeters of O	Number of cubic centimeters of N						
Various times.....	100	0	100.0*	50	++++	++++	764.1*	712.1*
Various times.....	80	20		8	++++	++++		
Various times.....	60	40		6	+++	+++		
Various times.....	40	60		7	++++	++++		
Various times.....	20	80		15	++++	++++		
Various times.....	15	85		6	++++	++++		
Various times.....	12	88		4	++++	++++		
Various times.....	10	90		6	++++	++++		
June 2, 1916.....	9	91		1	+++	+++		
Various times.....	8	92		6	++++	++++		
Various times.....	7	93		1	++	+++		
June 21, 1916.....	6	94		1	+	++++		
June 28, 1916.....	6	94		2	+	+++		
Dec. 11, 1916.....	6	94		1	0	++		
Dec. 12, 1916.....	6+	94−	6.6	1	+	+++	745.0	45.6
Dec. 19, 1916.....	5+	94+	5.63	1	0	+++	760.0	40.66
Dec. 14, 1916.....	5	95	5.0	1	0	++++	764.1	35.6
Various times.....	5	95		3	0	+++		
Various times.....	4	96		4	0	+++		
Various times.....	0	100	0.40*	50	0	++++		

*These figures represent but one determination made in the series but since the other experimental conditions were constant they are representative of the series.

of the latter is passed just underneath the surface of water contained in a small dish. After the air in the chamber has become entirely replaced by the gas to be tested and the medium of the culture may be assumed to have become saturated with this gas at atmospheric pressure, the culture chambers are sealed by fusing the small constricted portions of the tube, figure 1, b. The cultures are left in the incubator and

examined periodically under the microscope, which is fitted with a warm box. The maximum growth attained is recorded.

The measuring cylinder used in these experiments is a large one and the general method of measuring the gases is not very accurate. For this reason the measurements were in several instances checked by removing a sample of the gas and determining its oxygen content by the Haldane method. The pure oxygen and nitrogen gases were also tested by the same method (see table).[1]

It is also evident that in all these cultures another slight error must have resulted from sealing the tube and temporarily heating the air in the tube. This would make the actual pressure somewhat less than that recorded.

DISCUSSION AND CONCLUSIONS

Results of the experiments are given in the accompanying table. Tissues of chick embryos grow somewhat better in the spring and summer than in the winter. In the first column the date of the experiments is recorded. The growth is recorded in terms of the area of new cells which form about the fragment. Since there is no absolutely accurate method of measuring this, it is indicated by the relative term,+. In many cultures the cells grow in a single plane, while in others they grow in several planes and the density of the growth is not the same in one culture as in another.

As indicated by the corrected readings of the oxygen percentage in the gaseous mixtures, column 4 of the table, many of the readings in column 3 are probably slightly higher than the table indicates.

Including, however, these possibilities of inaccuracy in the measurements it seems quite evident that certain definite conclusions may be drawn:

1. Cells may grow in an atmosphere of pure oxygen.[2]

[1] The author is indebted to Mr. H. L. Higgins of the Department of Pediatrics for making these determinations.

[2] During the period of the development of the method the cells were found not to grow in pure oxygen. The gas attacked the rubber tubing used in connecting the cultures. The medium in the culture became orange red in color. This gas gave a definite test for ozone. It was not until the gas had been passed through olive oil that the results reported in the paper were obtained. Whether the ozone attacks the culture medium, liberating toxic substance, or whether it attacks the cells, was not determined. From the disintegrating rubber tubing SO_2 was liberated. The failure of growth may have been due to the presence of SO_2.

2. The growth in an atmosphere of pure oxygen, although often slightly more rapid, is not greater than the growth in a partial pressure of oxygen no more than 9 per cent or 10 per cent.

3. Although the growth becomes less when the partial pressure of oxygen is lower than 9 per cent or 10 per cent, very evident growth activity is seen in an atmosphere where the partial pressure of the oxygen is as low as 45.6 mm. Hg.

4. A method has been devised which in its full analysis will allow one to study in more detail many of those conditions which regulate oxidation in tissue cells and to study conditions which regulate oxygen pressure in the tissues.

These results have become of interest in that they show that the activity of the cells within the cultures is little influenced by changes in the oxygen concentration or partial pressure when it remains above a certain amount.

Again, they are interesting in that the lowest partial pressure in which growth took place is closely related to the venous oxgygen tension of mammals. The author does not know the venous oxygen tension of chickens. In the light of these facts an attempt is now being made to determine the venous oxygen tension of chickens, to study the effect of the addition of various substances to the plasma, and to measure with greater accuracy the lowest partial pressure of oxygen in which the cells may not only grow but show other forms of activity.

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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

III. The Influence of the Vagi and of the Sympathetic Nerves on its Rhythm-Forming Power

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INTRODUCTION

In a recent paper Laurens (1) has described the results of experiments on the rhythm-forming power of the atrio-ventricular connection of the turtle, Malacoclemmys geographica, when the connection is stimulated electrically. Haberlandt has recently published a series of articles dealing with funnel rhythm in the frog (2, 3 and 4) and in the last of these he includes experiments on the influence of the vagus on the production of funnel rhythm in the frog and turtle. He found that by the stimulation of the vago-sympathetic trunk the capacity of the A–V funnel to form automatic impulses, giving rise to fibrillation and to high frequent V contractions, is increased, so that long-lasting after effects are obtained which are not obtained by funnel stimulation alone. Atropin he found not to decrease this action of the vago-sympathetic trunk and concludes, therefore, that the effect must be due, in part at least, to the action of sympathetic fibers. Haberlandt draws a close comparison between his results and those that have been obtained on the production of "nodal rhythm" in the mammalian heart.

Laurens (1) found that it was impossible to induce by electrical stimulation of the funnel the automatic formation of rhythmic impulses in the intact heart of Malacoclemmys. It was his intention to carry this study of the rhythm-forming power of the funnel further by investigating the effects of vagal and sympathetic stimulation on its production and to attempt to see whether some difference between the nerves of the right and left sides could be demonstrated. The carrying out of this problem entailed an examination, more or less thorough, of the general influences of the vagus and sympathetic nerves on the tur-

tle heart, involving the repetition of much work that has been done already. It was necessary in the first place to find out whether the right and left vagus nerves could be shown to have a control over different parts and different functions of cardiac muscle—owing to the differential effects that have been described both for the turtle heart and the heart of the mammal—and whether any influence of the sympathetic nerves could be demonstrated. It seems best to consider briefly at this time the results that have been obtained by others.

HISTORICAL

Location of the pace-maker. Muskens (5) by suspending two parts of the sinus showed that the sinus and large veins of the turtle (Pseudemys rugosa and elegans) are a system of contractile units, the contraction wave, in most cases, being seen to start from the right vena cava. He also observed antiperistaltic contractions of the sinus and large veins in exposed hearts that were beating normally.

Garrey (6) concluded from inspection of the turtle heart that the beat arises at the junction of the right pre- and post-caval veins, the sinus contracting after this region. He claims that the right caval veins possess a greater rhythmicity than the other parts of the heart and determine the rate of the whole heart.

Meek and Eyster (7) have shown that the origin of the beat in the turtle heart is in the sinus and in a definite part of this, namely, the sino-auricular ring, somewhere to the right of the left venous valve (8), probably near the right venous valve, where the best connection between the sinus and the right auricle is found (9). Schlomovitz and Chase (10) also find the pace-maker to be in the right sino-auricular junction.

Action of vagus and sympathetic. The vagus has an inhibitory effect on the rate and strength of beat and on the conductivity and excitability of cardiac muscle. The effect of the sympathetic nerves is opposite to that of the vagus on rate and strength of beat and on conductivity. There is some doubt as to the action of the sympathetics on excitability. Recent work has indicated that, due to a difference in distribution, the nerves of the right and left sides do not exert their effects in equal degree. Garrey has most recently investigated this matter in the turtle. That the right and left nerves were not equally effective in stopping or slowing the turtle heart had been repeatedly observed by previous investigators.

According to Garrey (6 and 11) there is a preponderant inhibitory

action of each vagus upon the corresponding half of the heart, a homolateral distribution and function. The action of the right vagus is mainly negatively chronotropic and it has a preponderant action in stopping the heart, due to the fact that its effect is directly upon the right caval veins, where, as Garrey believes, rhythm is initiated. In many cases, in certain selected individuals, the left vagus was unable to affect the rhythm, but, by decreasing excitability, conductivity and contractility, blocked the impulse, thus producing or increasing S–A block. In cases of A–V block where the left vagus had no chronotropic influence, the effect was always to increase the degree of block, stopping or slowing the V, the A rate being unchanged. The right vagus, on the other hand, decreased the block, owing to its greater chronotropic effect. He obtained this effect also when the intracranial vagus was stimulated, thus showing that it was not due to sympathetic stimulation.

Greene and Peeler (12) agree with Garrey in so far as they found, in central cardiac inhibition, that when the left vagus was sectioned there was no change in the rhythm of the heart or in the state of inhibition, but that when the right vagus was cut, the heart resumed its normal rhythm.

Garrey pointed out that the depressing effect of the vagi upon conductivity and strength of contraction is sometimes wholly obliterated by the coincident slowing of rate. Dale and Mines (13) and Mines (14) have recently shown that in the frog the action of the vagus is primarily to produce increased resistance to transmission from A to V, decreasing the rate, (and to shorten the duration of the electrical disturbance in the V), while the action of the accelerators is to improve the rate of conduction between A and V (and to increase the duration of the electrical response in the V). These authors make no mention of a difference in degree between the action of the right and left nerves.

There is considerable evidence that in the mammalian heart there is a division of labor between the right and left nerves. Cohn (15 and 16) found a great qualitative difference in the action of the two vagi on the heart of the dog, the right controlling principally the Si node, and by its negative chronotropic influence the beat of all chambers, while the left has its greatest influence on the conduction of impulses over the A–V connection, producing either a delay, or incomplete block or complete cessation of V contractions. The left vagus may also have some influence over the A, and may rarely cause S–A block. Cohn and Lewis (17) showed that the left vagus has more effect on the A–V

junction than the right has, while the right effects the production of impulses in the A more than the left does. Lewis (18) has investigated the matter further and his results show that it is not so simple as at first might seem to be the case. He admits that S–A rhythm is more readily inhibited by the right than by the left vagus, but questions the view that the A–V connection is influenced chiefly by the left vagus. He finds that, while the left vagus may have more influence than the right in producing heart block, the right has a larger control than the left on "nodal rhythm" and that this control is even greater than the control of the right nerve over S–A rhythm. This latter in turn is about the same as the control of the left nerve over "nodal rhythm," while the control of the left nerve over Si rhythm is weakest of all. In brief the right has a greater control over both nodes, and the control of both vagi is greater over the A–V node than over the S–A node.

Rothberger and Winterberg (19 and 20) have reported that in the dog the right vagus and sympathetic influence particularly the S–A node, the left nerves particularly the A–V node. There are numerous exceptions due to the presence of fibers which go in each case to the other node, particularly in the left accelerator the presence of fibers which have a chronotropic influence on the S–A node, so that all parts of the heart are under the excitatory influence of the accelerators, the influence being strongest on the S–A node, the right increasing the rate more than the left (20). They were able to show, moreover, that the accelerators innervate their own side of the heart more or less separately, for in many cases of combined right stimulation, right extrasystoles appeared, and of combined left stimulation, left extrasystoles appeared, these breaking through the vagal standstill. In connection with other work on the production of "nodal rhythm" they found it impossible to isolate the inhibitory chronotropic fibers in the vagi which go to both the Si and A–V node because they are mixed, and that it is only exceptionally possible to demonstrate an inhibitory effect of the vagus on the A–V node, moreover that it is only exceptionally that one finds the negative chronotropic fibers for the S–A node exclusively in the right vagus. Sometimes also the right vagus produced complete standstill, while the left produced merely slowing of the A and dropping out of V contractions, thus showing that it was distributed to both nodes.

Ganter and Zahn (21) also report that the two vagi influence nodal tissue in unlike degree, the right being stronger than the left on stimulus formation in the Si, and the left being stronger than the right on

the A–V node, particularly in its effect on lowering conductivity. The distinction is by no means always a sharp one, the left vagus usually having a slight negative chronotropic effect as well.

In a still later paper Rothberger and Winterberg (22) give further evidence regarding the distribution of the right and left nerves. In cases of A flutter they found that strong accelerator stimulation increased the strength and the rate of contraction, the right having the greater effect, although the duration of A flutter and fibrillation is increased. The rate of the V contractions is increased, owing to improved conduction. The vagi also, when strongly stimulated, increase the rate of the A contractions, the effect of the right being stronger, and just so much stronger, as the left vagus has less of an inhibitory effect. By decreasing conductivity the rate of the V contractions is decreased.

Robinson (23 and 24) reports that the left vagus, just as it does not inhibit rhythm, does not inhibit A tachycardia and may only possibly inhibit A fibrillation. The right vagus has no influence on A fibrillation, but inhibits the A tachycardia which is coexistent with the A fibrillation, and changes the flutter into a fibrillation. The right vagus is the more effective in increasing the susceptibility of the auricles to faradisation, and in holding them in the abnormal activity. In some hearts vagal stimulation alone can initiate the same abnormal A activity caused by A faradisation. The left vagus is more effective than the right in replacing the abnormal auricular activity by the normal sequential beat.

The influence of the cardiac nerves on the production of nodal, or A–V rhythm. There are several references in the literature to this matter. These have to do particularly with the vagus nerve and Haberlandt in his papers has pretty thoroughly reviewed them. Rothberger and Winterberg also give a brief review and the last mentioned authors have most carefully and recently investigated this matter.

Winterberg (25) found that the accelerators have no influence on the origin or duration of V fibrillation and may shorten A fibrillation, while the vagi assist in the production of A and V fibrillation.

Rothberger and Winterberg (19 and 20) have shown that stimulation of the right accelerator cannot produce "nodal rhythm," while the left accelerator, which has only a slight chronotropic influence on the S–A node, did so in 30 per cent of their experiments, stimulation of the right accelerator causing this to disappear. The failure of the left accelerator to cause A–V rhythm is referred to the admixture, already men-

tioned above, of fibers which go to the S–A node. The stimulation of the vagus alone never produced V fibrillation; combined with the accelerator it did so in 10 per cent of their experiments.

Owing to the preponderant distribution, in certain cases, of the right and left vagi and sympathetics to the S–A node and A–V node respectively, they thought that combined stimulation of the left accelerator and the right vagus should give "nodal rhythm." Their early experiments (19) were negative. Later (20) by combining such stimulation, in those cases where the inhibitory fibers for the Si are practically only in the right and those for the A–V node in the left, they obtained results in two experiments. In a third (p. 361), combined stimulation of the left accelerator and either vagus produced "nodal rhythm," thus indicating an equally intense inhibitory effect of the two vagi. They also found in another case (p. 362) that the stimulation not only of the left accelerator, but of the right as well, produced in combination with stimulation of the right vagus a "nodal rhythm."

These various effects of the cardiac nerves bring up for consideration the question as to whether they really have any direct influence on the ventricle. Hering (26 and 27) and his collaborators hold that they do; Erlanger (28) does not believe that the vagi have any significant chronotropic influence on the ventricles and that the A–V bundle contains no inhibitory fibers for the ventricle.

Haberlandt (3) has recently described both positive and negative chronotropic and inotropic influences of the vagi on the slow automatic V contractions of the frog. He compares his results on the inotropic effect with those which Gaskell (29, p. 85) obtained, in one case, on inhibiting the independent auriculo-venticular rhythm through the coronary nerve by stimulating the right vagus. The positive influences which Haberlandt obtained would seem to be due to the excitation of sympathetic fibers in the vago-sympathetic trunk.

Tonus. It is conceivable that the rhythmical variations in tone of the turtle auricle may be influenced by the possible differentiation in function of the right and left nerves. The influence of the nerves on these tonic oscillations has been investigated by a number of workers, among them being Fano and Fayod (30), Bottazzi (31 and 32) and Oinuma (33), with the general result that the vagus increases the tone, while the sympathetic depresses or inhibits it. Gaskell (34) was of the opinion that the vagus nerve did not decrease the state of tonic contraction of the A, although he obtained a positive variation when

the vagus nerve was stimulated, as has recently been substantiated by Meek and Eyster (35) and Samojloff (36).

The supposed cause of the rhythmic variations in A tone is worthy of brief notice, particularly so since this matter has recently been given extensive consideration by Gesell (37 and 38). Rosenzweig (39), who found that vagal stimulation did not increase the tonus, came to the conclusion that the rhythmical variations in tone were due to the gradual death of the preparation. He suggested that they might be caused by the contraction of the smooth muscle cells lining the auricular cavity, an assumption which Bottazzi (32) confirmed, thus giving up his sarcoplasm contraction theory. Oinuma (33) agrees with this.

The anatomy of the sympathetics and vagi. The course of the sympathetic nerve and its innervation of the heart has been described for various species of turtles by various authors. According to Gaskell and Gadow (40) the rami cardiaci come chiefly from the first thoracic ganglion. In Testudo graeca they are sent off from the middle cervical ganglion and enter the heart, together with the vagus fibers, between the pulmonary vessels and the vena cava. In Chelone imbricata the ramus cardiacus from the middle ganglion enters the heart along the aorta, running close with the vagus of its side. In Emys europaea rami cardiaci likewise arise from the median ganglion and perhaps anastomose with the cardiac branches of the vagus. It has been shown by Bottazzi (31) that cardiac branches of the sympathetic in E. europaea arise from the middle and inferior ganglia, but chiefly from the latter. Oinuma (33) agrees with this. In Emys caspica Kazem-Beck (41) describes the main ramus cardiacus as arising in the first thoracic ganglion and running along the superior vena cava to the heart, although cardiac branches are also sometimes given off from the inferior and middle ganglia. According to Wesley Mills (42) the rami cardiaci in Pseudemys rugosa arise from the first thoracic and the middle ganglia, the former branches appearing to be the more important. Ranson (43) has recently studied the vagus in Chelydra serpentina. In this animal the sympathetic and the thoraco-abdominal branch of the vagus run together into the thoraco-abdominal cavity. The sympathetic leaves the vagus opposite one of the lowest cervical vertebrae and turns toward the inferior cervical ganglion of the sympathetic. At the point at which the two nerves separate there is developed the middle cervical sympathetic ganglion and the ganglion thoraco-abdominale vagi.

ANATOMICAL

A description of the sympathetic in Malacoclemmys geographica will not be out of place since it differs in some details from the conditions which have been described in other Chelonians. From the ganglion cervicale superior situated just under the base of the skull (fig. 1) a small branch passes anteriorly through the jugular foramen into the skull along with the vagus and accessory nerves. The cervical sympathetic arises from the posterior side of the ganglion and passes caudalwards with the vagus, just under the omohyoid muscle and median to the carotid artery. The two nerves run separately down the neck, and do not unite to form a common vago-sympathetic trunk nor have connections between them been observed. The sympathetic receives no rami communicantes from the first five spinal nerves. At the level of the fifth to sixth cervical vertebrae the sympathetic enters the ganglion cervicale medium. A small nerve may sometimes be seen connecting this ganglion with the vagus, but its presence is not typical. From the ganglion cervicale medium the sympathetic runs towards the median line and at the level of the seventh to eighth cervical vertebrae enters the ganglion cervicale inferior, which receives a ramus communicans from the eighth spinal nerve. It is continued downward to the first thoracic ganglion (*Th. G*) giving off a small branch to the plexus brachialis as it passes. Gaskell and Gadow have found considerable variation and fusion of two or more of the ganglia in this region, corresponding to the stellate ganglion in mammals.

That the main rami cardiaci arise in or pass through the first thoracic ganglion is corroborated by experiments. Gaskell and Gadow (40) have shown that stimulation of the sympathetic chain between the first thoracic and the middle cervical ganglion, or of the ramus cardiacus,

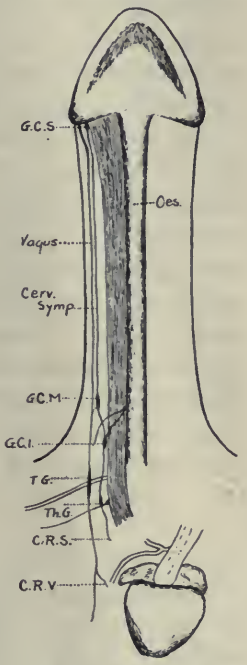

Fig. 1. Ventral view of neck of Malacoclemmys showing the course of the vagus and of the sympathetic nerve of the right side. *G.C.S.*, Ganglion cervicale superior; *G.C.M.*, Ganglion cervicale medium; *G.C.I.*, Ganglion cervicale inferior; *T.G.*, Ganglion thoracico-abdominale vagi; *Th.G.*, first thoracic ganglion; *C.R.S.*, cardiac ramus of the sympathetic; *C.R.V.*, cardiac ramus of the vagus.

in Testudo graeca "causes a most marked acceleration of the heart and augmentation of the auricular contractions." Kazem-Beck (41) pointed out that in Emys caspica stimulation of the ramus cardiacus from the first thoracic ganglion produces an acceleration of six to eight beats per minute following the stimulation. Dogiel and Archangelsky (44) found that in Emys europaea stimulation of the inferior ganglion caused an acceleration of about 4 beats, while stimulation of the first thoracic ganglion with a current of the same strength produced an acceleration of about 8 beats per minute. No mention is made by the last two authors of an augmentation of the A contractions. Bottazzi (31) has shown in E. europaea that stimulation of the sympathetic chain between the middle ganglion and the first thoracic ganglion produces an augmentation of the height of contraction as well as an acceleration. According to Greene and Peeler (12) the augmentatory apparatus of the turtle is poorly developed, if not in fact absent. Garrey (11) demonstrated what he considers an indirect augmentatory effect in cases of A–V block in the turtle after vagal stimulation, in that the block disappears for a while. Also that intraventricular block was improved after stimulation of the cervical vagus, but not of the intracranial vagus.

EXPERIMENTAL

Since Laurens (1) found that in the intact heart of Malacoclemmys a funnel rhythm could not be initiated by electrical stimulation of the A–V connection, it seemed interesting enough to see what the influence of stimulating the cardiac nerves at the same time would be. As has already been mentioned above, Haberlandt found that stimulation of the vago-sympathetic trunk in the frog and turtle had a beneficial influence on the formation of funnel rhythm. Since the work of Rothberger and Winterberg has shown that the vagus when stimulated has no ability to produce "nodal rhythm,"—whatever effect these nerves may have on such rhythm after it has been produced,—and that the stimulation of the left stellate ganglion could start "nodal rhythm," particularly when the vagus was also stimulated, it seemed that this matter might not be so simple as Haberlandt implies. The investigation, the results of which are now to be reported, was undertaken at the suggestion of Dr. Henry Laurens. My sincere thanks are due him for his assistance and helpful criticism at all times. A preliminary report of the results has already appeared (45).

METHODS

The turtle Malacoclemmys geographica was used in all of the experiments. The animal was rendered insensible by decerebration, anaesthetization, or by destroying the brain and spinal cord, decerebration proving the most satisfactory and therefore most generally employed. The plastron was removed and the fore limbs amputated after ligating the subclavian arteries. In all of the experiments care was taken to reduce the loss of blood to a minimum and to keep the circulation intact as much as possible. After the pericardium had been slit up the heart was fastened to a slender strip of cork by two small porcupine quills and suspended for mechanical registration from the right auricle and the apex of the ventricle. The cardiac nerves were exposed and shield electrodes placed on the thoracico-abdominal ganglion of the vagus and the first thoracic ganglion of the sympathetic, or between this and the inferior ganglion.[1]

The conclusions reached in this paper are based on results obtained during the first two to three hours of experimentation, and before the strength of Si impulse had markedly decreased.

Effect of sympathetic stimulation on the normal heart beat

Stimulation of the sympathetic by relatively weak interrupted currents produces no effect on the rate of beat. With stronger currents the action of the sympathetic becomes more apparent. Of 32 experiments, sympathetic stimulation produced a visible effect on the heart in 18. Of these, augmentation of the A contractions was produced in 10, augmentation and acceleration in 5, and acceleration alone in 3. The amount of augmentation, as calculated from the height of the registered contractions, was usually small (from 1 to 3 mm.), although an increase of as much as 6 mm. was not uncommon. The amount of acceleration varied from between 2-3 to 6 beats per minute. No difference between the effects of the right and left sympathetics could be noticed. The maximum effects became apparent fifteen to sixty seconds after the commencement of stimulation, though sometimes not until after it had been discontinued, and lasted for longer or shorter periods of time.

[1] In many cases, as observed by Gaskell and Gadow, the first thoracic and the inferior ganglion are fused. In such cases the electrodes were placed either on the "fusiform ganglion" or between this and the middle ganglion.

Effect of sympathetic and vagal stimulation on variations in A tone

The action of the sympathetic in abnormal conditions, such as increase in auricular tone, A-V block, V fibrillation and V-A rhythm is more striking than the augmentatory and acceleratory influences on the normal beat. Bottazzi (32) observed in one case that the right sympathetic was more effective in reducing A tonus than the left was, but that in all other cases the effect of both was the same.

In the intact heart of Malacoclemmys, variations in A tone rarely appear. In only one turtle used in this investigation did tonus oscillations spontaneously appear and then only in the left auricle. This animal had been in the laboratory aquarium for some time, and with the onset of cold weather had eaten little or no food and was in a very weakened condition, the heart beat being slow and weak. During the preparation for registration considerable blood was also lost.

Stimulation of either of the vagi with interrupted currents too weak to stop the heart caused an increase in the tone of the left auricle, the left vagus apparently being the more effective. Stimulation of the right or left sympathetic during a period of increased tonus, whether automatic or set up by vagal stimulation, caused the tone to decrease, the left sympathetic apparently again being the more effective.

Rosenzweig's view regarding the appearance of variations in tone seems to be substantiated by the fact that in only one turtle did marked variations in tone occur spontaneously. As is well known, and as Laurens has observed on the auricles of Malacoclemmys, marked variations in tone can be produced by mechanical or electrical stimulation, or by flooding the auricles with cold Ringer, etc. Laurens (1 and unpublished observations) found that in experiments on excised hearts nearly every one showed marked oscillations in tone, both in the beating and still (sinusless) heart.

Effect of sympathetic stimulation on A-V block

Block was produced by the method used by Laurens (46) of cutting through parts of the A–V connection. If the part cut is small, either no block is caused or a very transitory one of 3:1 or 2:1 rhythm. By cutting through a sufficiently large portion of the funnel severe and long-lasting partial or total block can be produced.

Strong faradisation of the sympathetic nerves has been found to improve the block. To describe a typical case: After complete block

had been produced (fig. 2) the right sympathetic was strongly stimulated. Soon after the stimulation was discontinued the V gave a single contraction, and then began to beat slowly as shown in the figure. By

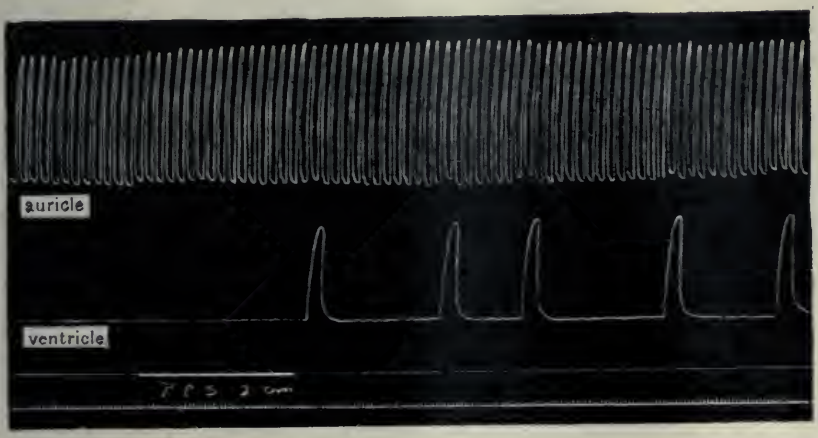

Fig. 2. Improvement of A–V block by sympathetic stimulation. Time in this and succeeding figures in seconds.

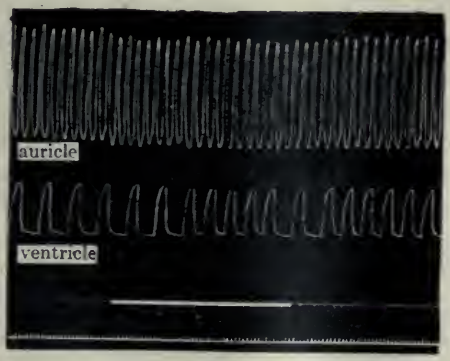

Fig. 3. Continuation of figure 2, showing continued improvement resulting from further sympathetic stimulation.

repeated stimulations the block was further improved, the A and V finally beating in a 2:1 ratio (fig. 3), although the block could never be brought to disappear entirely.

The action of the vagus and sympathetic on funnel rhythm

To produce funnel rhythm essentially the same method as employed by Laurens (1) was used. Two fine needle electrodes connected with the secondary of a Harvard inductorium were inserted into the V just below the A–V boundary so that they penetrated the A–V funnel. If strong interrupted currents are sent in fibrillation of the V is produced which in most cases spreads to the auricles. The fibrillation, however, does not persist after the current is broken, and is often interrupted by the normal sinus rhythm during the stimulation. Stimulation of the funnel sometimes produces a rapid V rhythm which also does not persist after the stimulation (fig. 4, *B*), Si rhythm reappearing almost immediately after the stimulation is discontinued.

If the vagus is stimulated with relatively strong interrupted currents at the same time that the funnel is stimulated, there is produced, in a few cases, V fibrillation, which, sometimes changing into a regular but rapid V–A rhythm, lasts over for varying lengths of time after the stimulation has been discontinued (figs. 5, 6, 7 and 8). In order to attain this result it has been found that the vagal stimulation must be of sufficient strength to completely inhibit the Si impulses, although, of course, even when this is so, automatic funnel impulses are not always set up. All cases of the rhythmic production of automatic funnel impulses were obtained by stimulation of the funnel and the right vagus. Conjoint stimulation of the funnel and the left vagus causes ventricular fibrillation during the stimulation as with right stimulation, but apparently the block produced by the left vagus is not sufficiently strong to prevent the reappearance of the normal sinus rhythm as soon as the stimulation ceases (see fig. 4, *A*). This may be due to the fact that the left vagus principally affects conduction between the A and the V and that the Si impulses, not having been weakened to any great extent, break through to the V.

If the vagus is stimulated during this funnel rhythm with a strength of current that would ordinarily stop the heart, it produces merely a decrease in the amplitude of the A contractions with no effect on the rate. Stimulation of either of the sympathetics, on the other hand stops the funnel rhythm, after which the normal Si rhythm reappears, the right and left sympathetics apparently being equally effective (figs. 5 and 6).

Conjoint stimulation of the funnel and of either sympathetic never produced funnel rhythm (see fig. 4, *C*). Nor did conjoint stimulation

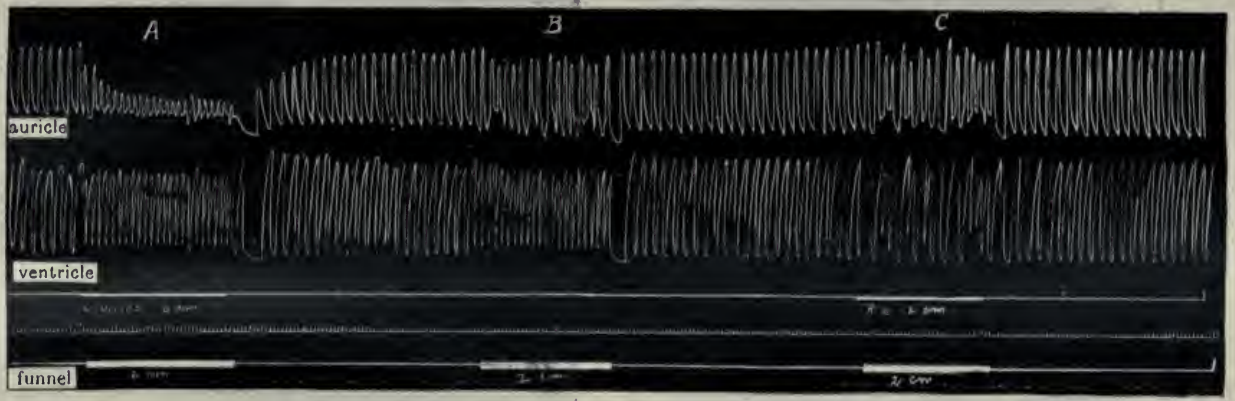

Fig. 4. Failures to produce funnel rhythm. *A*, Conjoint stimulation of funnel and left vagus. *B*, Stimulation of funnel alone. *C*, Conjoint stimulation of funnel and right sympathetic.

of either of the vagi and one or the other of the sympathetics result in the production of funnel rhythm. This latter subject is, however, worthy of a more detailed investigation than has been made.

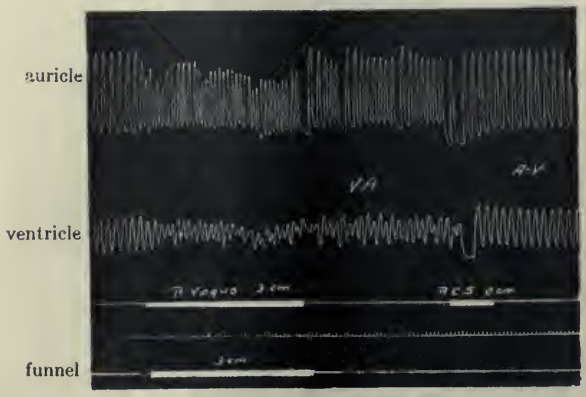

Fig. 5. Conjoint stimulation of funnel and right vagus resulting in the production of V fibrillation followed by V–A rhythm with partial block. Stimulation of the right sympathetic stops the funnel rhythm.

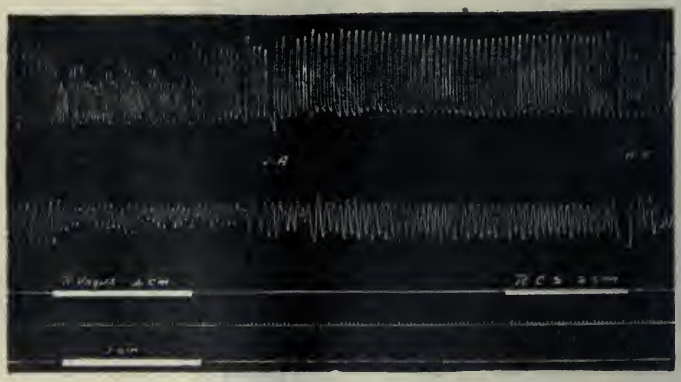

Fig. 6. Conjoint stimulation of funnel and right vagus resulting in V fibrillation followed by V–A rhythm showing partial block. The V shows pulsus alternans. Stimulation of the right sympathetic stops the funnel rhythm.

In a number of cases the funnel rhythm develops partial V–A block (see figs. 7 and 8, also 5 and 6). This block is characterised by the dropping out of A systoles after a gradually shortening V–A interval, evidenced most clearly by the gradually decreasing length of the suc-

cessive A–A intervals. The block, with the decrease in rapidity of production of the funnel impulses, eventually disappears. Laurens (1) has described such a case of block in detail. In the cases which I have observed, either the impulses are arising in groups characterized by a

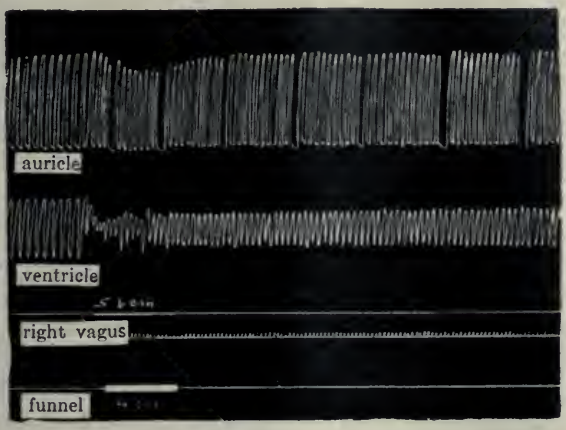

Fig. 7. Funnel rhythm (rapid V–A) showing V–A block following conjoint stimulation of funnel and right vagus.

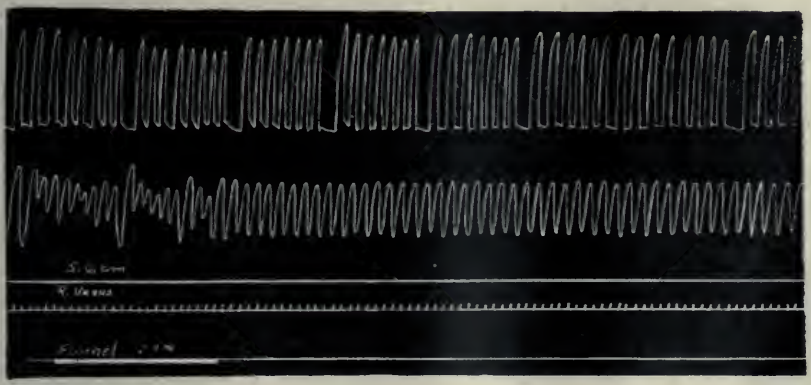

Fig. 8. Funnel rhythm, showing V–A block, following conjoint stimulation of funnel and right vagus.

gradual quickening of impulse formation, or the conduction towards the A improves from beat to beat until the impulse falls into the refractory period and an A systole is missed. Often there is a slight pause before the last As, which is then more vigorous (fig. 5).

The impulses for this V–A rhythm originate in the A–V funnel, probably below the level of the A–V boundary. They therefore spread in both directions, going to the V and upward to the A. There is no measurable variation in the lengths of the V–V intervals, and we are therefore obliged to admit that the impulses arise at regular intervals, but that they are conducted upward with increasing ease, until an impulse falls into the refractory period. The fact that in many cases there is a pause before the last As, which is then more vigorous, would seem to indicate that, in some cases at least, the dropping out of the As was due to a refractoriness of conduction.

THEORETICAL CONSIDERATIONS.

The results of my experiments have given no reason for believing that the distribution of the two sympathetic nerves is in any wise different, and have given only indirect evidence for the vagi. A series of observations on comparing the effects of right and left vagal stimulation on the right and left auricles respectively seemed to indicate that it is only extremely seldom that there is a difference between the influence of the two nerves.

In cases of A–V block, caused by cutting through parts of the A–V connection, stimulation of the sympathetic results in a partial removal of the block, decreasing it by improving the conductivity of the A–V connection as well as increasing the strength of the Si impulse. In the case illustrated (figs. 2 and 3), which is typical, the contractions of the V are at first at long intervals, and with each succeeding stimulation become more and more numerous, indicating a decrease in the degree of block which is dependent for the most part upon an improvement in conducting power of the remaining fibers of the A–V connection.

It seems certain when the A–V connection is cut that the irritability of the V is not reduced, but that the A impulse which is able to reach it over the intact but injured funnel fibers is decreased, so that it is subliminal and the V therefore fails to respond. The irritability of the V, owing to its inactivity, increases until the weakened A impulse getting through proves strong enough to elicit a contraction, after which the irritability of the V again falls to zero, and one or more V systoles are missed (partial block), or the impulse, although the irritability of the V is maximal, is always subliminal and the block is complete.

It has been shown above that sympathetic stimulation in the turtle causes an increase in strength of the sinus impulse, which coupled with

the increase in contractile power and the improvement in conduction causes an increase in strength of the A contractions. Owing to all of these effects of the sympathetic, the impulse reaching the V is increased in strength to such an extent that it elicits a contraction. We have not sufficient evidence to warrant an assertion regarding the effect on excitability. Owing to the increase in strength and the improvement in conduction the irritability of the V does not have to rise so high as it did before, in order to be excited to contract and it therefore contracts more often. In other words the strength of the impulse able to reach the V over the A–V connection is the important matter, and the strength of this impulse depends most on the conductivity of the connection fibers. Laurens (46) has recently discussed block with particular reference to cases produced by cutting the A–V connection, and therefore injuring the fibers remaining so that their conductivity is reduced. The argument that most depends on the improvement in conductivity gains support here from two factors of sympathetic improvement, namely, acceleration and the fact that the augmentation of the Si impulses is not very great in amount or in duration. If the conductivity of the A–V connection were not improved, an acceleration of the rate could have only the result of increasing the block. And if the conductivity were not improved permanently then the original degree of block would return soon after the stimulation ceased owing to the gradual decrease in the strength of the Si impulses.

The action of the vagus on funnel rhythm is due to its inhibiting effect on the normal pace-maker, or on the impulses originating there. Stimulation of the funnel in certain cases starts automatic impulse formation. If these are not interfered with they will continue for a longer or shorter time, arising automatically and rhythmically and initiating a beat of the heart which is characterised by a V–A sequence, owing to the fact that the V is nearer the point of origin of the impulses than the A is. The left vagus has been claimed to have, in certain cases, less of an influence on the normal pace-maker, in so far as the frequency of impulse formation there is concerned, and to have a much greater effect in reducing conductivity. It might be concluded from the failure of the left vagus, when stimulated conjointly with the funnel, to produce conditions favorable to the formation of a funnel rhythm that this was due to the fact that the Si impulses, weakened only slightly, were able to break through and reach the funnel and thus put a stop to the formation of impulses that might have started automatically to arise there.

Haberlandt, in certain cases, obtained funnel rhythm by stimulation of the vagus or of the funnel alone. This seems to have been in preparations which had previously been long and severely stimulated. It has also been observed in the present investigation that in old preparations the ability of the funnel to initiate rhythm is relatively increased, due to the weakened condition of the Si impulses. Haberlandt's results of obtaining funnel rhythm when the vagus alone is stimulated offers substantiating evidence of the relative increase in the ability of the funnel to form automatic and rhythmic impulses when the Si impulses are weakened. It speaks for the function of the funnel rhythm in cases where the Si impulses are not able to initiate the rhythm of the heart. It has also been observed during the course of the present investigation that as has often been observed before, the funnel rhythm may arise automatically in hearts which have been exposed for some time. Furthermore Laurens (1) has shown that in the still (sinusless) heart funnel rhythm is more frequently and easily accomplished than in the excised and beating heart.

The stopping of funnel rhythm by sympathetic stimulation (see figs. 5 and 6), falls in line with the explanation given above of the action of the sympathetic in improving A–V block, and the reverse of that just given for the influence of the vagus on producing funnel rhythm. As mentioned above the sympathetics stimulated conjointly with the funnel, or conjointly with the vagi, never led to the production of funnel rhythm. So far as our evidence goes, it points to an exclusive distribution of sympathetic fibers, so far as impulse formation is concerned, to the normal pacemaker, or sinus. By their stimulation the strength of the normal Si impulse is increased. Conductivity being also increased, the normal Si impulses come to the V with increased vigor, thus blotting out any attempts on the part of the funnel to automatically form rhythmic impulses, or when these impulses have been formed stopping them, and assuming again the initiation of the heart beat.

In this investigation sympathetic stimulation failed but once to reverse the sequential beat to normal.

It seems therefore that, in the turtle, vagal stimulation aids in the formation of an automatic funnel rhythm by inhibiting the Si impulses, either by decreasing them or decreasing the conductivity of the muscle. Sympathetic stimulation, by increasing the strength of the Si impulses and by improving conduction, prevents the formation of an automatic funnel rhythm by swamping with Si impulses the impulses which might begin to arise automatically in the funnel. Vagal stimulation does not

affect funnel rhythm after it has gotten started, but, by decreasing the conductivity and the contractility of the A muscle, decreases the strength of the A contractions. Sympathetic stimulation causes a reversal of funnel rhythm to a normal Si rhythm by its influence on the strength of the Si impulse and on the conductivity of cardiac muscle.

No very close comparison regarding the differential influence of the right and left nerves on "nodal rhythm" can, therefore, be drawn between the results that have been obtained on the mammalian heart and those obtained on the turtle heart.

SUMMARY

1. In Malacoclemmys geographica, the vagus and sympathetic nerves run separately in the neck and are not united to form a common vago-sympathetic trunk.

2. The sympathetic rami cardiaci come principally from the first thoracic ganglion.

3. Stimulation of the sympathetic results generally in an increase in strength of Si impulses, and an acceleration of rate of production, the former being more often obtained.

4. Stimulation of the vagus causes an increase in auricular tone. Stimulation of the sympathetic decreases and abolishes it. (Variations in tone obtained in but one case.)

5. Stimulation of the sympathetic, by improving conduction, decreases heart block produced by cutting the A–V connection.

6. Stimulation, in the intact heart, of the A–V funnel by strong interrupted currents causes V fibrillation, or rapid V rhythm, which stops when the stimulation is stopped.

7. Conjoint stimulation of the right vagus and of the A–V funnel with strong interrupted currents sometimes produces a funnel rhythm which lasts over, in different experiments, for varying lengths of time, after the stimulation has been discontinued.

8. Vagal stimulation during funnel rhythm with a strength of current sufficient to normally still the heart decreases the height of the A contractions but does not affect the rate. Stimulation of the sympathetic, by increasing the strength of the Si impulses and improving conduction, stops funnel rhythm, after which the normal sequential beat returns.

9. Conjoint stimulation of the funnel and sympathetic does not produce funnel rhythm, nor does conjoint stimulation of either sympathetic and one of the vagi do so.

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THE CONDITIONS DETERMINING THE RATE OF ENTRANCE OF WATER INTO FERTILIZED AND UNFERTILIZED ARBACIA EGGS, AND THE GENERAL RELATION OF CHANGES OF PERMEABILITY TO ACTIVATION

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In his recent book, "The Organism as a Whole," Professor Loeb raises an apparently serious objection[1] to my interpretation of the observation (recently made by me) that in dilute sea-water fertilized Arbacia eggs take up water by osmosis several times more rapidly than unfertilized eggs.[2] This phenomenon had seemed to me to indicate that the permeability of the egg-surface to water is decidedly increased by fertilization, and hence to confirm the view that a general increase of surface-permeability is an essential factor in the activation-process, a view which Professor Loeb appears to share; yet he dismisses the above observation in a footnote as unimportant, and suggests that the increased entrance of water after fertilization is due to the removal of the layer of viscous material or "jelly" which normally invests the unfertilized egg and disappears at fertilization. Apparently in his opinion it is this jelly—and not the relatively impermeable state of the egg-surface—which retards the entrance of water into the unfertilized egg; and he concludes that no inference can be drawn from this phenomenon with regard to any change in the condition of the egg itself. An objection of this kind is best removed by experimental evidence, which is presented below. I may remark, however, that the observation does not stand alone, but is supported by the results of a number of other investigators cited in my paper, all indicating that an increase of permeability is associated with fertilization. It seems therefore to me that this additional evidence ought not to be thus put aside on purely conjectural grounds; experiment

[1] The organism as a whole from a physico-chemical viewpoint. Putnams, 1916, 122.

[2] This Journal, 1916, xl, 249.

alone can decide in such cases; and the further observations which I have made during the past summer have shown definitely that the above objection has no basis; in point of fact the jelly does not interfere appreciably with the entrance of water into the egg.

To avoid misunderstanding regarding the essential nature of the question at issue, it seems necessary, before proceeding with the discussion, to distinguish clearly between two separate possibilities with reference to the relation which changes of permeability may bear to the activation-process. The first possibility is that after fertilization, and as one of the secondary consequences of this process, the general conditions of permeability in the egg are permanently modified, and that the protoplasmic surface-layer or plasma-membrane from that time on remains more permeable and more subject to changes of permeability than before; there is, in fact, considerable evidence that this is the case.[3] The second possibility—which is the one suggested by the resemblance between the conditions of activation of the resting egg and those of stimulation in general—is that the primary event in the activation-process, as well as in the stimulation-process, is a temporary increase of permeability; upon this initial change follow the other changes expressive of the general response or activation of the egg-cell. A *temporary* or initiatory increase of permeability is thus to be distinguished from a *permanent* alteration in the general properties of the plasma-membrane involving increased permeability. There is good reason to believe that both of these processes are concerned in the activation of the resting egg. This distinction, however, does not appear to have been made hitherto, and confusion may be avoided by recognizing it.

In Professor Loeb's earlier paper on the action of salt solutions upon fertilized and unfertilized eggs of Strongylocentrotus[4] he de-

[3] My own observations show that the rate of entrance of water from dilute sea-water remains several times greater in fertilized than in unfertilized Arbacia eggs, at least until the second or third cleavage, and probably later. Similar conditions hold for the electrical conductivity of the fertilized eggs of Echinus, according to the recent observations of Gray. After an initial increase of conductivity immediately following fertilization, the conductivity returns (within ten or fifteen minutes after fertilization) toward that of the unfertilized egg (Journ. Mar. Biol. Assoc., 1913, x, 50). Further investigation has shown, however, that this second change of conductivity is temporary and small compared with the original increase, and that the conductivity of the fertilized eggs always remains decidedly greater than that of the same eggs before fertilization (unpublished observations kindly communicated to me by Mr. Gray).

[4] Biochem. Zeitschr., 1906, ii, 81.

scribes experiments showing that pure solutions of NaCl are more toxic to fertilized than to unfertilized eggs; and he suggests, as a possible explanation of this fact, that fertilization may increase the permeability of the egg to NaCl or its ions; this explanation, however, he rejects in favor of one based upon the difference in the rate of oxidations in the two kinds of egg; the more rapidly metabolizing fertilized egg is more readily injured by any condition, such as lack of oxygen or presence of cyanide, which interferes with oxidations; and its greater susceptibility to injury by salt solutions probably has the same basis, since alkali is found to promote and cyanide to retard the toxic action. It is to be noted that these two explanations are in no sense inconsistent with each other; an increased rate of oxidations may well be associated with, or even dependent upon, an increased permeability of the egg-surface, *e.g.*, to oxygen. And in fact in a later publication Professor Loeb suggests that an increased permeability to oxygen or OH-ions, resulting from the surface-alteration, may be a factor in the increase of oxidations following fertilization.[5] This hypothesis is difficult to test experimentally and remains conjecture, although, for reasons to be given below, it seems probable that this condition actually does exist in the sea-urchin egg.

My own first experiments on the relation of permeability-change to activation were suggested by the idea of a general parallelism between the conditions of activation in resting eggs and of stimulation in irritable tissues. In stimulation there appears to be a temporary increase in the permeability of the plasma-membrane; it seemed therefore probable that a change of the same kind might be the critical event in activation. If this be so, a parallelism should exist between the permeability-increasing action of a substance and its general effectiveness as an activating agent. This view is therefore supported by the fact, previously shown by Loeb, that cytolytic substances are in general good parthenogenetic agents; obviously such substances increase surface-permeability. My own earlier experiments with Arenicola larvae had shown that pure isotonic solutions of Na and K salts increase the permeability of the pigment-containing body-cells and at the same time powerfully stimulate the musculature of these organisms; both effects are simultaneously prevented by the addition of $CaCl_2$.[6] It seemed therefore probable, if an increase of permeability is the initial

[5] Chemische Entwicklungserregung des tierischen Eies, Berlin, 1909, 16, preface.

[6] This Journal, 1909, xxiv, 14.

or critical factor in the activation of the resting egg, (1) that pure salt solutions would cause activation, and (2) that this effect would be prevented by the addition of $CaCl_2$ to the salt solution. In experiments with starfish and sea-urchin eggs both of these expectations were realized. As index of permeability-increase in Arbacia eggs the exit of pigment was employed; and it was found that those salts which freed pigment most rapidly were in general the most effective as activating agents; the order of relative effectiveness for both permeability-increase and activation, with pure isotonic solutions of Na and K salts, was (for anions): $Cl < Br < NO_3 < CNS$ and I.[7] In the presence of a little $CaCl_2$ (1 mol to 20 of alkali salt) both effects were prevented;[8] a similar though less complete prevention of this salt-action was later obtained by the use of various anaesthetic compounds[9]—a fact still further confirming the view that stimulation and activation of the resting egg are closely analogous processes.

Other evidence indicating that an increase of surface-permeability is the primary change in activation is briefly reviewed in my paper above cited,[10] which also again calls attention to the possible importance of the change of electrical surface-polarization presumably accompanying this increase. Negative electromotor variations are well known to result from the action of cytolytic (*i.e.*, parthenogenetic) agents upon other types of cell (*e.g.*, muscle or nerve) where observation can be made; and the same is probably true for cells in general, including the egg-cell; if so, we must assume that the activating agent causes a temporary electrical depolarization of the egg-surface. Electromotor variations appear to be common to all forms of stimulation and spontaneous cell-activity; and the universal action of external electric currents in accelerating, inhibiting, or otherwise modifying cell-activities shows that intracellular processes, metabolic and other, are profoundly influenced by changes in the electrical polarization of the cell-surface. Hence it seems reasonable to conclude that the initial electromotor variation induced by the surface-alteration is an essential if not the chief factor in the activation-process. This view does not deny the possible importance of other factors in the total complex process; it merely supposes that the sequence of events constituting activation is initiated or released by the primary surface-process with

[7] This Journal, 1910, xxvi, 106.
[8] This Journal, 1911, xxvii, 289; Journ. of Morph., 1911, xxii, 695.
[9] Journ. Exper. Zoöl., 1914, xvi, 591.
[10] This Journal, 1916, xl, 249.

its accompanying electromotor variation. Probably also, as already suggested, a general and permanent increase of permeability is one of the secondary consequences of activation, and itself becomes a factor in the later phases of the process, *e.g.*, by allowing readier interchange of oxygen and carbon dioxide (and possibly other substances), and so permitting a higher rate of metabolism. My own recent observations show that the increased permeability to water following fertilization in Arbacia eggs is not temporary, but remains (as indicated by the rate of swelling in dilute sea-water) at least until the second or third cleavage, and probably later;[11] similar conditions may exist with regard to the permeability to oxygen and carbon dioxide.

Early last summer Professor Loeb pointed out to me the possibility that the jelly surrounding the unfertilized eggs might interfere with the entrance of water; and I regret that I did not inform him of the results of the following experiments soon enough to prevent the appearance of this criticism in his book. The existence of a layer of viscous substance about freshly deposited Arbacia eggs, and its dissolution after fertilization, are well known facts; and quite conceivably the difference in the osmotic behavior of fertilized and unfertilized eggs might be explained in this simple manner, and not as due to a change in the properties of the egg itself. This jelly often has the thickness of an egg's diameter, but its consistency is so thin that it does not differ appreciably in refraction from sea-water nor prevent the eggs from coming in contact with one another; it is best demonstrated by the use of suspensions of India ink in sea-water; when mounted in this medium under a cover-glass each egg appears surrounded by a clear halo. One might doubt beforehand that any gelatinous coating of such slight consistency would decrease the rate of entrance of water to one-fourth of the value obtaining in its absence; such an effect would indicate that the jelly was more impermeable than the egg itself;[12] this consideration may help to account for my neglect to consider its influence in my former paper.

The jelly is easily removed from the eggs by washing in ordinary sea-water, but this treatment has no effect upon the osmotic behavior

[11] See footnote 3 above.

[12] Diffusion-rates in dilute jellies are known to be practically the same as in pure water (*cf.* Höber: Physikalische Chemie der Zelle und der Gewebe, 4th Edition, 1914, 346). A further indication that the jelly offers no obstacle to diffusion is the fact that there is little if any difference in the rate of staining of fertilized and unfertilized Arbacia eggs in solutions of neutral red in sea-water.

in the dilute medium. The following observations made last summer will illustrate:

September 4. Eggs were removed from several specimens of Arbacia at 9.30 a.m., washed as usual with two changes of sea-water, and examined in India ink suspension. Most eggs showed the presence of the jelly by the clear halo; the diameter of this halo was variable, in some cases equal to that of the egg, in others the jelly formed a thin film merely, while in a good many eggs it was absent. Out of 62 eggs examined at random at 10.30, 46 had a well-defined jelly; in 16 it was either absent or insignificant.

Part of these eggs were fertilized at 10.10, and the sea-water was again changed. Examination at 10.28 showed that the great majority but not all of the fertilized eggs had lost the jelly; about 5 per cent retained a thick coating like that of unfertilized eggs; these exceptional eggs all showed the typical fertilization-membrane, outside of which was the jelly; in the other eggs the India ink was in direct contact with the membrane.

It should be noted that the thickness of the jelly about unfertilized eggs is variable, but that there is no corresponding variability in the rate of osmotic swelling, which is uniform in all eggs. The same is true of fertilized eggs; although the jelly is retained in a few of these, all show the same rapid rate of swelling; evidently if the jelly hindered the entrance of water, a small proportion (the 5 per cent or so retaining the jelly) ought to swell slowly like unfertilized eggs.

Eggs from the above lot were washed by six successive changes of sea-water (with intervals of ten to fifteen minutes between washings); examination then showed them to be almost entirely free from jelly; a few, however, (less than 5 per cent), retained the typical coating. Control eggs from the same lot left unwashed and examined at the same time (11.30) showed jelly in the great majority. The jelly disappears slowly if the eggs are allowed to stand in sea-water; five hours after removal from the animals it remained in only about 35 to 40 per cent of all eggs.

That the change in the rate of swelling after fertilization is quite independent of the presence or absence of jelly is shown by the following experiment.

Part of the above eggs which had been freed from jelly by washing were fertilized at 11.43. At 12.05 unfertilized and fertilized eggs of this lot were placed separately in dilute sea-water (60 volumes tap-water *plus* 40 volumes sea-water). Within two minutes the difference in size was apparent, the fertilized eggs being distinctly the larger. After four minutes (at 12.09) eggs from the two lots were mixed and examined in a watch-glass as before; the contrast between the smaller denser unfertilized eggs and the larger paler fertilized eggs was then most striking; later as the osmotic equilibrium was neared in both eggs the difference became less marked. After twelve or thirteen minutes cytolysis had begun in the unfertilized eggs.

The essential results of these observations may be summarized as follows: (1) The jelly is variable in freshly shed eggs and absent in a good proportion; nevertheless the rate of swelling is remarkably uniform; the variability in the size of the eggs, after remaining several minutes in the dilute medium, appears no greater than in normal sea-water. (2) The jelly remains in a small proportion of fertilized eggs, yet all such eggs swell at the same rapid rate; no exceptional slowly swelling eggs are found. (3) After washing until the jelly is completely removed in most eggs the effect of fertilization on the rate of swelling is precisely the same as before.

It is clear therefore that the increased rate of entrance of water into the egg after fertilization is not due to the removal of an external impeding layer of jelly, but must be referred to a change in the egg itself, and most probably to a change in the properties of the limiting protoplasmic layer or plasma-membrane which controls the osmotic exchange. These observations indicate a change in the plasma-membrane in the direction of greater permeability to water. Professor Loeb remarks: "it is difficult to understand how this observation can throw any light on the mechanism of development, since water diffuses rapidly enough into the unfertilized egg." It *is* probable that the entrance of water and other substances is rapid enough for the requirements of the slowly metabolizing unfertilized egg; but the measurements given in my paper show that, as regards water at least, the rate of entrance is somewhat surprisingly low (see footnote, p. 257); and I regard it as probably a significant matter that the membrane should be thus relatively "waterproof" previously to fertilization. There is no known correlation between the permeability to water and that to oxygen and carbon dioxide; but if a general parallelism exists, as seems not improbable, it is clear that a relatively dense and impermeable plasma-membrane must at least restrict the possibilities of oxidation within the egg, since the rate of this process is limited by the rate at which oxygen can diffuse into the egg and carbon dioxide leave it. The presence of a relatively impermeable surface-layer will tend to keep down the rate of any intracellular process, like metabolism, which depends upon interchange between the cell and its surroundings. Resting eggs and other germs, *e.g.*, seeds and spores, in which the rate of metabolism is low—as shown by their ability to live through long resting periods—are very frequently enclosed in resistant or waterproof coverings; and the case of the unfertilized sea-urchin egg may belong in this general category.

A permanent increase of permeability to oxygen after fertilization may thus be necessary in many eggs in order to provide for the resulting increase in oxygen-requirements. According to Loeb and Wasteneys[13] the relative rates of oxygen-consumption in the unfertilized and fertilized eggs of Strongylocentrotus purpuratus are as one to four or five; artificial membrane-formation is followed by a similar increase. It is noteworthy that the increase of permeability to water following fertilization in Arbacia eggs is of a similar order, and that here also artificial membrane-formation has the same effect as fertilization; this correspondence suggests that permeability to water may be an approximate index of permeability to oxygen. Most probably the increase in oxidations is rather an accompaniment or consequence of activation than its necessary determining condition or cause. Activation appears to influence oxygen-consumption very differently in different eggs; thus in the starfish egg Loeb and Wasteneys found no significant change after fertilization,[14] while in Strongylocentrotus lividus Warburg found a tenfold increase.[15] A mere change in the conditions of permeability would seem insufficient to account for such an effect. It is more likely that the increased permeability after fertilization is merely one expression of a fundamental modification in the general properties of the protoplasmic surface-layer, and that this latter change of state forms the necessary condition of the subsequent changes in the physiological activity of the egg, including, besides the visible formative or developmental processes, the greater rate of oxidation and of general metabolism. We know that after fertilization the plasma-membrane is capable of undergoing those periodic variations in its physical condition which are associated with cell-division,[16] and even its mechanical properties appear to be different from before;[17] these changes of property and activity may largely determine or control the general physiological processes in the egg as a whole.

A brief reply to another of Professor Loeb's criticisms may be not irrelevant here, since the problems under discussion in his chapter are all closely interrelated, and the physiological significance of the various

[13] Loeb and Wasteneys: Journ. Biol. Chem., 1913, xiv, 469.

[14] Loeb and Wasteneys: Arch. f. Entwicklungsmech., 1912, xxxv, 555.

[15] Warburg: Zeitschr. f. physiol. Chem., 1910, lxvi, 305.

[16] The resistance of the plasma-membrane of Arbacia eggs to disruption in dilute sea-water undergoes a regular and marked decrease at the times of cytoplasmic division; *cf.* Journ. Exper. Zoöl., 1916, xxi, 369.

[17] *Cf.* footnote on page 264 of my paper in this Journal, 1916, xl.

processes under consideration is still largely obscure. In the part of his discussion dealing with the action of hypertonic sea-water[18] he adduces a number of facts which appear inconsistent with the hypothesis, formerly put forward by me, that this action is exerted essentially upon the plasma-membrane of the egg, and consists in restoring to this structure, whose permeability has been increased by the initial or membrane-forming treatment, the original or normal permeability.[19] According to this hypothesis, the treatment with hypertonic sea-water *reverses*, by means of its action on the plasma-membrane, the effect of the initial increase of permeability, which otherwise would lead to the disintegration of the egg; hence the "corrective" or life-saving effect of the treatment. The fact, however, that the exposure to hypertonic sea-water may precede, even by many hours, the membrane-forming treatment,[20] shows that for the assumed reversal of properties or restitution of the membrane a *subsequent* treatment is unnecessary; evidently the same kind of regenerative change takes place spontaneously in eggs which have been treated previously with hypertonic sea-water. I am of opinion that the hypothesis suggested in my recent paper on parthenogenesis in starfish eggs[21] may throw light upon this whole question of the mode of action of hypertonic sea-water; and that it will also explain, consistently with the hypothesis which Professor Loeb attacks—while upholding one which in certain essentials is very similar in character—why the treatment with hypertonic sea-water may either precede or succeed the membrane-forming treatment.

In considering this question it must be remembered that in some eggs, notably the starfish egg, no second treatment with hypertonic sea-water is necessary; a single properly timed exposure to acid-containing sea-water or high temperature results in complete activation.[22] The return of the plasma-membrane to the normal condition after the initial increase of permeability evidently takes place spontaneously in such eggs. In the sea-urchin egg, however, this is not the case; the egg undergoes cytolysis unless treated also with hypertonic sea-water. This suggests that in the latter egg the material required for the resto-

[18] The organism as a whole, iii, *seq.*; *cf.* 120.

[19] This Journal, 1911, xxvii, 289; Journ. of Morph. 1911, xxii, 695; Journ. Exper. Zoöl., 1913, xv, 23.

[20] Loeb: Artificial parthenogenesis and fertilization, Chapter xi; Arch. f. Entwicklungsmech., 1914, xxxviii, 409.

[21] Biol. Bull., 1915, xxviii, 260., *cf.* 300.

[22] Lillie: Biol. Bull., *loc. cit.*; Journ. Biol. Chem., 1916, xxiv, 233.

ration or regeneration of the surface-film is lacking, but may be produced under the influence of the hypertonic sea-water, *i.e.*, under conditions which abstract water from the egg. The question is why abstraction of water should favor the production of this material. A possible relation between dehydration-processes and the synthesis of structure-forming material seems indicated. In the paper cited above it was pointed out that an even partial dehydration of the egg-substance would in itself be a condition favorable to many intracellular syntheses, since such syntheses are for the most part dehydrolytic in character, and hence will be promoted by any condition tending to reduce the concentration of water at the regions where the condensation is taking place. For example, such a dehydrolytic synthesis as that of glycerol oleate from glycerol and oleic acid is greatly interfered with by the presence of water in the reaction-mixture;[23] hence abstraction of water favors this synthesis; and the same may be assumed to be true for other syntheses depending on dehydration.[24] The rapidity of such syntheses in cells indicate that some powerful dehydrative agency is active during life; the nature of this is unknown; one might conceive of alternate oxidation and reduction as one possibility; or the water-abstracting agency may be a physical process, or one of a more complex physiological kind (like the water-abstracting mechanism of the kidney). But whatever its nature, any other process working simultaneously in the same direction, like osmotic abstraction of water, will tend to supplement and hence to further its action—provided of course there is no interference between the two processes.[25]

It is possible that the dehydrating and hence the synthesizing power of the cell-protoplasm is temporarily below the normal in sea-urchin eggs in which artificial fertilization-membranes have been formed; and that a supplementary dehydration, such as that due to hypertonic sea-water, is required to enable the necessary syntheses to take place. In this way the materials needed for the reconstruction of the impaired or

[23] *Cf.* Armstrong and Gosney: Proc. Roy. Soc., Ser. B, 1914, lxxxviii, 176.

[24] The formation of polypeptide-like colloidal compounds by the condensation of mixtures of amino-acids and amides by means of dehydrating agents (P_2O_5, PCl_5) was accomplished long ago by Grimaux, Schützenberger and Pickering.

[25] In my paper in the Biol. Bull. (*loc. cit.*, 300 seq.) various biological facts are cited indicating that syntheses are promoted in other types of cell by abstraction of water. The results of Pavy and Bywaters on the synthesis of glycogen in yeast cells in differently concentrated sugar solutions seem especially significant in relation to the present hypothesis (Journ. of Physiol., 1907, xxxvi, 149).

over-permeable plasma membrane are furnished, and the latter regains its normal condition. It is only necessary to assume that a reserve of such materials is present in eggs that have previously been treated with hypertonic sea-water, in order to account for the beneficial effect of such treatment. Such eggs have already been brought into a condition in which they are no longer deficient in the necessary constructive materials; in this respect they are then similar to starfish eggs, which develop after membrane-formation without any second treatment. Hence in such pre-treated eggs the cell-division process proceeds normally after membrane-formation; while in untreated eggs it soon stops short, under conditions that suggest a lack of the material needed to reconstitute the plasma-membranes; this last is indicated by the fact that irregular changes of form and imperfect cleavage, soon followed by cytolysis, are characteristic of such eggs.

The characteristic behavior just described has been interpreted by Loeb as indicating that artificial membrane-formation "leaves the egg in a sickly condition in which the very processes leading to cell-division bring about its destruction;"[26] recovery from this condition is promoted by hypertonic sea-water. This conception seems to me especially interesting because of its suggestion that a change specifically associated with cell-division is the destructive factor. I have shown recently that during the time of normal cytoplasmic division the plasma-membrane of the Arbacia egg loses its former coherence and tenacity—so that the egg loses temporarily its resistance to destruction by dilute sea-water—and regains these properties after cleavage is completed.[27] In other words, at each cell-division the membrane passes through a cycle of physical or structural change involving first a loss and then a restoration of the properties which it possesses in the intervals between cleavages. The prompt return of resistance after the cleavage-furrow is complete indicates clearly that a reverse or reconstructive process takes place in the surface-film at the close of each division; there is evidence of a similar process after the initial surface-change of normal activation, as already pointed out. We may assume that if the material required for this reconstruction at any cell-division is insufficient or lacking only the first part of the division cycle can be carried out normally; the plasma-membrane then remains in the unstable and over-permeable condition shown during the formation of the furrow, with the result that breakdown soon follows;

[26] The organism as a whole, 115.

[27] Journ. Exper. Zoöl., 1916, xxi, 369.

such a view accounts for the failure of cell-division to continue in eggs which have been subjected to the membrane-forming treatment alone. The division-process itself, since it involves as its first phase a disintegrative or permeability-increasing change in the plasma-membrane, acts destructively on such eggs. The need of a synthetic process immediately succeeding each division may thus form the reason why the hypertonic treatment enables cell-divisions to continue. The material required to reconstitute the membrane after each division may be the same as that synthesized under the influence of the hypertonic sea-water; but it seems more probable that a new synthesis is necessary for each division, and that the beneficial action of the treatment upon the succeeding divisions is exerted somewhat indirectly. Presumably what is essential is that the initial reconstruction—that following the separation of the fertilization-membrane—should be *complete*, and should bring the plasma-membrane into an entirely normal condition. Then the succeeding divisions, each of which presumably has its own specific synthesis associated with it, may be carried out normally; and development proceeds as far as external and other conditions permit.

To recapitulate briefly: what is essential in the effect of the hypertonic treatment is not that it should follow or precede the membrane-forming treatment, but that it should rectify a deficiency in the supply of certain structure-forming materials in the egg, which are required for the reconstitution of the plasma membrane. This material is synthesized by a dehydrolytic process, hence is furthered by abstracting water from the egg.

I wish to point out here—since our conclusions regarding this subject have evidently been reached independently—that this hypothesis is quite consistent with one put forward by Professor Loeb in his book on Artificial Parthenogenesis and Fertilization. He suggests that the effect of the hypertonic solution is possibly "due to the formation of a definite substance which is retained by the egg and which is a preventive against the disintegration following membrane-formation."[28] But in pointing out this correspondence in our views I have no wish to disclaim responsibility for the more special features of the hypothesis summarized in the last paragraph. The view that the disintegration following membrane-formation is due to the persistence of a state of increased permeability resulting from the membrane-forming treatment, is essentially a corollary of the more general conceptions

[28] Loeb: Artificial parthenogenesis and fertilization, 121.

which I have long held regarding the characteristic properties and physiological significance of the plasma-membrane of cells. If an initial increase of permeability is the critical process in the activation of the resting egg—as apparently also in the analogous process of stimulation— it is clear that the condition of increased permeability cannot be permanent; a reverse change must follow, *i.e.*, a return to the semi-permeable and polarized state of the plasma-membrane, if the egg is to remain living. Otherwise diffusion-processes will soon cause the disintegration of the cell. It thus seems reasonable to infer that if the initial change in activation involves as its most essential feature a surface-alteration, so also must the second or "corrective" change. Loss of semipermeability in any cell must lead to cytolysis unless the semipermeable condition is regained soon—or at least before disintegration and loss of protoplasmic constituents have proceeded to an injurious degree. The view that the corrective effect of hypertonic sea-water is thus due primarily to its action upon the cell-surface, and consists in restoring to the latter its original semipermeability, is at least a logical one; evidently a reconstitution of the altered surface-film must result in some manner from the treatment; this effect may be indirect, as suggested above, but at least it is indispensable to the continued life of the egg, and for this reason must be regarded as the essential effect of the treatment.

The fact that anaesthetics and lack of oxygen prevent the hypertonic solution from exerting its usual action[29] is consistent with the foregoing view. Anaesthetics are well known to prevent growth and cell division,[30] and it is to be presumed that their interference with the action of hypertonic sea-water has some connection with their power of preventing growth. Probably in both cases they render impossible certain necessary constructive processes, both chemical and structural. There is now much evidence for the theory that anaesthetic action consists essentially in a certain reversible modification of the plasma-membrane; this structure is rendered temporarily more resistant to modification of any kind, hence all processes in which the membrane actively participates (stimulation, growth etc.) are retarded or pre-

[29] *Cf.* Loeb: Artificial parthenogenesis and fertilization, Chapter xi. That anaesthetics inhibit the action of hypertonic sea-water upon Arbacia eggs is shown in my recent paper in Journ. Exper. Zoöl., 1914, xvi, 591.

[30] *Cf.* Claude Bernard's experiments upon the anaesthesia of growth in "Leçons sur les phénomènes de la vie," i, 267. For the influence of anaesthetics upon cell-division in Arbacia eggs *cf.* my recent paper in Journ. Biol. Chem., 1914, xvii, 121.

vented.[31] The fact that anaesthetics prevent the action of hypertonic sea-water is a further indication that this action consists in some modification of the plasma-membrane; possibly both the synthesis of the materials needed for the reconstitution of the surface-protoplasm and their deposition in this layer are impossible in the presence of the anaesthetic. Some chemical combination evidently underlies the action of hypertonic sea-water, as Loeb first pointed out; this is indicated clearly by the temperature-coefficients; and the further fact that oxygen is necessary for this action and that it is prevented by cyanide indicates that oxidations are essentially concerned.[32] Possibly the syntheses are dependent on oxidation-processes, as in the oxidative syntheses of Schmiedeberg.

The fundamental physiological processes concerned in the activation of the resting egg are probably common to all living cells, although in the details of their manifestation they vary from cell to cell. In particular the existence of a highly developed regulative property in the plasma-membrane must always be assumed; if the integrity of the cell is to be preserved, any alteration involving injury to the membrane must be followed by a reverse or regenerative process which restores semipermeability. In all such membrane-processes metabolism enters; and the combination of metabolic construction and destruction postulated by Claude Bernard as essential to all life-processes is as necessary for the maintenance of the normal properties of the plasma-membrane as for that of any other living structure. Thus the assumption that in the fertilized egg-cell a reverse or reconstructive change takes place in the surface-film after membrane-formation is consistent with the facts of cell-physiology in general. Similarly at each cleavage the same condition appears to exist; during cytoplasmic division the plasma-membrane loses its former coherence and consistency, and

[31] The evidence for this theory is discussed at length in my recent article on "The theory of anaesthesia" in the American Yearbook of Anaesthesia, 1916; also in Biol. Bull. 1916, xxx, 311.

[32] *Cf.* Loeb: Artificial parthenogenesis and fertilization, Chaper xi. In considering the mode of action of hypertonic sea-water the fact that it may cause complete activation, acting alone, must not be forgotten. This fact, however, is not inconsistent with the above point of view; quite possibly, as Professor Loeb suggests (organism, 122), the initial effect of treatment with this agent may be membrane-forming (or what corresponds), and only the later effect "corrective." The sudden change of osmotic pressure when the eggs are first placed in this medium may act in a manner analogous to that of osmotic stimulation; later the general reparative or synthesis-favoring influence has time to assert itself, and the cycle of surface-change is completed.

recovers these properties when division is completed; this recovery is also to be regarded as the expression of a synthesis and redeposition of the necessary structural materials in the surface-layer.[33]

It may not be superfluous to call attention once more to the resemblance between these conditions and those of stimulation in general. In most irritable elements (nerve, muscle, etc.) the electromotor variation is the only indication of a surface-change associated with stimulation; but a reversible alteration of the surface-film must be assumed to occur in this case, just as in the dividing egg-cell; and presumably it is accompanied, as in the latter case, by a destruction and subsequent redeposition of structural material. In irritable elements the determining conditions for these two reverse processes appear to be electrical; when the living cell forms part of an electrical circuit, there is depolarization where the positive stream leaves the cell, and increased positive polarization (or repolarization) where it enters; at the former region excitation is aroused, at the latter it is inhibited. This general condition indicates that electrical factors—probably electrolytic in character since the two polar effects are opposite in character—play the determining part here.[34] Conditions in one type of cell often throw light upon those existing in another type where observation of the same kind is not possible; the recognized importance of the electrical factor in stimulation indicates that in other cell-processes, like the activation of the resting egg and cell-division, it may be equally important. Further discussion of this subject must be deferred for the present.

[33] The following quotation from Bernard's "Leçons sur les phénomènes de la vie" (i, 127) is singularly apposite here (I translate freely): "The process of disorganization or disassimilation uses up or destroys the living substance in functioning organs; the process of assimilative synthesis regenerates the tissue; it reassembles or restores the reserve-substances which are destined to be consumed in future activity. These two inverse processes of destruction and renovation are absolutely and inseparably interconnected, at least in the sense that the destruction is itself the necessary condition of the renovation. The phenomena of functional breakdown in living material are themselves the precursors and instigators of the renovation accomplished by the formative process, which works silently and obscurely in the interior of the tissue. Losses are thus repaired as rapidly as they are caused; and since equilibrium tends to re-establish itself as soon as it is destroyed, the normal composition of the living body is maintained." In this process of restitution, as Bernard points out elsewhere, both a chemical synthesis and a morphological synthesis—*i.e.*, a reformation of organized structure—take part.

[34] *Cf.* my recent paper on the physical chemistry of the conduction of the excitation-state, in this Journal, 1916, xli, 126.

THE EFFECT OF STARVATION ON THE CATALASE CONTENT OF THE TISSUES

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When an animal is starved or is supplied with an insufficient amount of food to meet the wear and tear and energy requirements of the body, the tissues themselves are consumed. The extent of this consumption differs very widely in the different organs. The heart, for example, loses very little in weight while the skeletal muscles lose much, the fat and glycogen completely disappear. The organs in which metabolism is most intense, such as the heart and central nervous system, preserve themselves best while the organs in which metabolism is less intense waste away. The preservation of the working tissues is thought to be brought about by the autolysis of the other less active tissues. The products of autolysis of these less active tissues pass into solution in the blood, are carried to the master tissues and used. The object of this investigation was to determine what change, if any, occurs in the catalase content of the heart, skeletal muscles and fat during starvation with the hope of finding an explanation for the fact that the heart muscle is not autolyzed during starvation while the skeletal muscles and fat are.

Twelve rabbits were placed in a cage and fed for six days on cabbage, turnips and apples. At the end of this time two of the rabbits were etherized and the blood vessels washed free of blood by the use of large quantities of 0.9 per cent sodium chloride. The heart, soleus muscle (red muscle) and fat around the kidney were removed and ground up separately in a hashing machine. The catalase content of these tissues was determined by adding one gram of the material to 45 cc. of hydrogen peroxide in a bottle. As the oxygen gas was liberated it was conducted through a rubber tube into an inverted burette previously filled with water. After the oxygen gas thus collected was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in one gram of the tissue.

A full description of the method is given in a previous publication (1). The catalase content of the heart, soleus muscle and fat of rabbits starved for two, four and six days respectively was determined as it had been for the normal rabbits. The results of these determinations are given in table 1. Each of the determinations represents an average for two animals.

TABLE 1

After heart, leg and fat are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 45 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of rabbits

RABBITS	NORMAL	STARVED TWO DAYS	STARVED FOUR DAYS	STARVED SIX DAYS
Heart	73	71	75	75
Leg	72	58	54	44
Fat	33	13	12	No fat

It may be seen in table 1 that one gram of the heart muscle of the normal rabbit liberated 73 cc. of oxygen in ten minutes from 45 cc. of hydrogen peroxide; that of the rabbits starved for two, four and six days liberated 71, 75 and 75 cc. of oxygen respectively; that 1 gram of the leg muscle of the normal rabbit liberated 72 cc. of oxygen; that of the rabbits starved for two, four and six days liberated 58, 54 and 44 cc. of oxygen respectively; that one gram of the fat of the normal animals liberated 33 cc. of oxygen, that of the animals starved for two and four days liberated 13 and 12 cc. of oxygen respectively while there was not sufficient fat in the animals starved six days for a determination.

By comparing the amounts of oxygen liberated by the heart of the animals starved for the different lengths of time it will be seen that starvation produced no effect on the catalase content of the heart muscle, that it reduced the catalase content of the leg muscle by 37 per cent as is indicated by the decrease from 72 cc. of oxygen, the amount liberated by 1 gram of the muscle of the normal animals to 44 cc., the amount liberated by the muscle of the animals starved for six days. It may also be seen that the catalase content of the fat was reduced during the first two days of starvation by about 61 per cent as is indicated by the reduction of oxygen liberated from 33 cc., the amount liberated by one gram of fat of the normal animal to 13 cc., the amount liberated by one gram of fat of the animal starved for two

days, and that the catalase content of the fat remained low during the rest of the period of starvation.

The preceding experiments show that the catalase content of fat and skeletal muscles which are autolyzed during starvation is decreased while it remains normal in amount in the heart which is not autolyzed during starvation. It has been shown that the amount of oxidation in a tissue is proportional to the amount of catalase present (1). From this it follows that oxidation is decreased during starvation in tissues such as fat and skeletal muscles in which the catalase is decreased, and remains normally high in a tissue such as the heart muscle. It is known that the autolyzing enzymes in common with other enzymes are destroyed by oxidation (2). The great resistance of the heart muscle to the digestive action of the autolyzing enzymes during starvation may be due to the intense oxidation in this organ, the assumption being that the autolyzing enzymes are oxidized and thus rendered inert. By the great decrease in the oxidative processes of skeletal muscles and fat during starvation the check on the autolyzing enzymes is removed and they are thus left free to digest these tissues.

Conradi (3), Rettger (4), and Effront (5) showed that when bacteria and yeasts were starved by being placed in a physiological salt solution, where there was no food, they were autolyzed. The explanation usually offered for this bacterial autolysis is that "the normal existing autolytic processes are not counteracted by synthesis of new protein material." A more plausible explanation would seem to be that by starvation the oxidative processes are decreased, thus removing the normal check on the autolytic enzymes, with resulting digestion of the cells.

Neuberg (6) found that when cancer tissue was exposed to radium rays the rate of autolysis of this tissue was greatly increased. He also found that the autolyzing enzymes of this tissue were not destroyed as were the oxidizing and other enzymes by the exposure. On the basis of the experiments reported in this paper it is assumed that the great increase observed in the activity of the autolyzing enzymes in the cancer tissue when exposed to radium rays was made possible by the decrease in oxidation in this tissue which in turn was due to the destruction of the oxidizing enzymes by the rays, thus leaving the autolzying enzymes free to digest the cancer tissue.

It has been shown that the resistance to the digestive action of trypsin of unicellular organisms, paramecia, living in a solution of this enzyme can be decreased by decreasing the oxidative processes so that

these organisms are literally digested alive and that they are revived, provided digestion has not proceeded too far, when normal oxidation is restored (7). From these and similar experiments (8) the authors conclude that the means by which living cells protect themselves from being digested by intracellular as well as extracellular enzymes is oxidation.

CONCLUSIONS

From the evidence presented in this paper the conclusion is drawn that the catalase content of the heart, which is not autolyzed during starvation, remains normally high while the catalase content of the fat and skeletal muscles, which are autolyzed during starvation, is greatly decreased. In view of the fact that the catalase content of a muscle is directly proportional to the amount of oxidation in the muscle and that the autolyzing enzymes are destroyed by oxidation, the further conclusion is drawn that the heart is not autolyzed during starvation because oxidation in this organ remains normally intense and thus provides for this oxidation of the autolyzing enzymes and the maintenance of the normal balance between oxidation and autolysis; on the other hand the fat and skeletal muscles are autolyzed during starvation because of the decreased oxidation which leaves the autolytic enzymes free to digest these tissues.

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CHANGES IN THE AMOUNT OF SALIVARY SECRETION ASSOCIATED WITH CEREBRAL LESIONS

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The secretion of an abnormally large amount of saliva has been observed frequently in various organic paralyses and is sometimes considered symptomatic, but no accurate determination of the quantity of secretion in such cases has been made nor has the condition of the salivary reflexes been studied. As it seemed possible that a study of such conditions might contribute something to our understanding of the cortical control of secretion, I gladly took advantage of the opportunity to examine the salivary reflexes in a number of organic cases available at the Government Hospital. The secretion of the parotid gland alone was studied, as there is no satisfactory technique for determining the reflexes of the submaxillaries. The method used for collecting the saliva was that which I have fully described in an earlier paper.[1] Briefly this is accomplished by having a drainage tube attached by suction, over the mouth of Stenson's duct so that the saliva may run out unimpeded through a small tube. The drops falling from the tube vary only slightly in size and a count of their number gives an accurate measure of the quantity of saliva secreted, except in cases where there is a pronounced change in the viscosity of the secretion.

Observations were made upon nine patients with more or less fully developed hemiparesis or hemiplegia. They included (1) tests of the volume of secretion without stimulation; (2) tests of the reflex secretion to ordinary gustatory stimuli; (3) various attempts to induce inhibition of secretion; and (4) tests of possible conditioned or "psychic" reflexes. Brief descriptions of the cases studied are given below, only the important and more pronounced symptoms being included.

[1] Reflex secretion of the human parotid gland. Journ. Exper. Psych., 1916, i, 461–493.

J. E. Female, colored, aged 72. Right hemiplegia of eight years standing. The paralysis was of gradual onset. At the time of the tests the subject showed contracture of the right arm with atrophy of disuse, right leg only slightly involved, hyperactivity of the muscles of the right side of the face during speech, the tongue protruded in the median line, no defect of speech. Wassermann reaction was negative, no history of convulsions. The patient is well nourished and shows no trophic disturbances.

W. S. Male, aged 24. Crossed hemiplegia (syphilitic) of four years standing. The first symptom was a right facial paralysis which cleared up almost at once and was followed in two months by paralysis of the left arm and leg with gradually developing contractures. Tongue is protruded in the median line, the muscles of the left side of the face are atrophied, those of the right somewhat stiff so that only the left side of the mouth contracts when smiling. Both sides are retracted equally in voluntary movement. There are no marked trophic disturbances.

J. S. Male, aged 46. Hemiplegia (syphilitic) of eighteen years standing. Contractures of left arm and leg. There is stiffness and only slight voluntary control of the muscles of the left side of the face. Tongue in voluntary movement is protruded to the right, speech is thickened with occasional drooling of saliva. There have been no convulsions in recent years. The patient is somewhat emaciated.

A. W. Male, aged 35. Hemiplegia (syphilitic) of eight years standing, with aphasia, now partly recovered. There are contractures of the right arm and leg, with but little evidence of facial involvement; the tongue is protruded in median line. The patient is excessively obese but shows no other trophic disturbances.

E. P. Male, aged 37. Hemiplegia (syphilitic) of eight years standing, with complete aphasia. The first symptom was a paralysis of the right side of the face, following a convulsion, from which the patient partly recovered. A second convulsion was followed by a right hemiplegia with left facial paralysis, involving the tongue and soft palate, and with aphasia. At present there are contractures of the left arm and leg, stiffness of the muscles of the right side of the face with tremor of the left. The tongue deflects to the right and can be protruded only slightly. The patient drools saliva continuously. He has had one or two convulsions per year but they are beconing more infrequent. He is excessively obese.

J. E. Male, aged 51. Traumatic spastic paralysis of right side, of seven years standing. History of many convulsive seizures extending to the present time. The right side of the face, the tongue and soft palate are involved in the paralysis. There is occasional drooling of saliva.

J. H. Male, aged 46. Right hemiparesis following trauma, of seven years standing. Convulsions involving right side occurred during the first two years after onset. There is no evidence of facial paralysis. The patient shows a frequent spasmodic laughter during which there is occasional drooling of saliva. The patient is large, well nourished, and shows no trophic disturbances.

V. S. Male, aged 34. Hemiparesis (syphilitic) with contracture of right biceps, of four years standing. Progressive development of weakness of right side. Slurring speech and spasmodic laughter. No evidence of facial involvement. No trophic disturbances.

P. M. Male, aged 35. Hemiplegia (syphilitic) involving left side, of seven years standing. Deteriorated, with restriction of speech to stereotyped sentences but without appreciable motor speech defect. Left side of face and palate show spastic paralysis, and there is a slight atrophy of the left side. Voluntary movement of lips is defective. The patient has an excess of abdominal fat.

All the patients showed an exaggeration of reflexes, with varying degrees of hypertonicity. None showed any malnutrition or evidence of ill health beyond the paralysis or paresis. J. E. had a convulsive seizure two weeks after the examination, but none of the others had given any symptoms of central irritation for a year or more.

Unstimulated secretion. The rate of secretion of all the patients without exterostimulation was determined. After the attachment of the drainage tube and a wait of five minutes or more to allow the effects of the stimulation of the mouth from the attachment of the tube to subside, the number of drops falling from the tube during each of twenty or more successive minutes was recorded. Only one drainage tube was placed in position at a time, as it is difficult to record the flow from two tubes simultaneously. This introduces a slight source of error in the comparison of the secretion of the two glands, since the rate of secretion may be modified to some extent by bodily fatigue, etc. But in each case where there was a pronounced difference in the secretion of the two sides, this difference persisted irrespective of which of the drainage tubes was applied first, and the ratio of the secretion on the two sides was not altered even by considerable differences in the conditions of stimulation during the two determinations.

The size of the drops falling from the drainage tube is fairly uniform, about 0.06 to 0.07 cc., and for readier comparison with the data obtained by other observers the rate of secretion has been expressed in terms of cubic centimeters per hour, computed from the number of drops secreted in twenty minutes. The rates of secretion without extero-stimulation, determined for the nine subjects, are given in table 1.

The average secretion of a single parotid gland in normal individuals varies from 0.5 to 8.0 cc. per hour, never more in any case which has been recorded. The secretion of the majority of the pathological subjects, as recorded above, falls well within these limits. In two, perhaps three, of the nine patients tested there was, however, a secretion significantly above the mormal. Subject E. P. showed the most pronounced excess of secretion. If we estimate the quantity of fluid given off by the submaxillary and sublingual glands as half that of the parotids

(probably a very considerable underestimation) we may find that this subject secretes almost two liters of saliva daily, exclusive of the additional flow excited by food. The patient J. M. secreted an almost equal quantity. That the heightened secretion in these patients is characteristic and not the result of temporary accidental stimulation is shown by its persistence day after day. Thus the rates of secretion shown in table 2 were obtained with E. P. in tests extending over two weeks.

TABLE 1.

The amounts of secretion of parotid saliva in cases of paralysis. The figures express the total secretion in cubic centimeters per hour with no stimulation of the mucosa of the mouth

SUBJECT	RIGHT PAROTID	LEFT PAROTID
V. S.	4.64	4.20
W. S.	1.08	2.52
J. E. (talking constantly)	1.19	1.44
J. H. (almost constant laughter)	5.42	4.20
A. W.	3.36	1.68
J. S.	1.26	1.52
E. P.	21.29	32.34
P. M.	14.17	27.82
J. E.	8.82	6.82

Aside from the exaggerated secretion in the three subjects the data on the rate of secretion without stimulation shows little abnormality The differences in the quantities secreted by the two glands are, except in the three patients showing abnormal secretion, not greater than those found in normal men and lie well within the probable error of the measurements. The relation of the differences in secretion of the two glands in these three patients will be discussed below.

Reflex secretion. Tests were made of the reflex secretion of all the subjects to dilute acid and other gustatory stimuli and of reflex inhibition by strong muscular effort. In all but four subjects (J. H., E. P., J. M. and J. E.) these reactions seemed in every respect normal. The reflexes were prompt, inhibition of secretion occurred both during normal muscular activity and during attempts to move the paralyzed limbs, and the quantity of secretion obtained was proportional to the concentration of the stimulating solutions used (HCl, from 0.2 per cent to 5.0 per cent). Further tests showed the normal relation of secretion to stimulation of the two sides of the tongue, unilateral stimu-

lation exciting the gland of the same side to a greater extent than that of the other. Evidence for the existence of conditioned reflexes was obtained in but few cases; the difficulty of exciting conditioned reactions in normal subjects, however, makes their absence in these cases insignificant. Each of the remaining subjects showed variations from the normal which it seems advisable to consider separately.

The salivary reflexes of J. H. seemed to be very much reduced. Stimulation of normal subjects with a 2 per cent solution of HCl excites a secretion of from 10 to 30 drops (0.7 to 2.1 cc.) from each parotid gland. Only one of the other pathological subjects gave less than 8 drops of secretion in response to this stimulation. J. H., however, never gave more than 5 drops of saliva after stimulation with the acid and usually he secreted only 2 or 3 drops. The patient is partly anosmic, unable to distinguish the most common odors, and his sensitivity to protopathic acid stimuli seems reduced, although he retains sensitivity to the four primary taste substances. The reduction of the reflexes may be the result of this sensory defect.

The second abnormal characteristic of the patient's reflexes was an extremely slow reaction time. The exact reaction time of the salivary glands is difficult to determine, owing to the fact that the secretion must be forced through a somewhat elastic duct, but with normal subjects under moderately strong stimulation I have never seen it exceed 10 seconds. The reaction time of J. H. was very much greater than this. His reflex secretion, which appeared always as several drops of the saliva coming in rapid succession, never appeared in less than twenty seconds after the application of the stimulus. His average reaction time determined from twenty observations was 32.2 seconds, with a range from twenty to seventy seconds. The time of reaction was independent of the subject's spasmodic laughter, there was no indication of abnormality in the ducts which might retard the flow of the saliva, and the rate and duration of the reflex secretion, once initiated, were not abnormal. It seemed probable, therefore, that the long reaction time was the result of some defect in nervous organization. In other respects the patient's reflexes were normal, equal on the two sides, and showed the usual relations to stimulation of different parts of the tongue.

Since E. P. showed such a marked excess of secretion in the absence of stimulation he seemed particularly favorable for study and an extensive series of tests was undertaken in the hope of revealing some relation between the increased salivation and the paralysis. The data given in table 2 show that the excess secretion is a characteristic condi-

TABLE 2.

The amounts of parotid secretion obtained from E. P. in tests on different days. The quantities are given in cubic centimeters per hour

DATE	RIGHT PAROTID	LEFT PAROTID
August 21	18.5	20.2
August 30	21.3	32.3
August 31	21.9	35.7
September 1	15.8	25.2
September 3	16.8	32.8
September 4	18.1	33.6
Average	18.7	29.9

tion of the patient and that the difference in the secretion of the two glands is likewise constant. The patient has a residual paralysis of the left side of the face with involvement of the tongue and soft palate. The quantity of unstimulated secretion of the left parotid is about 60 per cent

TABLE 3.

Reflex secretion of the parotid glands to gustatory stimulation with dilute acid. The quantitives secreted during one minute preceding and one minute following stimulation are given. Subject E. P.

RIGHT PAROTID		LEFT PAROTID		
Before	After stimulation	Before	After stimulation	
HCl, 1 per cent (1 cc.)				
drops	*drops*	*drops*	*drops*	
4	8	7	8	
4	8	6	10	
4	11	8	9	
5	11	7	9	
4.2	9.5	7.0	9.0	Averages
HCl, 2 per cent (1 cc.)				
drops	*drops*	*drops*	*drops*	
4	11	6	14	
4	15	7	13	
4	15	6	12	
3	16	7	16	
4	20	6	17	
3.8	15.4	6.4	14.4	Averages

greater than that of the right. In contrast to this the reflex secretion of the two glands is approximately the same. The left parotid gave little evidence of reaction to weak stimuli so that its rate of secretion after stimulation with dilute taste substances (1 per cent HCl, for example) was no greater than that before the application of the stimulus. The threshold of reaction of the right parotid was much less than that of the left and its reactions increased with increased strength of stimulation until it equalled the quantity secreted by the left. The relative reactions of the two glands in response to 1 per cent HCl are shown in figure 1. To stronger stimulation both glands reacted about equally with perhaps the more intense reaction in the right. The reactions of the two glands to 1 and 2 per cent solutions of acid are given in table 3.

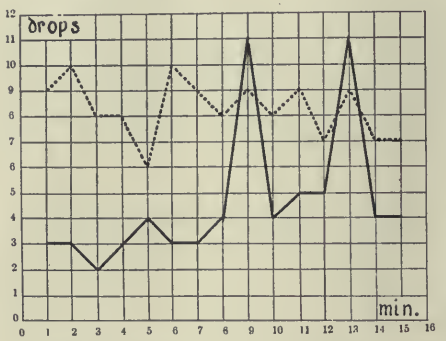

Fig. 1. The relative intensity of reflex secretion of the right and left parotid glands under the same conditions of gustatory stimulation.——Right parotid; - - - - left parotid. Stimulus applied during 9th and 13th minutes. (Patient E. P.)

The patient was aphasic and badly demented so that he did not coöperate well in the experiment and the results are not so clear as they might have been made if it had been possible to control the distribution of the stimulating substance in the mouth and to avoid disturbing movements. The results suggest, however, that weak stimuli did not affect the patient's left parotid until they reached an intensity sufficient to excite a secretion of the right gland equal to the constant secretion of the left. The reactions of his left parotid to unilateral stimulation of the tongue also differed from those of the right. With equal stimulation applied first to one side of the tongue, then to the other, the right parotid gave uniformly about twice as much secretion to stimulation of the right as to the stimulation of the left. The left parotid gave, on the contrary, almost exactly the same amount of secretion in response to stimulation on either side of the tongue.

The tremendous spreading of excitation in these paralytics when they attempt to move the paralyzed limbs suggested the possibility that an increase in secretion might occur during such effort. In this patient and in all the others tested, however, strong muscular effort or attempt

at movement resulted in inhibition of secretion, just as it does in normal individuals. The inhibition of secretion resulting in E. P. from a series of attempts to grip strongly with the paralyzed hand is shown in figure 2. A part of the reduction in secretion may be the result of constriction of the ducts by contraction of striped muscle, but extensive experiments on normal individuals (op. c.) have failed to reveal any mechanism by which such a constriction can be brought about voluntarily.

Various attempts were made to obtain evidences of conditioned reflexes at the sight and odor of food. Fairly clear evidence for an increase in the secretion of the right parotid at the sight of food was obtained and no evidence of a similar excitation of the left parotid was found in a long series of tests with a variety of foods. The data obtained indicated an abolition of the conditioned reflexes of the left parotid and not of the right. It is difficult, however, to obtain conditioned secretion even in normal subjects under laboratory conditions and the failure to get it in this deteriorated patient is therefore scarcely significant.

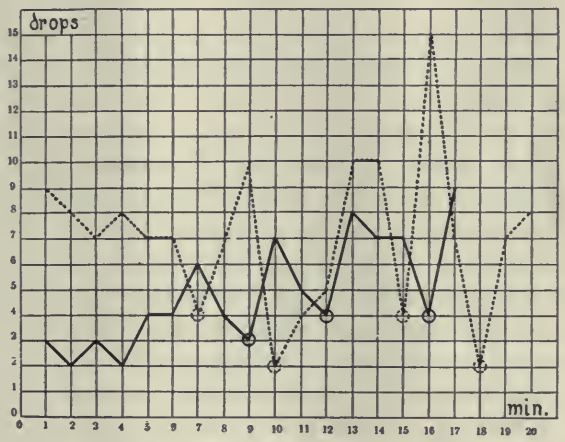

Fig. 2. The inhibitory effect of strong muscular effort upon the rate of secretion of the parotid gland. During the minutes indicated by circles the patient (E. P.) gripped a dynometer with his paralyzed hand.——Right parotid; - - - - left parotid.

Patient P. M. did not coöperate well, talked constantly and became restless under the restraint of the experiment. As a result, tests could be continued for a total of only four hours, which permitted only a determination of the secretion without stimulation, the reactions to two intensities of gustatory stimuli, and the inhibitory effects of attempts to move the paralyzed limbs.

The rate of secretion without stimulation did not vary greatly during the two days on which it was tested. The difference in the secretion of the two glands noted above also remained constant. The rates of secretion determined for the two days were:

September 15. Right parotid, 14.2 cc. per hour
Left parotid, 27.8 cc. per hour
September 17. Right parotid, 19.7 cc. per hour
Left parotid, 23.7 cc. per hour

The reactions of the left parotid to acid stimuli were somewhat greater than those of the right, the averages being the following:

HCl 1 per cent. Right parotid, 12 drops
Left parotid, 15 drops
HCl 2 per cent. Right parotid, 14 drops
Left parotid, 22 drops

There was no indication of a difference in the threshold of the two glands and reactions to unilateral stimulations of the tongue seemed normal.

The only abnormalities of secretion shown by J. M. were exaggerated secretion in the absence of stimulation with inequality of the secretion of the two glands. The difference in the reflexes of the two glands may also be a pathological condition. I have found a similar inequality of reflexes in one normal subject who shows, however, a slight clumsiness of the side having the greater reflexes. The greater secretion and more pronounced reflexes of J. M. were given by the gland on the side showing motor paralysis. Slight inhibition of secretion on both sides followed muscular effort.

Patient J. E. was like the preceding case in that he did not coöperate well, so that only a few tests could be made. His salivary reflexes showed two characteristics which I have not noted in normal individuals. The reaction time was usually prolonged, averaging about fifteen seconds, and the reflexes of the right were much more pronounced than those of the left. The average reflex secretions were:

HCl 1 per cent. Right parotid, 2 drops
Left parotid, 5 drops
HCl 2 per cent. Right parotid, 3 drops
Left parotid, 8 drops

The intensity of the reflexes was also considerably below the normal. Owing to the patient's failure to coöperate, his sensitivity to the acid could not be tested in other ways, and the possibility of a sensory defect is not excluded.

The abnormalities of secretion found in these four patients may be summarized as follows:

J. H. Subnormal salivary reflexes on both sides, with greatly prolonged reaction time.

E. P. Exaggerated secretion in the absence of stimulation, with greater glandular activity on the side the musculature of which was involved in the paralysis (left), a higher threshold of excitability on this side than on the other, absence of differential reaction to stimulation of the two sides of the tongue for the left parotid, and, possibly, loss of conditioned reflexes on the left side.

P. M. Exaggerated secretion in the absence of stimulation with inequality of unstimulated secretion and of reflex secretion.

J. E. Prolonged reaction time with inequality of reflex secretion.

The relation of the quantity of secretion to the drooling of saliva in these patients may be of some clinical interest. Drooling was occasionally noted in J. H., J. S. and J. E., and was almost continuous in E. P. Only one of these patients has a constant secretion of parotid saliva greatly in excess of the normal. All have other defects which lead to a slight difficulty in swallowing: E. P., a marked paralysis of the tongue and palate; J. S., a slight paralysis of the soft palate; J. H., spasmodic laughter which inhibits swallowing; and J. E., a serious dementia. None of the other patients was seen to drool, although in one case (P. M.) the secretion of the unstimulated glands is far in excess of the normal. From this it seems that the drooling of saliva (unless excessive) is to be regarded as indicative of affection of the swallowing mechanism and accessory muscles rather than of heightened secretion.

The data at hand are scarcely extensive enough to justify any general conclusions as to the relation of the abnormal secretion to the other symptoms of motor paralysis. There seems to be no constant relation between the abnormal secretion and facial paralysis since somewhat like conditions of the face, tongue and throat appear in patients with and without abnormal secretion. There is little evidence for an increase in salivary reflexes, resulting from the lesions of the nervous system, which might be comparable to the increase in tendon reflexes. The reflexes to dilute acids in no cases exceeded that which I have found in normal individuals.

In one of the two cases where there was a great excess of secretion there was a history of paralysis involving both sides of the face, and in the other case one side was involved, while the heightened secretion was bilateral. The greater secretion in every case appeared on the side showing the more thorough involvement in the paralysis.

The mechanism by which the heightened secretion is excited presents an interesting problem. It is not a "paralytic secretion" such as is obtained by section of the nerve supply of the glands, as is shown by the long continuance of the secretion after the onset of the paralysis and by its viscosity. It must rather be explained as the result of a continuous excitation of the glands, and in this respect it seems to call for much the same explanation as the contracture appearing in the striped muscles. In this case, since the glands are wholly under the control of the autonomic system, the excess of secretion lends some support to the view that the contractures are the result of autonomic excitation of postural or tonic contraction.

STUDIES IN THE PHYSIOLOGY OF THE RESPIRATION

I. The Capacity of the Air Passages and the Percentage of Carbon Dioxide in the Alveolar Air During Rest and Exercise

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The expired air is made up of two portions: the dead-space air which remains in the bronchi and bronchioles at the end of an inspiration, and the alveolar air which has actually suffered a change by being exposed to the respiratory membrane in the alveoli. If, under the same physiological condition, a normal and a deep expiration following a normal inspiration are measured in a spirometer and the percentage of CO_2 in the two determined, it will be found that the larger expiration contains the larger percentage of CO_2. This is because it contains the larger percentage of air which has been in contact with the pulmonary epithelium. Therefore, the deeper the expiration, other things being constant, the more nearly does the percentage of CO_2 in the expired air approach that present in the alveolar air, while the relative content of dead-space air decreases.

Under the same physiological conditions we may rightly assume that the amount of air in the dead space and the percentage of CO_2 in the alveolar air do not vary when expirations of different depth are made, providing the time consumed in the expiration remains fixed. This being the case, we can determine the amount of air in the dead space and the percentage of CO_2 in the alveolar air by combining the results of the analysis of a deep and a normal expiration in a binomial equation. If we let:

A = amount of air in a large expiration,
A_1 = amount of air in a normal or lesser expiration,
B = the percentage of CO_2 in the expired air of large expiration,
B_1 = the percentage of CO_2 in the expired air of small expiration,
x = the capacity of the dead space, and

y = the percentage of the CO_2 in the alveolar air, then
$AB = (A-x)y$, and
$A_1B_1 = (A_1-x)y$.
Solving this for x, y remaining constant under the same physiological conditions, we have:

$$x = \frac{AA_1(B-B_1)}{AB-A_1B_1}$$ = Dead space air; or solving for y we have:

$$y = \frac{AB-A_1B_1}{A-A_1}$$ = Percentage of CO_2 in the alveolar air.[1]

The details of an actual experiment are as follows: While at rest I breathed normally through membrane valves having a dead space of about 10 cc. The outlet valve led into a T-tube, one end of which was connected with a Krogh spirometer of 1500 cc. capacity and capable of being read to within 5 cc., while the other end remained free. The nose was closed with a spring clothes-pin. In order to keep the attention from the breathing a simple problem of multiplication was solved. The breathing thus became automatically adjusted to the changed condition which the introduction of any respiratory device produces.

After breathing for some time in this way, the tube to the spirometer was opened during inspiration while the free end of the T was closed, and the air from a normal expiration was collected. This was accurately measured and a portion was analyzed for its CO_2 percentage. Under the same conditions of rest, an expiration somewhat deeper than a normal one was made, but the rate of the expulsion of the air was quickened so that the time of the expiration remained about normal although the amount expired was increased. This was measured and the CO_2 content determined as in the previous sample.

The following figures give the results of five determinations.

NUMBER OF OBSERVATION	CUBIC CENTIMETERS OF AIR IN EXPIRED SAMPLE	PER CENT OF CO_2 IN EXPIRED SAMPLE	CUBIC CENTIMETERS OF CO_2 IN EXPIRED SAMPLE
1	487	3.86	18.80
2	540	4.10	22.15
3	1,000	4.73	47.30
4	1,050	4.75	50.00
5	1,300	4.88	63.40

[1] This formula has been used by the author for some time in estimating the dead space and the CO_2 percentage in the alveolar air. Recently it was discovered that A. O. de Almeida (Brazil-Medico, June 24, 1916) has also used the above formula in estimating the percentage of CO_2 in the alveolar air.

By combining the above results in the equation for the dead space, when we compare observations 1 and 5 we have:

$$\frac{1300 \times 487\,(4.88 - 3.86)}{63.4 - 18.8} = 145 \text{ cc.} = \text{dead space.}$$

By combining the above observations in all the possible ways we find the following values for the dead space during rest:

	cc.
Nos. 1 and 3, the dead space	= 149.0
Nos. 1 and 4, the dead space	= 149.0
Nos. 1 and 5, the dead space	= 145.0
Nos. 2 and 3, the dead space	= 140.0
Nos. 2 and 4, the dead space	= 134.0
Nos. 2 and 5, the dead space	= 132.0
Average	= 141.5
Dead space in valves	= 9
Actual physiological dead space	= 132.5

If we combine the results of the above experiments in order to estimate the percentage of CO_2 in the alveolar air, we have in the case of observations 1 and 5:

$$\frac{(1300 \times 4.88) - (487 \times 3.86)}{1300 - 487} = 5.45 \text{ per cent,}$$

the percentage of carbon dioxide in the alveolar air. Combining the observations as follows, we find the following values for the CO_2 percentage in the alveolar air:

	per cent
Nos. 1 and 3	= 5.54
Nos. 1 and 4	= 5.50
Nos. 1 and 5	= 5.45
Nos. 2 and 3	= 5.45
Nos. 2 and 4	= 5.43
Nos. 2 and 5	= 5.40
Average	= 5.46

The CO_2 percentage in the alveolar air was estimated at the same time by the classic method of Haldane and Priestley, and was found to be 5.8 per cent.

In order to determine the effect which exercise has upon the capacity

of the dead space and the percentage of CO_2 in the alveolar air, I made the following experiment. The nose was closed and, breathing through the same valves which I used in the resting experiments, I walked up and down the corridor of the laboratory at the rate of about $3\frac{1}{2}$ miles per hour. I found that I could not walk faster than this rate without being conscious of respiratory effort when breathing through the valves. After continuing the exercise for about five minutes and when breathing was perfectly regular, I stopped and without delay collected air from the beginning of expiration, the amount collected being varied by a valve in the opening to the spirometer. Otherwise the experiment is exactly the same as the resting experiment.

NUMBER OF OBSERVATION	CUBIC CENTIMETERS OF AIR IN EXPIRED SAMPLE	PERCENT OF CO_2 IN THE EXPIRED AIR	CUBIC CENTIMETERS OF CO_2 IN THE EXPIRED SAMPLE
1	500	3.61	18.0
2	1,000	4.72	47.2
3	475	3.60	17.1
4	975	4.70	45.8
5	1,125	4.75	53.5
6	1,600	5.14	82.2

By combining the above results we find the estimated dead space in these observations is as follows:

	cc.
Nos. 1 and 2, the dead space =	188
Nos. 1 and 4, the dead space =	188
Nos. 1 and 5, the dead space =	179
Nos. 1 and 6, the dead space =	189
Nos. 2 and 6, the dead space =	190
Nos. 4 and 6, the dead space =	187
Nos. 3 and 2, the dead space =	178
Nos. 3 and 4, the dead space =	178
Nos. 3 and 5, the dead space =	172
Nos. 3 and 6, the dead space =	180
Average =	182.9
Dead space in valves =	9
Actual physiological dead space =	171.9

If the above results are combined to estimate the percentage of CO_2 in the alveolar air, the following results are obtained:

	per cent
Nos. 1 and 2	= 5.85
Nos. 1 and 4	= 5.80
Nos. 1 and 5	= 5.70
Nos. 1 and 6	= 5.83
Nos. 2 and 6	= 5.80
Nos. 2 and 3	= 5.75
Nos. 3 and 4	= 5.70
Nos. 3 and 5	= 5.67
Nos. 3 and 6	= 5.75
Nos. 4 and 6	= 5.80
Average	= 5.76

The carbon dioxide in the alveolar air during exercise of the same degree as estimated by the Haldane-Priestley method was 6.2 per cent.

The dead space during exercise in the above experiments evidently increased from the resting figure of 141.5 cc. to 182.9 cc., or about 30 per cent. Whether this is an actual fact or simply due to the experimental error in the methods involved, cannot be determined at present. However, there are at least two reasons for believing that there is an increase in the capacity of the bronchial tree. These are: (1) the fact that the calculations individually do not vary greatly from the mean, during either exercise or rest; and (2) that in the passage of air through any conduit, in this case the bronchial tubes, there is a tendency towards the formation of a film of inert or still gas next to the walls of the tube. This film in effect decreases the effective cross section through which the air is flowing. In the present instance it tends to decrease the dead space. Conversely, when the air velocities through the tubes are increased, the more rapid flow tends to scrub away the film so that the effective dead space increases.

In the hope of showing the possible effect of this phenomenon, the dead space was figured from high-velocity data. The results were compared with dead-space values computed from low-velocity data, all results applying to the condition of rest. This comparison showed no appreciable difference in dead space as a result of difference in air velocities in the bronchial tree. This is not conclusive, however, since the expected increase in the dead space from the increased air velocity might indeed be approximately offset by compression of the bronchioles incident to the somewhat forced expiration producing the higher velocities.

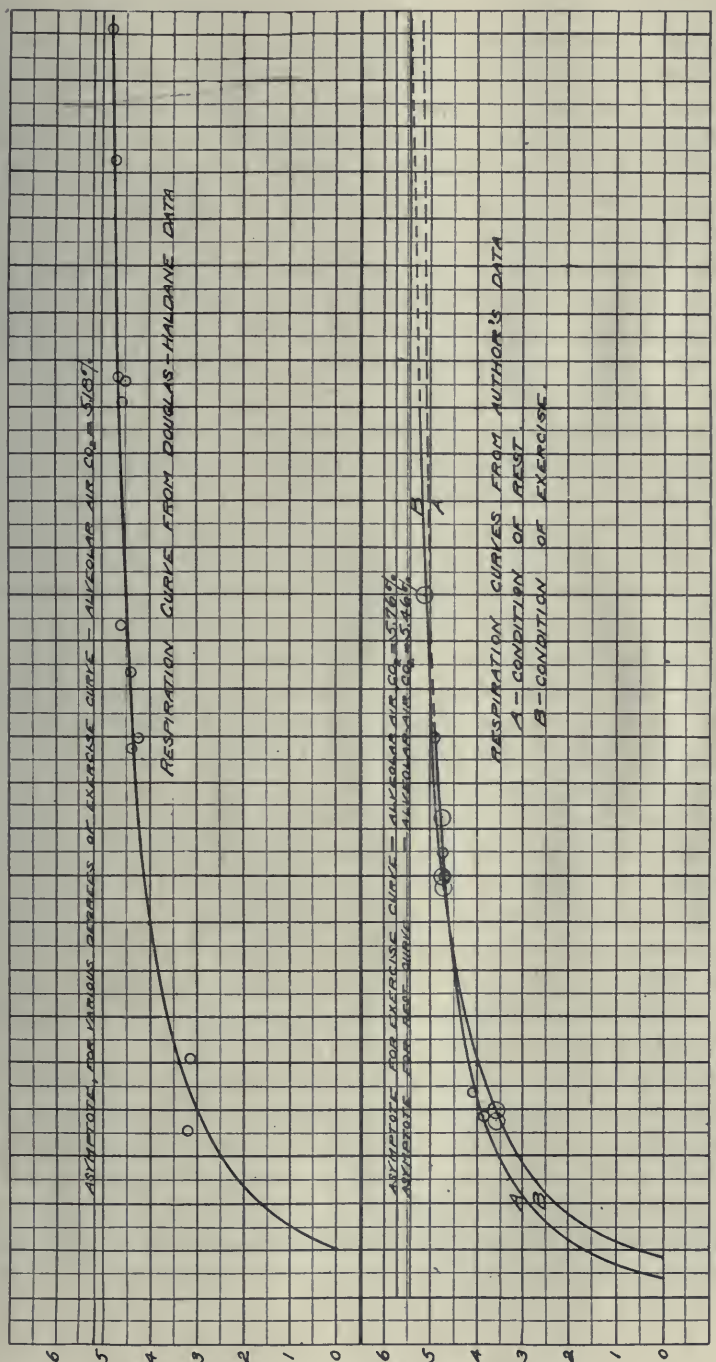
An inferential proof that the bronchial tree can increase or decrease

in capacity during exercise lies in the known fact that the bronchioles are supplied with muscles which contract if their nerves are stimulated.

The CO_2 in the alveolar air during the exercise increased over that present during rest by 0.32 per cent. This figure agrees very well with the observations of Campbell, Douglas, Haldane and Hobson (1), which show that a rise of 0.22 per cent in the pressure of the CO_2 in the alveolar air is sufficient to increase the depth of the respirations 100 per cent. In the above experiments the depth was increased during exercise about 200 per cent. The significance of the difference in the percentage of CO_2 found by the Haldane and Priestley method and the method described above will be pointed out in the discussion.

The results of the above observations can be plotted in the form of a curve, the ordinates of which represent the percentage of CO_2 in the expired air, and the abscissæ the cubic centimeters of expired air. The curve starts at the point where the dead-space air just equals the expired air. As a matter of fact, this is a theoretical point and not actual, for Krogh and Lindhard have determined that it is necessary to expel at least twice the volume of the dead space to be sure that this moiety is displaced. The horizontal asymptote of the curve is the percentage of CO_2 in the alveolar air.

The above results are of interest since they throw some light upon the unsettled question of the relation of work to the capacity of the air passages and the percentage of CO_2 in the alveolar air. In 1905 Haldane and Priestley (2) developed their method for the determination of the percentage of CO_2 in the alveolar air and the dead space. By the use of this method they showed the importance of the tension of CO_2 in the blood and the alveolar air in the regulation of the respiration. In the calculation of the dead space they use three figures, viz.: the cc. of air expired in a single breath, the percentage of the CO_2 in the expired air, and the percentage of CO_2 in the alveolar air. The latter figure they obtain by taking the average percentage of CO_2 in samples of expired air taken at the end of a quick expiration following a normal inspiration and a forced expiration following a normal inspiration. While it is possible to obtain very consistent figures for the percentage of CO_2 in the expired air, and it is easy to collect the air of an entire expiration, it is very difficult to obtain by this method figures representing the percentage of CO_2 in the alveolar air which do not vary by less than 0.2 to 0.3 per cent in duplicate estimations. Since the alveolar-air CO_2 is a basic figure in the calculation of the dead space by the method of Haldane and Priestley, it is very important that it should be correct.


ASYMPTOTE FOR VARIOUS DEGREES OF EXERCISE CURVE = ALVEOLAR AIR CO_2 = 5.18%
RESPIRATION CURVE FROM DOUGLAS-HALDANE DATA
ASYMPTOTE FOR EXERCISE CURVE = ALVEOLAR AIR CO_2 = 5.76%
ASYMPTOTE FOR REST CURVE = ALVEOLAR AIR CO_2 = 5.46%
RESPIRATION CURVES FROM AUTHOR'S DATA
A - CONDITION OF REST
B - CONDITION OF EXERCISE
PERCENT OF CO_2 IN EXPIRED AIR
EXPIRED AIR - CUBIC CENTIMETER
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800
0 1 2 3 4 5 6

Krogh and Lindhard (3) have criticised the Haldane and Priestley method for the determination of the alveolar-air CO_2. They point out that the average of the two determinations made does not represent the average composition of the expired alveolar air, since the actual time required to make the forced expirations following a normal inspiration and expiration is sufficient to allow CO_2 to accumulate in the supplemental air of the lung and thus raise the percentage of the CO_2 in the last portions expelled.

When the metabolism is very low, as it is during rest in bed, this factor of error may be of little importance, but when metabolism is increased, as it is during exercise and fevers, then the percentage of CO_2 in the alveolar air as estimated by the Haldane and Priestley method is much too high.

Using the Haldane and Priestley method for the determination of the alveolar air and the dead space, Douglas and Haldane (4) investigated the effect which exercises have upon the alveolar-air CO_2 and the capacity of the dead space. They found that when a person walked at the rate of 5 miles an hour, the capacity of the air passages increased by 400 per cent over that present while resting. This increase was accompanied by an increase in the CO_2 percentage in the alveolar air. Krogh and Lindhard believe that alveolar air CO_2 does not increase to nearly the extent that Douglas and Haldane's figures indicate, and that the error in the Haldane and Priestley method is sufficient to account for the great changes which Douglas and Haldane found to occur in the dead space during exercise.

Krogh and Lindhard, using an entirely different method for the determination of the alveolar air, were unable to confirm the observation of Douglas and Haldane. Later they developed a method in which they were able to obtain, automatically, samples of air from different and known portions of an expiration. In these experiments they also took samples of the alveolar air by the Haldane and Priestley method, and found that these contain on the average about 13 per cent more CO_2 than that shown by the average of their curve.

If one takes the average of the CO_2 percentage in the alveolar air as given by Douglas and Haldane in the table of their experiments on rest and exercise (4), and reduces this figure by 13 per cent, as suggested by Krogh and Lindhard, he obtains 5.25 per cent in place of 6.04 per cent. Using this figure to calculate the dead space in the various degrees of increasing exercise, one fails to find a change in the dead space as claimed by Douglas and Haldane.

It may be questioned whether it is correct to recalculate the figures of Haldane and Douglas on the assumption that the percentage of CO_2 remains constant during exercise. In the experiments which are reported in the first part of this paper, the author shows that there is a slight increase in the percentage of CO_2 in the alveolar air while one is walking $3\frac{1}{2}$ miles an hour. This method used by him has in it a portion of the error which Krogh and Lindhard point out to be present in the Haldane-Priestley method for the estimation of the alveolar-air CO_2. The increase in the percentage of CO_2 in his experiments, however, is much less than that recorded by Haldane and Douglas for the same degree of work. Moreover, if we recalculate the results given by Douglas and Haldane, using the binomial equation described in this paper, we can determine the dead space and the percentage of CO_2 in the alveolar air without reference to the alveolar CO_2 pressures given by the authors. In this formula the assumption is made that the dead space and percentage of CO_2 in the alveolar air remain constant for the same physiological condition. If the dead space and CO_2 percentage in the alveolar air do increase during exercise, then a great difference in the value of the dead space and the percentage of CO_2 in the alveolar air will occur when we make various combinations in the equation of figures representing the expired air taken during different amounts of work.

If we obtain like values for the dead space and the percentage of CO_2 in the alveolar air when we combine the data obtained during different amounts of exercise and rest, or when we combine the results of experiments in which the amount of work is different, it is fair evidence that the capacity of the air passages and the percentage of the CO_2 in the alveolar air have not changed during the exercise. The results given by Douglas and Haldane in the table referred to above are especially adapted to use in the binomial formula for determining the value of the dead space and the percentage of CO_2 in the alveolar air. In these experiments they were careful that the respiratory center controlled the depth of the respiration, and the observations extended over a period of time. The depth of each respiration was estimated by counting the total number of respirations and measuring the air expired during the observation period. The CO_2 content of the expired air was also determined. Figures representing average results should be subject to less variation than those in which only one expiration is measured, for unless great care be exercised, any conscious attention to the breathing modifies it, and samples of air collected under this condition are

not reliable. In the following table is found an abstract of the essential figures found in the table of results given by Douglas and Haldane. From these data we obtain the figures for the application of the binomial equation. In order to facilitate expression, the observations are numbered in the table, and these numbers are used to designate the combination used.

TABLE 1

Abstract of the table given by Douglas and Haldane on the effect of exercise on the dead space

NUMBER OF OBSERVATION	OBSERVATION	VOLUME OF EACH BREATH	CO_2 PER CENT IN EXPIRED AIR	CO_2 CONTENT OF THE EXPIRED AIR IN CUBIC CENTIMETERS	PER CENT OF CO_2 IN THE ALVEOLAR AIR (HALDANE AND PRIESTLEY)	EFFECTIVE DEAD SPACE OF AIR PASSAGES (HALLDANE AND PRIESTLEY METHOD)
1	Rest	457	3.19	14.5	5.97	160
2	Rest-standing	612	3.14	19.2	5.70	222
	Walking					
2	2 miles per hour (Lab.)	1,296	4.25	55.1	6.04	331
4	2 miles per hour (Grass)	1,271	4.39	56.0	6.04	293
5	3 miles per hour (Lab.)	1,433	4.38	62.7	6.14	358
6	3 miles per hour (Grass)	1,535	4.62	71.0	6.10	320
7	4 miles per hour (Lab.)	2,010	4.55	91.6	6.23	488
8	4 miles per hour (Grass)	2,064	4.67	96.2	6.36	497
9	4.5 miles per hour (Lab.)	2,055	4.50	92.5	6.44	565
10	4.5 miles per hour (Grass)	2,524	4.72	119.0	6.20	549
11	5 miles per hour (Lab.)	2,810	4.80	135.0	6.28	609
12	5 miles per hour (Grass)	3,145	4.79	150.8	6.10	622

Average for the CO_2 in alveolar air as estimated by Douglas and Haldane 6.13 per cent.

According to Krogh and Lindhard this is about 13 per cent too high. The corrected value is therefore 5.30 per cent.

Applying the figures in the above table to the binomial formula for the calculation of the percentage of CO_2 in the alveolar air we have made all possible combinations of the observations. The following table gives the percentage of CO_2 found in the alveolar air as found by the combinations as indicated. The observation numbers refer to the Douglas-Haldane table given above.

TABLE 2

The percentage of CO_2 in the alveolar air

OBSERVATION NUMBER	1	2	3	4	5	6	7	8	9	10	AVERAGE
3	4.95	5.28									5.12
4	5.10	5.55									5.32
5	4.95	5.48	5.55								5.32
6	5.25	5.58	(6.70*)								5.41
7	4.95	5.20	5.13	4.90	5.10						5.06
8	5.20	5.33	5.40	5.25	5.30						5.29
9	4.90	5.05	4.95	4.70	4.70						4.86
10	5.10	5.23	5.20	5.05	5.25	4.85	5.32	5.00	5.7		5.19
11	5.10	5.32	5.18	5.15	5.30	4.85	5.40	5.25	5.70		5.27
12	5.10	5.25	5.20	5.05	5.15	4.88	5.25	5.08	5.90	4.80	5.16
Average	5.06	5.32	5.23	5.01	5.17	4.83	5.32	5.11	5.77	4.80	

General average for all 5.18

* Not in general average.

The dead space calculated by the binomial equation from the figures given by Douglas and Haldane as tabulated in the table given above, and in the various combinations as was done above in the calculation of the alveolar air, is as follows:

TABLE 3

The capacity of the air passages

OBSERVATION NUMBER	1	2	3	4	5	6	7	8	9	10	AVERAGE
3	154	240									197
4	168	234									210
5	160	210									185
6	176	267	(460*)								221
7	162	237	237	119	168						184
8	164	247	270	182	256						243
9	158	230	178	78	118						152
10	174	190	242	168	184	83	320	105	397		207
11	171	218	255	185	235	120	330	196	410		235
12	168	238	228	168	209	140	258	153	320	184	206
Average	165	233	226	150	195	114	203	151	375	184	

General average for the capacity of the dead space during experiments.....206.5 cc.
Dead space in the valves..... 58.0 cc.
Actual capacity of the air passages.....148.5 cc.

* Not in general average.

With a few exceptions the percentages of CO_2 found in the alveolar air in the above calculations differ from one another by less than 0.2 per cent. Since the per cent of CO_2 found is not conditioned by the manner in which we combine the observations, we are forced to conclude that if the percentage of CO_2 in the alveolar air increases during exercise, the increase is relatively small and is overshadowed by the experimental error in the observations.

If the corresponding averages in the ordinate and abscissa columns are examined it will be seen that, with the exception of observations Nos. 6 and 9, they correspond very closely. This lack of agreement can be readily explained by reference to the curve plotted from the data given by Douglas and Haldane. The percentage of CO_2 in the expired air in observation 6 lies above the curve and in 9 below the curve. In observation 1, the dead-space air has not been completely displaced, hence the percentage of CO_2 in the expired air is a little above the curve. With these exceptions the curve and the actual calculations agree very well.

The curves serve to bring out the relative accuracy of the Douglas-Haldane experiments and the author's experiments. It will be noted that there is a marked deviation of the Douglas-Haldane points from the smooth curve, whereas the author's data are not only more uniform, but they are sufficiently consistent and accurate to permit the clear though slight distinction between the rest and exercise curves; all points practically coincide with the average value curves.

Another point of importance is the fact that when observations are combined in the binomial equation in which the percentage of CO_2 in the expired air or the number of cubic centimeters in the expired air is nearly equal, the experimental error in the analysis is very important and the variations are more pronounced. Attention must also be called to the close agreement of the average figures for the percentage of CO_2 obtained by the binomial equation and the average of Douglas and Haldane's estimations when corrected by the per cent of error found present by Krogh and Lindhard in the Haldane and Priestley method.

What has been said regarding the percentages of CO_2 in the alveolar air as determined by the binomial equation in the experiments of Douglas and Haldane may be applied to the calculation of the dead space. The average value for the dead space found by this manner of calculation, as shown by table 3, is about the accepted normal figure given for the dead space, and compares very favorably with the dead space found by the author in himself. The variations from the mean

figure are within 30 per cent of the mean in all the observations except in observations 6 and 9. It has been pointed out that these two observations are probably unreliable. If these two observations are cast aside, then the variations from the mean are less than 20 per cent. The differences found in the dead space bear no relationship whatsoever to the kind of exercise being performed during the observation period, and moreover if the dead space did increase during the exercise, the change is less than the percentage of error in the method of obtaining the expired air and the percentage of CO_2 it contains. The results fail absolutely to confirm the conclusion which Haldane and Douglas drew from the same data, namely, that the dead space increases *greatly* during exercise, and do confirm the work of Krogh and Lindhard, who found that the dead space does not vary appreciably during exercise.

The method outlined for the determination of the CO_2 percentage in the expired alveolar air may also be applied to the estimation of the oxygen percentage in the alveolar air. In reading over the account of the observations on the cause of mountain sickness, etc., made on Pike's Peak by Haldane, Douglas, Henderson and Schneider (5), one is impressed with the idea that the determinations of the oxygen pressures in the alveolar air made then by the Haldane-Priestley method are as much too low as the CO_2 pressures are too high when estimated by the same method. Unfortunately, the data given are not sufficient to recalculate their results with the binomial equation.

SUMMARY

Methods for calculating the dead space and the percentage of CO_2 in the expired alveolar air are proposed, in which the necessary data are obtained by determining the amount of air and the percentage of CO_2 in the air of a normal and deep expiration.

By the use of these methods, only a small variation in the dead space or the percentage of CO_2 could be determined between the conditions of rest and exercise consisting of walking $3\frac{1}{2}$ miles an hour.

A recalculation of the data given by Douglas and Haldane, using the proposed method, fails to confirm their conclusion that the capacity of the dead space increases to anything like the extent they claim.

The Haldane-Priestley method for the estimation of the CO_2 percentage in the alveolar air gives results that are too high.

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THE GRADIENT IN SUSCEPTIBILITY TO CYANIDES IN THE MERIDIONAL CONDUCTING PATH OF THE CTENOPHORE MNEMIOPSIS

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By way of introduction to the experimental data it is necessary to call attention briefly to certain anatomical and physiological features of the ctenophore body and to make clear the point of view from which the experiments were undertaken. The motor organs of the ctenophore consists of series or rows of so-called swimming plates, each plate consisting of a number of strong cilia arranged in a single plane and more or less fused basally, thus forming a flat paddle-like organ which beats as a whole. In *Mnemiopsis leidyi* eight rows of swimming plates, four longer alternating with four shorter rows, extend along eight meridians of the body-surface from points near the apical or aboral pole toward the oral end. Each row consists of numerous plates arranged in regular order and spacing and with the plane of each plate at right angles to the direction of the row.

The question whether the ctenophores possess a definitely differentiated nervous system has never been finally answered. At the apical pole is a static sense organ and certain other specialized, apparently sensory areas, and various authors have asserted that a central nerve mass is also present in this region. Under normal conditions the swimming plates of each row beat metachronically, the beat beginning at the oral or apical end of the row and progressing as a wave which can be followed by the eye. Normally also the beats follow each other in a regular rhythm which can be accelerated, retarded or inhibited in various ways.

The presence of a specialized nerve underlying the row of swimming plates has not been demonstrated, but Engelmann (1, 2) and others have supported the hypothesis of neuroid transmission, i.e., of an impulse resembling the nervous impulse passing from cell to cell along the row of plates, while Verworn (3) maintained that meta-

chronic action in cilia generally is the result of direct mechanical stimulation of one cilium by another. Parker's more recent work (4) supports the neuroid rather than the mechanical hypothesis of transmission, at least as regards the case of the ctenophore, and Baglioni (5) and Bauer (6) also conclude that the evidence indicates neuroid transmission in the ctenophore. Moreover, it would be extremely difficult to interpret in terms of mechanical transmission the experimental results described below. Evidently then, although a morphologically differentiated nerve has not thus far been found, the weight of the evidence supports the view that the metachronic beat of the swimming plates of the ctenophore is determined by the passage over a more or less definite path, doubtless from cell to cell, as Chun (7) suggested, of an impulse resembling the nerve impulse. This impulse must originate in the central complex of the apical region and since the rhythm is at least usually synchronous in the two rows of plates on each quadrant of the body, in *Mnemiopsis* a longer and a shorter row, while in the rows of different quadrants this is not the case, there must be, as Chun pointed out, four centers, one to each quadrant.

Tashiro (8-12) and Tashiro and Adams (13–15) have shown that metabolic activity is in some way associated with the nerve impulse, stimulation of the nerve increasing and narcosis decreasing its carbon dioxide output. Moreover, Tashiro (10, 11, 12) has also shown that a gradient of CO_2-production exists in the unstimulated nerve and that the normal nerve impulse passes down this gradient, i.e., from a point of higher to one of lower CO_2-production. If this conclusion is correct and if transmission along the plate-row in the ctenophore is neuroid in character, we might expect to find a gradient in metabolic rate or in rate of oxidation with its highest point at the apical or aboral end.

Experimental investigations concerning the nature of the physiological axes of organisms have led me to the conclusion that such axes in their simplest form are essentially gradients which may be regarded from one point of view as gradients in metabolic rate or in the rate of certain fundamental metabolic reactions and from another as gradients in protoplasmic condition, irritability or whatever we choose to call it, associated with such differences in metabolic rate (16-28). In the gradient which represents the primitive, main or polar axis of the body the region of highest metabolic rate becomes the apical end or head region and in other axes the positions of organs are definitely related to the gradient. Moreover, according to this point of view the dominance and subordination of regions or parts along an axis result from

and are dependent upon this gradient, the region of highest rate dominating regions or levels of low rate within a certain variable limit of distance (23, chap. IV). Such an axial gradient originates in the final analysis from the establishment of a region of high metabolic rate or high irritability by the differential action of external factors upon the cell or cell mass from which the axiate organism develops. If the metabolic differential thus determined is sufficiently great, transmission of some sort of dynamic change from the region of high rate occurs, and the intensity or the effectiveness of the transmitted change decreases with increasing distance from the point of origin. If such transmission is continued long enough or repeated often enough a more or less permanent gradient in metabolic rate and protoplasmic condition associated with it is established, and this represents the physiological axis in its simplest form. Development and differentiation along this axis result primarily from different conditions at different levels of the gradient, and the central nervous system where it is present is both morphologically and as regards its conducting and integrating function, the final expression of the primitive quantitative metabolic axial relations. Of course such a gradient may be complicated by many factors and changes may occur during development, but it is possible, at least in the simpler organisms, to trace the continuity in the sequence of events. Moreover, a gradient once established may and often does persist through many cell generations and through other forms of reproduction. The various lines of evidence which support this conception of the physiological axis cannot be considered here but are discussed in the publications referred to above.

In the course of these investigations it has been found that a relation between susceptibility to cyanides, anesthetics and various other toxic agents and the general metabolic rate or protoplasmic condition associated with it exists (17, 18, 22, Chap. III). This relation is briefly as follows: To concentrations or intensities of such agents which kill so rapidly that acclimation or acquirement of tolerance to them does not occur, the susceptibility, as measured by the survival time or in various other ways, varies in general with the metabolic rate and with other factors associated with it. To very low concentrations or intensities to which more or less acclimation occurs, the susceptibility varies in general inversely as the metabolic rate. These relations can of course be altered experimentally, but the evidence indicates that they are nevertheless of general significance. The question of their nature, their relation to permeability, colloid state, etc., need not be considered here.

Differential susceptibility to a great variety of agents is a characteristic feature of physiological axes, as I have shown for both animals and plants (16–28), not only of the axes of polarity and symmetry of the organism as a whole but of the axes of special organs and parts, at least so far as they have been investigated. It has even been possible to demonstrate in certain nerve fibers by means of differential susceptibility, a gradient in the structural death changes (28) corresponding to the metabolic gradient discovered by Tashiro (10, 11, 12).

If Tashiro's conclusions and my own are essentially correct, the nerve fiber, at least in its more primitive form, is not fundamentally different from other physiological axes, even the axes of polarity and symmetry of the whole organism. Both the nerve fiber and the body axis are metabolic, associated with protoplasmic gradients, and in both the highest metabolic level of the gradient dominates lower levels, because dynamic changes transmitted from it are effective in regions of lower metabolic activity, i.e., the dynamic change transmitted along a general protoplasmic axis, and the nerve impulse transmitted along a highly specialized path both pass, at least mainly and under ordinary conditions, from higher to lower metabolic levels of the gradient.

This conception of the physiological axis constitutes the point of departure for the experiments on the ctenophore. The row of swimming plates represents not only a path along which transmission of impulses, apparently neuroid in character, takes place, but also a direction along which a definite spatial and temporal morphological order or pattern develops. In short, it possesses the general characteristics of a physiological and morphological body axis, and of a specialized nervous axis. In view of these facts it is of interest to determine whether there is any similarity between this axis and a general body-axis on the one hand and a specialized nerve on the other.

METHOD

In order to determine whether a differential susceptibility to cyanide is present along the row of swimming plates the ctenophores were placed in a concentration of KCN determined by preliminary experiment as high enough to inhibit all movement within at most a few hours, but not high enough to inhibit movement at once. Concentrations ranging from approximately m 25 x 10^{-6} to m 5 x 10^{-5} were found to be most satisfactory. A concentration of m 1 x 10^{-5} produced no other effect after five hours than a slight retardation of the rhythm. At the other extreme a concentration of m 1 x 10^{-4} can be used, but in

concentrations much above this the complete cessation of rhythmic movement occurs too rapidly to permit prolonged observation of the various stages. In *m* 25 x 10^{-4} movement of all plates ceases almost instantly and within a minute or two the state of aggregation of the protoplasm of the plate rows changes, and the plates become opaque white instead of almost transparent. This change probably indicates the moment of death. In a concentration of *m* 25 x 10^{-6} the animals may be kept for some hours and still be capable of complete recovery after return to water. In this way the changes occurring during recovery after more or less complete inhibition can be determined.

In the experiments described below only KCN was used as inhibiting agent. I am of course aware of the desirability of comparing the effects of other agents, such for example as the anesthetics in the stricter sense with those of KCN and hope to be able to extend the investigation along these lines in the future. The results obtained with KCN, however, are very clear and definite and are sufficient to permit certain conclusions and to show that these forms are valuable material for certain lines of experimentation.

EXPERIMENTAL

The first effect of the concentrations of KCN *m* 25 x 10^{-6} to *m* 5 x 10^{-5} is a retardation of the rhythm which begins within a few moments. After fifteen to thirty minutes impulses are still evidently originating at the aboral (apical) end of the plate-row, but the amplitude of vibration is least at the aboral end and increases in the oral direction along the row, until in the oral half more or less of the row it is indistinguishable from the normal. After one-half to one hour movement in the most aboral plates of the row is almost imperceptible, but increases orally and may still be normal in amplitude over a longer or shorter distance at the oral end. After one hour in KCN all movement of the plates in about the aboral fourth or third of the row has ceased, but the single plates of this region still respond to direct mechanical stimulation by one or a few beats of almost or quite normal amplitude. Such response may be limited to the plate stimulated, or the stimulus may be transmitted to the two or three plates adjoining on the oral side of the plate stimulated. Where this response is rhythmical, the rhythm is independent of and usually more rapid than that in the more oral regions of the row where rhythmic movement still persists.

At about this time (one hour) or a little later another effect makes its appearance, particularly in the four longer plate-rows. This is

the appearance of an independent rhythm over a longer or shorter distance at the oral end of the plate row, sometimes involving the oral third or even more of the row.

The manner in which this rhythm makes its appearance is of interest. In some cases, for example, in KCN m 25 x 10^{-6} movement ceases in the most aboral plates, while in the middle half of the row the plates are still beating with a rhythm much below the normal. In this case this rhythm does not usually extend into the oral fourth more or less of the row, but this region shows an independent and more rapid rhythm alternating irregularly with periods of complete quiescence. Such periods of quiescence are interrupted from time to time by the passage of an impulse from the more aboral region into the oral fourth.

It is evident that at this stage the impulses from the middle region do not ordinarily pass into the oral fourth and this is developing an independent rhythm more rapid than that of the more aboral regions. This rhythm, however, is still intermittent and in the periods of quiescence summation of the impulses from the middle region may occur at the boundary between the two and a single impulse or sometimes two or three may pass all the way to the oral end. After this the oral portion may again become quiescent or may resume its independent rhythm.

In some cases four regions of different behavior are distinguishable along a single row: the most aboral where there is no movement; a second or middle region in which rhythmic movement is proceeding with a slow rhythm; a third region which sometimes beats with the rhythm of the second and sometimes independently with a more rapid rhythm; a fourth region at the oral end of the row in which the rhythm is completely independent of all more aboral regions and most rapid of all. In such cases the most oral region has become completely independent of other parts and has developed its own rhythm, while the region next to it is at times independent of and at times subordinated to the region next aboral to it.

In still another case five distinct regions appeared along a plate-row; an aboral region in which movement had ceased; a second showing a slow rhythm; a third showing mostly an independent rhythm more rapid than the second but occasionally becoming subordinate to it and showing the same rhythm for a short time; a fourth region showing a still more rapid independent rhythm; a fifth region, the most oral portion of the row with a still more rapid independent rhythm. In this case the fifth region became independent first, then the fourth and then the third.

In general the effect of the inhibition at this stage—after one hour—is to make a longer or shorter portion at the oral end of the plate-row independent of the impulses originating at the aboral (apical) end or at the most apical level which is still active. For some reason the impulse coming from aboral regions is no longer effective in controlling the oral region and as time goes on other regions in succession from the oral end become independent. The appearance of independent, more rapid rhythms in the oral regions of the row shows further that these regions, when they have attained a certain degree of independence of more apical regions are capable of initiating and maintaining a rhythm of their own or even several different rhythms at different levels, that of the most oral region being the most rapid.

In some cases the oral portion of the plate-row not only becomes independent of impulses from aboral regions but the direction of transmission of the rhythmic impulse actually undergoes reversal from aboral-oral to oral-aboral. This reversal has been observed in three cases in different animals. In such cases the reversed impulse begins at the extreme oral end of the row and travels a longer or shorter distance in the aboral direction to a point where it meets an aboral-oral rhythmic impulse with a different rhythm, and there it ceases to be effective. The boundary between these two different rhythms proceeding in opposite directions is at times perfectly distinct, one of two adjoining plates beating with one, the other with the other rhythm. At other times there may be an intermediate region of a few plates where impulses sometimes travel in one direction, sometimes in the other. As will be pointed out below, these cases of reversal of the direction of conduction are of particular interest.

If the action of the cyanide is continued beyond this stage of complete aboral inhibition and oral independence with or without reversal, complete quiescence of the plates gradually progresses from the aboral end of the row in the oral direction and the rhythms in these parts of the row which are still active become slower and slower, the retardation at any time being greatest in the most aboral and least in the most oral region. After one and a half to three hours according to concentration and age, the older animals being less susceptible, movement has ceased over the aboral three-fourths to seven-eighths of the row and only the oral one-fourth to one-eighth still shows rhythmic movement, the direction of conduction being either normal or reversed, and the rhythm being much slower than when this region first became independent. Finally, movement ceases in this region. Here as else-

where the single plates remain capable of response by one or a few beats to direct mechanical stimulation for some time after rhythmic movement has ceased, but such stimulation is usually not transmitted or at most affects only two or three plates.

This condition may persist for an hour or more after cessation of movement but finally the plates become whitish and opaque and are certainly dead. This final change usually begins at the aboral end of the row and proceeds orally, but the difference in time between death of the plates at the two ends of the row is very much less than the difference in time of cessation of movement and frequently the change extends over the whole row almost at once. Not infrequently single plates suddenly begin rapid vibration just before they turn white. In no case has transmission of this vibration to another plate been observed and it usually continues only a few seconds, but occasionally for a minute or two. It is evidently the result of a stimulation connected with the changes immediately preceding death.

If the animals are returned to sea-water after one to one and a half hours in KCN, or if the dish is left open so that the KCN gradually escapes, more or less complete recovery may occur. The changes along the plate-row in recovery are essentially the reverse of those in KCN. The rhythms become more rapid, this change being much greater aborally than orally. Plates near the aboral end of the row, which had ceased to move gradually resume movement with increasing amplitude of vibration until finally the whole row is again active.

The most interesting phase of recovery is the subordination of the independent oral portions of the row to the aboral impulse. Where no reversal of direction of conduction has occurred in the oral region it can be observed that the aboral rhythm gradually impresses itself on the oral region, at first only intermittently but with increasing approach to continuity, until finally the oral region is again under control. In cases where two or three independent regions with different rhythms arise in the oral region of the row, the subordination or control of these regions in recovery progresses in the oral direction until a single rhythm again extends over the whole length of the row.

In cases where the direction of conduction has undergone reversal in the oral region, this region retains its independence for a longer time than otherwise, sometimes until the death of the animals, which usually occurs after a day or two in the laboratory. The reversal in direction of the impulse, or conditions associated with the reversal, have somehow made this region more independent of the other impulse.

A few other incidental experimental data and observations are briefly mentioned. The effect of cutting across a row of plates is like that observed by Parker (4). Recovery on both sides of the cut occurs rapidly and on the aboral side of the cut the usual rhythm synchronous with that of the other row of the same quadrant is maintained, while oral to the cut an independent rhythm arises. Direct transmission across a cut as recorded by Eimer (30) and Verworn (3), was not observed in *Mnemiopsis*.

Several cases were found in which a plate-row had been separated into two parts by some injury, and the wound had healed, leaving a distance of one to several millimeters between the two parts. In all such cases observed, the part oral to the injury showed a rhythm independent of parts aboral to it, even though the separation of the parts by the injury was not more than one or two millimeters.

If the plate-row is divided into several independent regions by cuts across it at different levels and the animal then placed in KCN the retardation of rhythm, decrease in amplitude of vibration and cessation of movement occurs in general most rapidly in the most aboral portion and less rapidly in each succeeding portion in the oral direction. In such cases, before inhibition has proceeded too far, several different rhythms are present, the slowest rhythm in the most aboral portion and successively more rapid rhythms in each successive portion in the oral direction. In this respect these cases where the plate-row is separated by cuts are like those described above, in which one or several different rhythms appear in the more oral regions of the row without any mechanical interruption of continuity. In the one case a physical, in the other a physiological isolation has occurred.

In general the susceptibility of the plate-rows as well as of the whole body-surface of *Mnemiopsis* is greatest in the youngest animals and decreases with advancing age as in other animals (22). In the very young animals, where the plate-rows consist of only six to ten plates the gradient in susceptibility along the rows is slight and independence of the oral portion has not been observed. Apparently in those stages the length of the row is so short that the impulse undergoes but little decrement. In the large old animals independent rhythms appear in the four long rows more frequently than in the four short rows, another fact indicating the existence of a spatial decremental factor in the transmission of the impulse.

DISCUSSION

The nature of transmission in the plate row. Considering first the question of the nature of transmission, the experimental data do not support the theory of direct mechanical transmission. All the phenomena of inhibition by cyanide are essentially similar to those observed in metabolic gradients along the main axes of simple organisms (23, Chaps. IV, V). The ability of the plates to respond to direct mechanical stimulation long after rhythmic metachronic movement has ceased and the fact that this response is either not transmitted at all or only to two or three plates make it probable that mechanical transmission does not play any very important rôle. Before the plates cease to move the amplitude of their vibrations gradually decreases, while transmission still occurs and is effective to a certain limit of distance. But the appearance in KCN of independent rhythms in the oral regions of the plate-row before transmission and movement of plates have ceased in more aboral regions presents the greatest difficulties to the mechanical hypothesis. If transmission is mechanical it is impossible to understand how one plate can beat with a certain retarded rhythm while the plate next to it orally develops an independent more rapid rhythm, or how, in the absence of the impulse from the apical region, any level of the plate-row may become the point of initiation of a new rhythmic impulse. The only conclusion possible in view of all the facts is that reached by Parker (4) that, while direct mechanical transmission may occur to some extent or under certain conditions, it is not the fundamental or chief method of transmission. The hypothesis of neuroid transmission is the only one which will account for the facts.

The susceptibility gradient. Assuming then that transmission is neuroid in character it is necessary to interpret the various phenomena of inhibition by cyanide on this basis. The first and perhaps the most conspicuous feature is the gradient in susceptibility to KCN along the plate-row. Cessation of movement begins in all cases at the aboral end of the row and progresses in general in the oral direction. Cessation of vibration of the plates, however, does not mean loss of the ability to vibrate, for plates that have ceased to vibrate in the regular progress of inhibition may still be induced to vibrate by direct mechanical stimulation. Evidently cessation of movement in KCN is the result of cessation or decrease below the threshold of the transmitted impulse.

Decrease in amplitude of vibration of the plates precedes complete cessation of movement, but the plates when stimulated mechanically after complete cessation of movement may show the full normal amplitude of vibration. This decrease in amplitude of vibration may result in part from decrease in the irritability of the plate itself in KCN, but the fact that vibrations of full amplitude may follow mechanical stimulation even after the cessation of natural movement, proves that another factor must also be concerned. There seems at present to be no escape from the conclusion that a decrease in the intensity or physiological effectiveness of the transmitted impulse must be at least in part responsible for the decrease in amplitude of vibration. If this conclusion is correct amplitude of vibration must be a function of intensity of impulse, at least up to a certain limit and the decrease in amplitude and final cessation of the rhythmic movement in KCN must mean that the intensity of the impulse gradually approaches the threshold and finally falls below it.

In the decrease in amplitude of vibration the same gradient along the plate-row as in cessation of movement appears. In this gradient several factors may conceivably be concerned: there may be a gradient in rapidity of decrease in intensity of the transmitted impulse with a decrease in rapidity in the oral direction; or a gradient in the same direction in the rapidity of decrease of irritability of the plates, or the threshold of stimulation may be highest in the most aboral plates of the row and may decrease in the oral direction or possibly all these factors may play some part in the result.

Another effect of KCN is the progressive retardation of the rhythm, the decrease in frequency of impulse. This effect also appears in the form of a gradient, the retardation being most rapid aborally and decreasing in the oral direction. This gradient, however, becomes visible only when the plate-row is separated by section at several levels into several independent portions, or when one or more oral regions become physiologically independent in KCN. In all such cases at the proper stage the rhythm is slowest in the most aboral portion and increases in each successive portion in the oral direction. In later stages of course complete inhibition occurs in the aboral region and retardation progresses in the oral direction.

All these facts indicate the existence of a gradation of some sort in the path of conduction along the plate-row. As regards the amplitude of vibration, the retardation of rhythm and the cessation of movement, the action of KCN is most rapid at the aboral end of the

plate-row and decreases progressively to the oral end. Moreover, the aboral end of the plate-row, the region of highest susceptibility, is the region where the normal impulse originates and the transmission of this impulse is from levels of higher to levels of lower susceptibility. On the basis of the general relation between susceptibility to KCN and many other agents and metabolic activity or physiological condition mentioned on p. 89 above and discussed in various earlier publications (see especially 17, 18, 22, Chap. III, 23, Chap. III, 25), we are forced to conclude that the susceptibility gradient along the plate-row of the ctenophore is an indicator of a gradient in general metabolic activity, or irritability, i.e., the potentiality of metabolic activity as expressed in the condition of the protoplasmic system. According to this conception the aboral or apical region is the region of highest metabolic rate and the rate decreases in the oral direction along the plate-row. The normal impulse then is transmissible down the gradient as in the case of the nerve fiber according to Tashiro (10, 11, 12), in other words the region of highest metabolic rate dominates or sets the pace, so to speak, for other levels, and in isolated portions of the plate row the level of highest rate becomes the dominant or controlling region.

The fact that essentially similar relations between a metabolic gradient and physiological dominance and subordination are characteristic features of general body-axes in both animals and plants (23) must at least suggest the possibility that a certain fundamental similarity exists between physiological axiation and order in the development of the organism and in the transmission of impulses along a conducting path, whether protoplasmic, "neuroid" or of the highly specialized nervous type. In fact, if we conceive the general organic axis as a dynamic or metabolic gradient established by the general protoplasmic transmission, with a decrement in intensity, of dynamic changes from a region of high metabolic activity, which is itself determined in the final analysis by the differential action of external factors upon the protoplasm concerned—if we accept this conception of the organic axis, we can trace a genetic relation between the general physiological axis in its simplest form and the highly specialized nerve-axis. The one represents in fact the most generalized, the other the most specialized condition of the same thing.

Physiological Isolation. Under the usual conditions the impulse is transmitted over the whole length of the plate-row, and conclusive evidence for a decrement in intensity or effectiveness in the normal

animal is lacking. It has seemed to me, however, that a distinct gradual decrease in amplitude of vibration of the plates toward the oral end of the plate-row could sometimes be seen when the animal was not strongly stimulated, but I cannot state this as a positive fact. Whatever the condition in the normal animal, it is evident that sooner or later in KCN the impulse from the aboral end loses its effectiveness at some point near the oral end, especially of the long plate-rows, and the oral region thus set free from the control of the impulse transmitted from more aboral levels soon initiates a rhythmic impulse of its own. Later, a second and in some cases even a third region may be thus set free from aboral control and develop its own independent rhythm. Stated in slightly different terms, one or more regions at the oral end of the plate may be successively physiologically isolated as the effectiveness of the original impulse decreases in KCN.

Conditions on both sides of the point where the aboral impulse ceases to be effective probably play a part in determining the position of this point. The action of the cyanide is most rapid in the aboral portion of the row and the retardation of rhythm is greatest there. Since the oral region is less affected by KCN it is capable, if isolated from the aboral impulse, of initiating a much more rapid rhythm. It seems probable that when the aboral rhythm has been retarded to a certain degree an independent more rapid rhythm may arise at some point in the oral region in the intervals between the aboral impulses. If, however, the impulse from the aboral region retains its original intensity or effectiveness and is merely retarded in rhythm we should expect the independent rhythm of the oral region to be interrupted or modified by the passage of the less frequent aboral impulses over the region. This does occur in some cases in the early stages of independence but later the oral region becomes entirely independent and the aboral impulse does not produce any effect upon it. The only possible conclusion is that the aboral impulse has lost in intensity or been weakened in some way, so that its effective range, i.e., the length of path over which it is effective, is decreased.

The fact that the effective range of the impulse is limited and undergoes a progressive decrease in KCN indicates very clearly that a decrement in intensity or effectiveness occurs in transmission, at least under these conditions, and that consequently a spatial range of effectiveness exists, which decreases as the inhibitory action of KCN proceeds. In short, the impulse behaves like a wave in a physical medium in that at a certain distance from its point of origin it dies out, so far

as the characteristic physiological effect is concerned, and this distance decreases progressively with the action of KCN. This decrease may conceivably be due either to a decrease in intensity of the impulse at the point of origin or to a decrease in conductivity of the path or more probably to both factors. As noted above, the facts indicate that a decrease in intensity does occur in KCN and it is probable also that a decrease in conductivity of the path occurs, in fact a decrease in rate of conduction is clearly visible. In any case it is evident that the aboral end of the plate row is most susceptible to the inhibiting action and that even while the oral portions of the plate-row still retain their irritability and conductivity the impulse transmitted from the aboral end becomes ineffective at a greater or less distance from the oral end. Using the physical analogy we may say that KCN decreases the height of the wave at its point of origin and the conductivity of the medium, and so decreases the distance it travels before becoming ineffective.

These cases of the escape of one or of successive regions at the oral end of the plate-row from the control of the impulse transmitted from the aboral end are cases of physiological isolation similar in character to cases of physiological isolation observed and experimentally produced in the axes of the simpler organisms (16; 22 p., 228; 23, Chap. V). Physiological isolation of parts or regions in a metabolic gradient may be brought about in four ways: first, by growth of the protoplasmic or cell mass so that the length of the mass in a given axis is greater than the effective range of control of the dominant region; second, without altering the actual size of the mass, by decreasing the metabolic activity in the dominant region and so decreasing the effective range of control so that those portions of the mass most distant from the dominant region are no longer affected by it; third, by decreasing the conductivity of the path along the gradient, i.e., by decreasing its excitability, and in this way decreasing the effective range of the transmitted change; fourth, by excitation of a subordinate region to such a degree that it becomes independent of the excitation transmitted from other regions. Physiological isolation occurs in nature and can be induced experimentally in these four ways.

In the physiological isolation of the oral region of the ctenophore plate-row the second factor, decrease in the activity of the dominant region, and the third, decrease in conductivity of the path, are undoubtedly concerned and in addition there is in consequence of the differential susceptibility to KCN a relative increase in the metabolic activity of the oral, as compared with the aboral region which is essen-

tially similar to the fourth factor, excitation of the originally subordinate region. But whatever the rôle of these three factors in any particular case, the fact of physiological isolation of the oral region or of two or three regions successively is sufficiently evident.

In the plate-rows of small young individuals physiological isolation of the oral region in KCN has not been observed, and in the four shorter plate-rows of large individuals it is much less frequent than in the four longer rows. Apparently, as might be expected if the impulse undergoes a decrement in effectiveness in the course of transmission, the longer the plate-row the more frequent is physiological isolation at its oral end.

The effect of physiological isolation. In the conducting path of the ctenophore plate-row, as in the axis of the simpler animals and plants, the effect of physiological isolation is essentially similar to that of physical isolation by section, viz., the reproduction of a new individual order, the development of a new individual. Physiological isolation in the chief axis of the lower organisms is the necessary condition for many if not all the processes of agamic reproduction, fission, budding, etc., and in the minor axes, of reduplicative reproduction of parts. The reproductive process in the physiologically isolated part of the ctenophore plate-row consists in the initiation of an independent rhythmic impulse which begins at one end of the isolated portion and is transmitted over its length. This physiologically isolated region then becomes a new individual essentially similar to the previously existing individual—the whole plate-row—of which it was originally a part. The result is the same as the result of physical isolation of a part of the plate-row by cutting across the conducting path. Not only one but two or three such individuals may arise successively, beginning at the oral end of the row, as the original impulse becomes progressively weaker and its effective range decreases. In some of the simpler animals, e.g., *Planaria* (16) series of new individuals arise at the posterior end of the body in essentially the same way, either as the result of increase in the length of the body or depression or removal of the anterior end.

These physiologically isolated regions of the plate-row, particularly in the earlier stages of their isolation, are sometimes temporarily subordinated again to the original impulse, either in consequence of summation of excitations at the boundary between the two rhythms, or perhaps by unusually intense impulses which have a greater effective range and so are able to pass this boundary. The same relations ap-

pear between anterior and posterior zoöids in *Planaria*. Summation of impulses or strong stimulation of the anterior body-region may bring the posterior zoöids under complete control of the anterior region for a time, but as the animal returns to the usual condition they soon become physiologically isolated again to a greater or less degree.

This initiation of a new and independent rhythm in the physiologically isolated, oral portion of the plate-row is then in the broad sense a case of reproduction, differing from various agamic reproductive processes in the simpler organisms chiefly in its somewhat specialized character. The occurrence of this reproductive process depends upon the fact that these portions of the plate-row, while they do not ordinarily initiate a rhythmic impulse but are subordinated to the rhythmic impulse transmitted from the aboral end, still retain the capacity to initiate such an impulse where the original impulse is prevented by any means from reaching them or when its intensity falls below a certain level. Similarly the agamic formation of new individuals from parts of the body of *Planaria* and other forms depends upon the fact that these regions, while physiologically and morphologically parts of an individual still retain the capacity to become new individuals when physiologically or physically isolated from the control of the dominant region of the original individual.

The effect of recovery from KCN. Where recovery is permitted to occur, the physiologically isolated region where a new individual has developed may be again subordinated to the dominance of the aboral end of the plate-row and so may lose its independent rhythm, i.e., its individuality, and again become what it was originally, a part of a larger individual. This is a process of reintegration, the reverse of reproduction. This reintegration evidently results from an increase in the intensity and so of the effective range of transmission of the aboral impulse during recovery, until it dominates and obliterates the independent rhythm in the part which was before physiologically isolated. Similar reversal of the reproductive process and reintegration may be brought about by fundamentally similar methods after agamic reproduction has begun in the lower animals. For example, in *Planaria* and other forms new "zoöids," i.e., new developing individuals in the posterior body-region, may be made to disappear by removing the anterior half or more of the original or parent individual and permitting a new head to regenerate from the cut end, which is much nearer the new zooid than was the original head. Since the distance between the regenerated head and the new zoöid is much

less than that between the original head and the new zoöid, the zoöid is no longer physiologically isolated and disappears as an individual, becoming again a part. In some forms this reintegration may be brought about even after a considerable degree of morphological development of the new individual has occurred. This case differs from reintegration in the ctenophore plate-row merely in that in the one the intensity and so the effective range of the transmitted impulse is increased by recovery from KCN, while in the other the dominant region is actually brought nearer to the physiologically isolated region which then falls within the effective range of the transmitted dynamic changes.

The rhythmic period in physiologically isolated regions. When a region at the oral end of the plate-row is physiologically isolated and develops an independent rhythmic impulse, the rhythmic period, i.e., the interval between impulses is shorter than the period existing at the same time in the more aboral region and when two or three regions are successively isolated physiologically and develop independent rhythms, the rhythmic periods in all these regions are shorter than in the aboral regions, but that of the most oral region is the shortest of all, that of the second region longer and that of the third region still longer.

These differences in the independent rhythmic periods at different levels of the plate-row undoubtedly depend at least in part upon the general metabolic gradient which appears in KCN as a susceptibility gradient. The aboral end of the row is most susceptible to KCN and the rhythm is most retarded there, while the oral end is least susceptible and the rhythm is therefore least retarded in the stages of KCN-action under consideration. Between these two extremes are intermediate degrees of susceptibility, and when the aboral impulse becomes so weak that one or more oral regions are physiologically isolated the rhythmic period in each such region must depend in part upon its level in the general gradient and so upon the degree to which KCN has already affected it. But the rhythmic period in these physiologically isolated oral regions of the row are often very short, even shorter than those in normal animals and the rhythmic activity may be irregularly intermittent. The behavior of these regions when physiologically isolated suggests the possibility that some factor which regulates and orders the rhythmic period in normal animals is not fully developed in these regions which are suddenly made independent. In fact, to state the case in unscientific terms, they behave as if they were not accustomed to independence. Until we know more of the dynamic conditions which determine rhythmic activity, interpretation of its changes under experimental conditions can not go very far.

The direction of transmission in physiologically isolated regions. In the physiologically isolated regions of the plate-rows the direction of transmission is, in the majority of cases, aboral-oral like that of the original impulse, but sometimes a reversal of direction occurs sooner or later in the extreme oral region, and the impulses run in the oral-aboral direction. If the direction of transmission is connected in any way with the metabolic gradient, as both Tashiro's and my experimental data indicate, a reversal in direction of transmission must be associated with a reversal of the gradient, and I believe that such reversal of the gradient has occurred in these cases. Since the levels of higher metabolic rate in a metabolic gradient are more susceptible to KCN in sufficiently high concentration than levels of lower rate, the general effect of KCN on such a gradient must be first a levelling down, a decrease in the metabolic differences at different levels, which may lead to complete obliteration and even to reversal of the gradient. This is true not only for KCN but for many other inhibiting agents and such reversals have been experimentally induced in the polar axes of organisms through differential inhibition, as experimental data soon to be published will show.

If a wave of increased metabolic activity is an essential feature of the transmission of excitation in protoplasm, it is probable that the effective range of such a wave is greatest in the downward direction along a metabolic gradient, less along a metabolic level and still less in the upward direction along a gradient. If this be true then the levelling down and reversal in KCN of a metabolic gradient along a conducting path must be an important factor in decreasing the effective range of an impulse transmitted along that path. A metabolic wave probably can not be transmitted up a gradient beyond the point where the metabolic rate or the protoplasmic condition before excitation is the same as that in the wave of excitation. Moreover, in a metabolic gradient in which rhythmic impulses originate, as in the conducting path of the ctenophore plate-row, it is evident that normally the impulses originate in the region of highest metabolic rate in the gradient and are transmitted down the gradient. There is every reason to believe that the same relation between gradient and rhythmic impulse exists in the physiologically isolated oral regions. Where the direction of transmission remains aboral-oral the original gradient still persists, and where transmission is in the opposite direction the gradient has been reversed by the differential action of KCN on different levels of the original gradient. The limitation of reversal to the

oral region of the plate-row in my experiments is probably due at least in part to the fact that metabolic activity in the more susceptible aboral regions of the plate-row is inhibited so rapidly and to such an extent that these regions become incapable of initiating or transmitting impulses by the time a well marked reversal of the gradient has occurred in them. With less toxic agents or perhaps with lower concentrations of KCN it may be possible to reverse the direction of transmission throughout the whole length of the plate-row.

In some species of ctenophores reversal in the direction of transmission occurs or can be experimentally induced in normal animals. Parker (4, p. 411) states that in *Pleurobrachea* a rapid wave in the aboral-oral direction is sometimes "reflected" at the oral end of the plate-row and is transmitted in the oral-aboral direction, but rarely over more than one third the length of the plate-row. Much earlier Eimer (29, p. 226) observed reversal in the direction of transmission in *Beroe* and Chun (7, p. 182) records similar observations on *Beroe* and other species, while Verworn (3, p. 167; 30, p. 440) observed that such reversal can often be induced by stimulating mechanically the oral end of a plate-row.

I believe that all such cases of reversal in the direction of transmission are dependent upon temporary reversal of the metabolic gradient along the path, or that part of it where reversal occurs. In the case of "reflection" recorded by Parker the excitation reaching the oral end of the row increases the metabolic rate there so rapidly and so far above the level of adjoining parts that an impulse is initiated there and is transmitted backward to a greater or less distance. Reversal after mechanical stimulation of the oral end of the row as observed by Verworn is evidently due to the increase in metabolic rate at that end in consequence of the stimulation and so the initiation of an impulse sufficiently intense to travel some distance in the oral-aboral direction.

All such cases of temporary reversal in the direction of transmission are in reality temporary reversals of the physiological polarity of the conducting path and the readiness with which they occur or can be induced undoubtedly varies with the slope of the metabolic gradient and with the degree of permanency or irreversibility of the record in the protoplasmic condition of the dynamic gradient in different species.

Physiological polarity in the lower organisms shows similar possibilities of reversal and alteration by very similar methods (23, pp. 96, 117, 132, 142) and, as in the ctenophore plate-rows, the readiness with which reversal occurs or can be induced in different species depends

upon the degree of permanency or irreversibility of the record in protoplasmic condition and differentiation of the preexisting dynamic gradient.

THE GENERAL SIGNIFICANCE OF METABOLIC GRADIENTS

The conception of the physiological axis as consisting in its simplest form of a metabolic gradient together with the gradient in protoplasmic condition in the broadest sense which must be associated with a dynamic gradient has proved a fruitful working hypothesis in its application to normal processes and experimental modifications of development in both animals and plants (16–28). Many different lines of evidence indicate the existence of such axial gradients, and it is possible to control and modify development to a high degree through the differential action of external agents upon such gradients and to interpret in terms of gradients modifications produced in nature and experiment. If the conception is correct it means that the first step in the physiological integration which constitutes what we call the axiate individual or organism consists in the establishment, or the inheritance from a preexisting individual of one or more such gradients. The primary gradient represents the primary or chief axis and the region of highest metabolic rate in that gradient becomes the apical or head region, and in other axes the morphological and physiological order or pattern shows a definite relation to the metabolic gradient in those axes. In any such gradient the region of highest metabolic rate dominates or controls regions of lower rate, and is the primary factor in establishing the gradient, because in consequence of its activity dynamic excitatory changes of some sort are transmitted through or over the limiting surfaces of the protoplasm to other less active regions and are more effective in determining their metabolic activity than excitatory changes transmitted from regions of lower rate. In short the highest level of the gradient is to a greater or less degree physiologically dominant because the excitatory changes initiated in it are greater or more intense than those initiated at other metabolic levels.

Since in general protoplasmic transmission a decrement in intensity or effectiveness occurs, such a transmitted change has a limited effective range which varies in general with metabolic activity and protoplasmic condition, and this effective range determines the spatial limit of such physiological dominance.

According to this conception the unity and order, the physiological integration of the organism is primarily dependent upon the transmission of dynamic changes rather than upon the transportation of

chemical substances. In other words, physiological integration in the axiate organism is primarily "neuroid" in character rather than a matter of so-called chemical correlation, though I prefer "transmissive" and "transportative" to "neuroid" and "chemical" as denoting the character of the fundamental condition in such an integration.

As I have shown elsewhere (23) localization and differentiation arise in relation to different levels of the axial metabolic gradients, and in a system so complex as even the simplest living protoplasm, there is no difficulty in accounting for the origin of qualitative from quantitative differences. As soon as differentiation begins, transportative or chemical correlation begins to play an essential rôle and is of course of great importance in further development. It is evident, however, that transportative or chemical correlation cannot be the starting point of physiological integration in the individual because a definite unity and order, a definite organization, in short an integration, must be present before such correlation is possible. It is this primary, fundamental organization that the conception of metabolic gradients attempts to account for. This conception is in no sense a substitute for the conception of chemical correlation which plays so important a rôle in present-day physiology. It is merely an attempt to establish the basis upon which chemical correlation becomes possible. The gradient is merely the starting point and as soon as the production of different substances at different levels of the gradient begins, which must be very early, transportative correlation becomes an essential factor in determining the further course of events. The gradient merely determines the primary pattern, and chemical factors may play the chief, or at least a very large rôle in determining the character of the different parts of the pattern.

If we conceive the organism merely as a complex of specific chemical correlations we cannot account for the origin and development of the nervous system. No adequate reason can be given for the transformation of a system in which correlation is primarily transportative or chemical into a system with transmissive correlation. The transmissive factor must be, as we know it is, a fundamental property of living protoplasm, and if this is true, this factor must play a fundamental part in physiological integration. If the organism is primarily a transmissive integration the origin, development and functional dominance of the nervous system become at once intelligible. Moreover, the central nervous system develops in the regions of highest metabolic rate in each of the primary axial gradients of the organism (23, p. 175).

This fact is highly significant as indicating that the nervous system is merely the final morphological and physiological expression of the relations which in their simplest terms are represented by metabolic gradients.

If this conception is correct we must expect to find that the nerve fiber is primarily a metabolic gradient and Tashiro's observations (10, 11, 12) indicate that this is actually the case. The existence of a gradient in the "neuroid" conducting path of the ctenophore plate-row is also to be expected, and the similarity between its behavior and that of the chief axis of the simpler organisms follows as a matter of course.

We must expect, moreover, to find that other organs in which rhythmic impulses are transmitted in a definite direction are likewise primarily metabolic gradients. The vertebrate heart, for example, is such an organ, and all the experimental data which we possess concerning its rhythmic activity indicate the presence of a metabolic gradient. The sinus-region which normally initiates the beat and so is physiologically dominant must be primarily the region of highest rate in this gradient. It is a familiar fact that when this region is inhibited by cooling or otherwise, the beat may begin in the uninhibited region nearest to the sinus and reversal of the direction of the beat by means of inhibition of the sinus end and stimulation of the bulbus end has even been induced. This is essentially similar to what occurs in the ctenophore plate-row and also in the axes of the simpler organisms.

In the ascidian heart reversal of the direction of beat occurs periodically under natural conditions probably in consequence of differential fatigue, the region of high rate in the gradient at any given time, which is the dominant region, the initiator of the beat at that time, becoming fatigued more rapidly and to a greater degree than regions of lower rate. In consequence of this differential fatigue the metabolic activity of the dominant initiating region decreases more rapidly than that of other levels and this leads sooner or later to cessation of the beat in the original direction. The existing gradient is also levelled down or perhaps reversed by differential fatigue and the region which was formerly the low end being least fatigued recovers more rapidly and so contributes further to reversal of the former gradient. In this way the low end of the gradient of one series of beats becomes the high end and the initiator of the next period, and in this manner periodic reversal of the direction of beat continues. According to this interpretation the ascidian heart is simply a reversible gradient, while in the heart of the higher vertebrate the gradient is much more stable, and reversal therefore less readily induced.

It is, of course, not necessary to assume that transmitted impulses or changes along a metabolic gradient are always rhythmical, they may be tonic, irregular or rhythmical. In the chief physiological axes of the organism they may be largely tonic with irregular, or more or less rhythmic changes as metabolic changes in the dominant region occur.

This conception seems at first glance to disagree with what we know concerning transmission in the medullated nerves of vertebrates. It has been stated repeatedly that in these nerves transmission under normal conditions shows no decrement in intensity or effectiveness and the further assertion has been made that the "all or none law" applies not only to the primary excitation but also to transmission in such nerves. If there is actually no transmission-decrement in such nerves then transmission to an infinite, or better an indefinite distance, would occur in a nerve fiber of infinite or indefinite length. It seems highly improbable that any physico-chemical medium is capable of such transmission; moreover, as regards the medullated nerve, it is a familiar fact that under various experimental inhibitory conditions such as partial anesthesia, cooling, etc., a decrement in effectiveness and a limit of effective range appear. It is difficult to believe that anesthesia or low temperature or other inhibitory conditions alter so fundamentally as this the nature of transmission in the medullated nerve. That they decrease the conductivity or the intensity of the impulse or both and so increase the decrement and decrease the effective range can readily be understood, but that they determine a decrement and a limit of effective range where none is present normally, it is difficult to believe. There is much evidence for the normal existence of a decrement and a limit of effective range in the more primitive protoplasmic and neuroid conducting paths, and in view of this fact, the only logical conclusion seems to be that the medullated nerve is simply a so much better conductor of impulses than these primitive paths that within the lengths of nerve fiber available or ordinarily used for experiment, the decrement is slight or inappreciable. The evidence in the case taken as a whole seems to point very clearly to this conclusion as the only one possible, and if we accept this conclusion, the medullated nerve presents no difficulties to the general conception of metabolic gradients.

The attempt has been made in this paper to show, on the basis of experiment upon a relatively primitive "neuroid" conducting path that there are adequate reasons for believing that the body axes of organisms in their simplest terms, and the most highly specialized axes in the organism, the nerves, as well as other physiological axes intermediate between these extremes are fundamentally similar in certain

respects. Considered in the light of its value as a basis for experimental investigation, analysis and synthesis, the hypothesis justifies itself, and while it will undoubtedly undergo modification as time goes on, I believe it will serve to throw light on many physiological and morphological problems. Objection may be made to the term "metabolic" as applied to the axial gradient. This term means no more than that differences in the degree of metabolic activity are associated with and serve as an indicator of the gradient. Since function and structure are indissociable, it goes without saying that a metabolic gradient cannot persist or even exist without associated differences in protoplasmic condition corresponding to different levels of the gradient and those who prefer to emphasize the structural or physical rather than the dynamic or chemical aspects of the gradient may prefer to call it something else than a metabolic gradient. But whatever we call it, the facts indicate that a gradient in activity or in reactive capacity, determined in the final analysis by the differential action of factors external to the protoplasm concerned, represents the first step in not only the physiological integration of the axiate individual or organism, but in that of axiate organs and parts.

It must be noted, however, that such a gradient represents only one possible type of integration or individuation. Even in the organism many other kinds of integration undoubtedly occur, such as, for example, molecules, molecular complexes, colloid particles, crystals, etc. The conception of the gradient is concerned only with that sort of integration which expresses itself as a definite, controlled, progressive order of events in space and time, occurring in living protoplasm, whether cell or cell mass, with a definite relation to certain directions or axes in the protoplasm. The specific protoplasm or even the cell, with all the possible kinds of integration or individuality which may be present in it is regarded as given, and the conception of the gradient is merely an attempt to answer the question, what is the nature of a definite physiological axis in a specific protoplasm, whether cell or cell mass?

SUMMARY

1. In the conducting path along the row of swimming plates of the ctenophore, *Mnemiopsis leidyi* a gradient in susceptibility to KCN exists. This gradient is indicated by the fact that decrease in amplitude of vibration, increase in rhythmic period and cessation of rhythmic movement occur first at the aboral end of the plate-row and show a regular progression toward the oral end. Since the plates remain

capable of responding to direct mechanical stimulation by beats of full amplitude after the rhythmic beat has ceased, decrease in amplitude and cessation of rhythmic movement must be due primarily to changes in the transmitted impulse rather than in the plates themselves.

2. This susceptibility gradient is an indicator of a gradient in general metabolic rate and in protoplasmic conduction associated with it. According to the relation between susceptibility and general metabolic condition the aboral end of the plate-row is the region of highest metabolic rate in this gradient and from this the rate decreases in the oral direction.

3. The effective range of the rhythmic impulse decreases in KCN until it may be less than the length of the plate-row. Under such conditions a longer or shorter region at the oral end, or two or three regions successively, became physiologically isolated and develop independent rhythms which have a shorter period than the more or less inhibited impulses from more aboral regions. This difference in rhythmic period is another feature of the gradient and results from the fact that the less susceptible oral regions are less inhibited and their rhythmic period less retarded than the aboral region.

4. Occasionally a physiologically isolated oral region shows reversal in the direction of transmission at a certain stage of KCN action. Such reversal is undoubtedly associated with reversal of the metabolic gradient through the differential susceptibility to KCN.

5. In recovery the effective range of the aboral impulse increases, and regions previously physiologically isolated are brought again under control. Amplitude of vibration also increases and rhythmic period decreases during recovery.

6. The behavior of this physiological axis under the conditions of experiment is fundamentally similar to that of the main body axes of organisms, which are also in their simplest terms metabolic gradients, and the experimental data serve as a basis for consideration of the general significance of metabolic or dynamic gradients as physiological axes, both in the organism as a whole and in its parts, even in axes so highly specialized as nerve fibers.

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CARBON DIOXIDE ACIDOSIS, THE CAUSE OF CARDIAC DYSPNEA

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This paper deals with the discrepancies observed in a series of cases in a comparison of the carbon dioxide of the alveolar air and that of the plasma. Comparisons of a similar nature have been made by Van Slyke (1), Peabody, and Walker and Frothingham (2).

METHODS

For the plasma CO_2 the Van Slyke method (1) was used. In this method plasma is saturated with an atmosphere containing about 6 per cent CO_2, the tension usually obtaining in alveolar air. The plasma is then introduced into the Van Slyke pipette and rendered strongly acid to release CO_2 from the carbonates. This carbon dioxide is pumped out by means of a Toricellian vacuum and measured volumetrically. Corrections are made for temperature and atmospheric pressure and the volumetric reading reduced to the mgm. CO_2 chemically bound, which it represents. Finally, multiplication by an empirical constant, 35, converts it to terms of the alveolar carbon dioxide tension that should correspond with the determined concentration of carbonates in the plasma.

The blood for the carbonate determinations was withdrawn within fifteen minutes of the time when the last alveolar specimen was obtained and always after the alveolar work had been completed. The latter precaution was taken to eliminate the possible action on the respiratory center of the excitement caused by the venous puncture. The blood was drawn directly into a centrifuge tube containing a small amount of neutral, recrystallized potassium oxalate, removed at once to the laboratory and centrifugated. In most cases the blood was drawn and centrifugated under a layer of albolene. The plasma was

aerated in a 250 cc. separating funnel with the author's alveolar air[1] and the carbon dioxide content determined immediately. All determinations were made in duplicate. All studies were made just before meals, either between 11 and 12 a.m. or 3.30 and 4.30 p.m.

For the determination of the alveolar carbon dioxide the Fridericia (3) method was employed. It seemed best to use some modification of the Haldane method and to make the comparison with arterial carbon dioxide, both because the original work of Van Slyke was done in this way and also because arterial readings might be expected to bring out discrepancies more clearly. (The work done by Peabody and by Walker and Frothingham shows that, for clinical purposes, this is an unnecessary precaution). Of the arterial methods the Fridericia is the simplest. It is impossible to use it unless the patient is intelligent enough to coöperate; it is also uncertain in the presence of marked respiratory irregularities, such as Cheyne-Stokes breathing; and it obviously demands a certain minimum respiratory capacity to clear out the dead space of the machine. In view of these possible errors the cases here reported have been chosen with the greatest care and much interesting material has been omitted. Repeated determinations were made in all cases and none have been accepted in which the readings varied by more than 0.2 to 0.3 per cent.

In all the tables Column I shows the observed alveolar carbon dioxide, Column II the alveolar carbon dioxide calculated from the carbonate determination and Column III the ratio of the actual to the calculated value.

OBSERVATIONS

Of course the deductions made from such a study are largely dependent for their value upon the accuracy of Van Slyke's constant. In Walker and Frothingham's paper (2) 116 observations are reported on 100 cases. When these cases are compared on the basis of the ratio of alveolar to plasma CO_2, it is found that 92 out of the 116 ratios fall between 0.90 and 1.10; 102 between 0.85 and 1.15. If 1 mm. Hg. is subtracted from each alveolar reading in an attempt to reduce venous to arterial figures, 105 out of 116, or 90 per cent lie between 0.85 and 1.15.

Table 1 gives the results in five normal cases (the author, three mem-

[1] Determinations of my own alveolar carbon dioxide repeated over a period of fifteen months have shown a maximum variation of 3 mm. Hg., between 44.40 and 47.60, under normal conditions.

TABLE 1

Part 1

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
1	Normal adult	June 10		45.5	46.2	0.99
2	Normal adult	August 31		47.2	47.6	0.99
		September 4		44.1	43.1	1.02
3	Normal adult	August 21		45.8	42.0	1.09
		August 31		47.6	45.0	1.06
		September 4		46.0	41.7	1.10
		March 20		44.8	42.4	1.06
		June 2		44.4	45.5	0.98
		November 7		42.7	43.4	0.98
		November 8		44.3	43.9	1.01
		November 8		42.9	40.3	1.06
		November 8		46.1	42.7	1.08
		November 9		38.8	38.5	1.01
4	Normal adult	November 16		46.3	45.2	1.03
		November 16		43.0	42.5	1.01
		November 17		40.2	42.7	0.94
		November 17		48.5	45.2	1.07
5	Normal boy	April 15		42.4	42.7	0.99
6	Diabetes mellitus	May 19		43.3	46.9	0.92
		May 24		45.3	44.1	1.03
7	Diabetes mellitus	November 14		44.8	48.0	0.93
8	Diabetes mellitus	November 25		41.3	37.8	1.09
9	Diabetes mellitus	October 7		37.1	42.2	0.88
10	Diabetes mellitus	November 2		47.5	41.7	1.14
11	Diabetes mellitus	November 9		37.6	38.5	0.98
12	Diabetes mellitus	September 4		37.0	33.6	1.10
		September 10		43.0	44.1	0.97
13	Diabetes mellitus	December 13	No hyperpnea	38.1	37.1	1.03
14	Chronic deforming arthritis with obesity	June 9	Considerable expiratory dyspnea of the asthmatic type, with rather marked cyanosis	41.6	43.6	0.95
15	Acute nephritis	May 23	No hyperpnea	38.3	42.5	0.90
16	Acute nephritis	April 17	No hyperpnea	41.1	43.1	0.96
		May 16		47.1	43.8	1.08
17	Acute nephritis, nephrolithiasis	April 21	No hyperpnea	36.3	38.7	0.94
18	Acute nephritis, saphenous thrombophlebitis	April 17	No hyperpnea	33.1	32.6	1.02

TABLE 1—Continued

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
19	Acute nephritis	June 10	Considerable hyperpnea with long, deep respirations. No cyanosis	29.5	34.0	0.87
20	Chronic nephritis	October 20	No hyperpnea	38.4	41.0	0.94
21	Chronic nephritis	June 12	No hyperpnea	39.3	41.7	0.94
22	Chronic nephritis	June 2	Respiratory rate slightly increased, respiration short and regular. No cyanosis	37.2	42.4	0.88
		June 5	Respiratory rate normal	40.5	43.4	0.93
23	Chronic nephritis	June 13	Respirations slow, fairly deep and slightly irregular	41.9	41.0	1.01
24	Chronic nephritis		No dyspnea nor cyanosis	39.7	44.1	0.90
25	Chronic nephritis, fibrinous pericarditis. Uremia	April 26	Respiratory rate increased with long deep respirations and marked subjective dyspnea	27.1	27.3	0.99
26	Chronic nephritis, uremia	June 1	Respirations rapid and deep, but without subjective dyspnea. No cyanosis	21.6	21.4	1.00
27	Chronic nephritis	May 27	No hyperpnea nor cyanosis	34.5	37.8	0.91

Part 2

28	Diabetes mellitus		756 grams of bicarbonate administered in the first thirteen days			
		December 8	Evident dyspnea with long, deep respirations	28.7	30.5	0.94
		December 10	Hyperpnea increasing	26.6	26.6	1.00
		December 16	Hyperpnea very marked, almost Kussmahl	15.1	19.6	0.76
		December 17	No recognizable hyperpnea	23.5	35.4	0.66
		December 18	No recognizable hyperpnea	22.9	35.4	0.65
		December 19	No recognizable hyperpnea	30.6	49.8	0.61

TABLE 1—Continued

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
28	Diabetes mellitus	December 20	No recognizable hyperpnea	33.8	52.5	0.64
		December 22	No recognizable hyperpnea	33.0	52.6	0.63
		December 23	No recognizable hyperpnea	36.1	46.2	0.78
		December 24	No recognizable hyperpnea	37.7	41.0	0.92
		December 26	No recognizable hyperpnea	36.8	40.3	0.91
		December 30	No recognizable hyperpnea	33.7	42.4	0.80
29	Diabetes mellitus	October 28	No hyperpnea evident. Received large doses of bicarbonate just before admission	31.8	39.8	0.80
		October 29		28.8	43.9	0.66
		November 1		33.5	44.8	0.75
30	Diabetes mellitus	October 7	Respiratory rate 22, respirations short and without effort	34.1	28.4	1.20
31	Vomiting, starvation acidosis, diabetes	December 29	No hyperpnea	35.7	29.1	1.23
		December 31	No hyperpnea	29.4	30.5	0.96
		January 3	No hyperpnea	37.4	33.6	1.11
32	Chronic gout, acute attack	June 12	No hyperpnea nor cyanosis evident	35.2	47.3	0.74
33	Chronic nephritis	October 2	No hyperpnea	33.6	36.6	0.90
		October 7	No hyperpnea; after bicarnate, 20 grams	39.7	34.5	1.15
		October 11	No hyperpnea	36.5	35.0	1.01
		October 12	No hyperpnea	41.0	38.5	1.07
34	Chronic nephritis, mild uremia	June 2	Respirations increased in rate, short and regular slight cyanosis	32.2	33.6	0.95
		June 7	Respirations rapid, regular, short. Has nocturnal attacks of paroxysmal dyspnea	30.0	33.6	0.89
		June 14	Respiratons regular and short. Rate 20. No cyanosis. Has received some bicarbonate	31.5	36.1	0.87
		June 22	Respirations short, not quite regular, rate 24. Still has occasional attacks of nocturnal dyspnea Still receiving bicarbonate	30.8	42.7	0.72

TABLE 1—Concluded

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
35	Chronic nephritis, renal tuberculosis, uremia	September 3	Slight hyperpnea, rather deep respirations	32.4	32.2	1.01
		September 10	Dyspnea more marked	30.0	33.7	0.89
		November 7	No dyspnea. After bicarbonate	45.5	50.9	0.89
		November 24	Respirations quiet. Still taking bicarbonate	37.2	31.1	1.20

bers of the Presbyterian Hospital staff and one boy of fourteen who appeared to have no pathological condition), eleven diabetics, one case of starvation acidosis, one gout, one arthritis deformans with obesity, five acute nephritics, two cases of mild chronic nephritis, eight cases of advanced chronic nephritis, and one of renal tuberculosis and chronic nephritis; in all thirty-five cases, with seventy-six determinations.

Fifty-two ratios fall between 0.90 and 1.10; 60 or 79 per cent between 0.85 and 1.15. In all but three persons a normal relation was at some time observed and in two of these only single studies were made. The fifteen abnormal readings were distributed among seven cases: three diabetics, one starvation acidosis, one gout and three chronic nephritics. The alveolar readings in those with normal ratios varied from 19.6 to 47.6 mm. Hg. while the plasma values varied from 21 to 48. The relation, therefore, seems to be independent of the carbonate content of the plasma. In the 5 normal persons the 18 ratios obtained all fell between 0.94 and 1.10 giving a maximum variation of 0.10 or a mean variation of 0.04.

In the majority of cases, then, there is a very close correspondence between the two, so close as to suggest that a ratio below 0.85 or one above 1.15 denoted some abnormality in the respiratory mechanism. It may be that even this is too wide a range, as no such variation has been found in any healthy person.

Table 2 presents an entirely different series of cases. In all there was cardiac decompensation with more or less dyspnea, and usually some degree of cyanosis. The first five cases had hypertensive nephritis with cardiac decompensation. All had very definite dyspnea, in some very severe, while only one, No. 38, showed a definite diminution of blood alkalinity, and that of very mild degree. In other words, the

TABLE 2

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
36	Chronic nephritis, cardiac decompensation	May 13	Considerable dyspnea and orthopnea throughout the period of observation	37.4	46.9	0.80
		May 22		36.2	42.0	0.86
		June 3		32.7	44.3	0.74
		June 17		32.8	42.0	0.78
37	Chronic nephritis, cardiac decompensation	May 25	Marked dyspnea and considerable cyanosis	33.5	38.2	0.88
		June 1	Dyspnea and cyanosis continued	32.3	43.1	0.75
		June 13	Very little improvement	30.8	41.0	0.75
38	Chronic nephritis, cardiac decompensation	May 7	Dyspnea severe, chiefly expiratory, some cyanosis	28.6	38.7	0.74
		May 11	Dyspnea improved	31.3	33.8	0.93
		May 26		32.8	32.9	0.99
39	Chronic nephritis, cardiac decompensation	December 21	Extreme dyspnea with rapid, short respirations Massive right hydrothorax. Some cyanosis	27.5	37.1	0.74
		December 23		28.9	37.5	0.77
		December 24	Dyspnea greatly improved	34.6	37.1	0.93
		December 26	Dyspnea still less	38.0	37.8	1.00
		January 3	No dyspnea	37.7	38.7	0.97
40	Chronic cardiac valvular disease, chronic nephritis	February 24	Extremely dyspneic and cyanotic	27.1	36.4	0.74
41	Chronic cardiac valvular disease	December 29	Very cyanotic. Respirations short and rapid	39.5	48.0	0.82
		December 31	Dyspnea and cyanosis slightly diminished	38.9	47.6	0.82
42	Chronic cardiac valvular disease, acute bronchitis	February 24	Respirations quite rapid. Some cyanosis	32.9	46.6	0.71
		March 3	Respirations much improved	38.5	40.3	0.96
		April 4	Dyspnea considerable	29.5	41.7	0.71
43	Chronic cardiac valvular disease	April 14	Respirations somewhat rapid, apparently deep, somewhat irregular. Subjective dyspnea in excess of objective evidences	40.9	47.6	0.86

TABLE 2—Continued

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
43	Chronic cardiac valvular disease	April 22	Respirations slightly increased in rate, not very deep	45.2	48.0	0.94
		May 3	No subjective dyspnea, rate slightly rapid.	38.3	47.6	0.80
		May 17	No subjective dyspnea nor cyanosis. Respirations somewhat rapid and fairly deep	43.1	46.9	0.92
		June 2	Respirations rather rapid, deep and regular. No cyanosis	41.5	44.1	0.94
44	Chronic cardiac valvular disease	August 12	Respirations rapid and short	30.4	38.5	0.79
		August 13	Dyspnea improved	36.8	39.9	0.92
		August 17	Respirations still somewhat rapid	30.4	38.2	0.80
		March 28	Respirations short and rapid; no cyanosis	30.7	41.7	0.73
		April 6	Dyspnea improved	36.9	48.0	0.76
		April 24	Respirations still rather rapid	42.3	48.3	0.88
		December 30	Patient able to do light work. No subjective dyspnea while at rest	43.7	44.5	0.98
45	Chronic cardiac valvular disease	May 25	Moderate increase of respiratory rate with short breaths	39.9	43.9	0.91
46	Chronic cardiac valvular disease	May 30	Respirations rapid and quite short. Some cyanosis	34.6	46.2	0.75
		June 20	Respirations very rapid, short and slightly irregular. Some cyanosis	36.3	45.2	0.80
47	Chronic cardiac valvular disease	June 8	Respirations somewhat rapid and short; slight cyanosis	29.4	43.1	0.68
48	Chronic cardiac valvular disease	May 13	Extreme cyanosis. Respirations rather rapid Right hydrothorax	35.4	45.2	0.79
		May 16	Respirations still rapid. Cyanosis considerable	31.3	46.2	0.68

TABLE 2—Concluded

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
48	Chronic cardiac valvular disease	May 20	Respirations slightly improved. Cyanosis diminished	36.2	42.4	0.85
49	Chronic cardiac valvular disease	June 6	Considerable dyspnea and cyanosis	31.4	36.8	0.86
		June 19	Dyspnea slight	34.9	37.0	0.94
50	Chronic cardiac valvular disease	August 24	Marked dyspnea and cyanosis. Double hydrothorax	28.1	37.1	0.76
		August 31	Dyspnea and cyanosis somewhat diminished	32.6	46.9	0.70
51	Chronic cardiac valvular disease	August 17	Extreme dyspnea and cyanosis. Right hydrothorax	23.9	33.3	0.71
		August 21	Dyspnea improved. Thorax has been aspirated. Still some cyanosis	30.4	38.5	0.79
		August 25	Dyspnea still considerable. Cyanosis slight. After bicarbonate	37.2	49.4	0.75
		September 3	Dyspnea very much diminished	39.7	43.4	0.91
		October 8	Breathing rapid and very short with some cyanosis	32.9	43.1	0.76
52	Chronic cardiac valvular disease	April 5	Marked dyspnea, with short rapid respirations orthopnea and cyanosis	27.0	38.2	0.71

dyspnea was not due to an acidosis. The remainder of the cases were simple cardiac cases with dyspnea. Of these only one, No. 51, showed an acidosis in the plasma determinations, and this was very slight. All but two, however, showed an alveolar/plasma ratio below 0.85 at some time during their course. Of these, one, No. 49, went as low as 0.86 and the other, No. 45, showed no definite decompensation. In almost all cases with marked improvement or where the studies were continued into the period of compensation the ratio increased, approaching or attaining the normal. In some cases, Nos. 44 and 51, with an

interval of improvement a normal relation was established, only to fall away again with the development of a new insufficiency.

In most cases the plasma readings during the decompensated period were slightly lower than those found after recovery, denoting a relative diminution of blood alkalinity. In returning to the normal, as a general rule, both alveolar and plasma figures at first rose simultaneously, with a continuation of the abnormal ratio. With this rise there was an improvement in the dyspnea. With continued improvement the alveolar CO_2 rose to meet the plasma. Occasionally the plasma reading fell slightly. Probably a part of the dyspnea was due to fixed acidosis of slight degree.

In No. 38 the opposite was found. This patient seemed to recover from his cardiac difficulty very rapidly, losing his dyspnea and cyanosis, but his nephritis increased and the fall in plasma carbonates was accompanied by a rapid rise in the blood urea. He developed a mild degree of fixed acidosis after recovery from his acute cardiac insufficiency.

Table 3 presents three cases with extreme pulmonary disease that show a similar disturbance of the alveolar plasma ratio.

DISCUSSION

If the three tables were put together and the results analyzed there is no doubt that the close relation indicated above would be entirely destroyed and it may seem arbitrary to divide the cases as we have. We are certain, however, that a further accumulation of normal material would soon restore our percentage of close agreements, but have considered it unnecessary to undertake such a time-consuming task when the work has been so thoroughly carried out by Van Slyke and others. Besides, we believe that a careful analysis of the methods used and of collateral evidence offers a sufficient justification for such classification.

In view of the fact that the two methods do not agree, the question naturally arises whether one should consider the plasma or the alveolar reading as the true index of blood reaction. A careful consideration of the chemical factors involved leaves no doubt that, as far as the fixed acids are concerned, the determination of the carbonates of the plasma must give the more correct figures. If this were not the case the use of the alveolar CO_2 as an index of blood reaction would be fallacious. The original application of the alveolar methods to this study depends on the fact that the alveolar CO_2 tension is the same as that in the arterial blood and this, in turn, varies with the concentration of carbonate in

TABLE 3

NO.	DIAGNOSIS	DATE	REMARKS	CARBON DIOXIDE		
				Alveolar	Plasma	Alveolar/plasma
53	Diabetes mellitus, pulmonary tuberculosis, left pneumothorax	September 25	Respirations rapid (24) and rather short. Considerable subjective and objective dyspnea. Suggestion of cyanosis. The whole left chest is tympanitic except for some fluid at the base Intrathoracic pressure found greatly increased	33.7	44.5	0.76
		September 28	Respirations and pulmonary condition unchanged	32.3	39.9	0.81
		October 6	Condition unchanged	29.2	36.9	0.79
54	Carcinoma of the lungs	October 5	Very marked dyspnea, with short, rapid respirations. Extreme anemia and suggestion of cyanosis Autopsy disclosed massive carcinomatous involvement of the left pleura with complete collapse of the lung. The right lung was practically solid from carcinomatous infiltration	25.8	41.3	0.63
55	Thyroid carcinoma with lung metastases	December 9	Respirations always rapid and very short extreme cyanosis	27.8	49.8	0.57
		December 13		33.4	48.3	0.69
		January 1		36.6	47.6	0.77
		February 11	Dyspnea and cyanosis seem less marked Patient died of cerebral thrombosis. No autopsy could be obtained. X-ray showed both lungs practically solid.	40.2	49.2	0.82

the blood. In a balanced solution such as the blood, under a fixed CO_2 tension, no change of reaction can occur without a corresponding change in the total carbonate concentration. This has been practically demonstrated by Van Slyke by a comparison with the H-ion concentration determined by the gas-chain method, and by a study of the effect of the addition of acids and alkalies to the blood. The latter work we also repeated. We found that the addition of acids or alkalies of equivalent strength gave definite changes regardless of the type of acid or alkali used and that these changes varied quantitatively according to the amount of acid and alkali added and the amount of carbonate in the plasma.

In order that the alveolar carbon dioxide tension may be used as an expression of blood reaction, certain factors must be normal. (1) The facilities for the exchange of gases between the blood and the air in the alveoli must be unimpaired. (2) The respiratory center must be in a state of normal sensibility to the reaction of the blood and under the usual physico-chemical control. (3) There must be an adequate means of aerating the alveoli. The last factor may be neglected for all practical purposes, as it is obviously impossible to study the alveolar air with any degree of certainty in persons with obstruction of the external air passages sufficient to prevent the collection of alveolar specimens.

If one can be certain that there is no physical interference with the gaseous exchange, the study of the discrepancies between the carbon dioxide values obtained by the two methods should give an accurate index of the sensibility of the respiratory center to the natural chemical stimulus. If the respiratory center is hypersensitive to H-ions the pulmonary ventilation will be increased above the normal. This will pump carbon dioxide out of the alveoli and the blood. The amount of fixed carbonate, however, will not be disturbed and the Van Slyke readings will be found normal. In other words the alveolar reading should be lower than that of the plasma if the sensibility of the respiratory center to acid is increased.

If there is an inadequate means for the exchange of gases between the blood and the air in the lungs the same result will be obtained, but by a different mechanism. In this case the carbon dioxide will be dammed back into the blood stream, where it will increase the concentration of H-ions. This will produce an increase in the pulmonary ventilation and the alveoli will be pumped out. The blood carbon dioxide, however, will not maintain a normal level unless there is sufficient difference between the alveolar and the blood tension to overcome the impedi-

ment to the gaseous exchange. Again one will find a lowered alveolar-plasma ratio. Such a condition might be produced by general venous stasis, an interference with the pulmonary circulation, injury or diminution of the alveolar surface or an impairment of the alveolar ventilation.

We believe that the few discrepancies obtained in Table 1 represent respiratory center changes, those in tables 2 and 3 changes in the mechanism for gas exchange. We can not adduce definite direct proof for either, but believe that the latter, at least, can be substantiated by indirect evidence.

We have assumed 0.85 and 1.15 as the limits of normal because, when all the ratios in table 1 are arranged in order of magnitude, there seems to be a rather sharp break at these points. Abnormal cases may be included among the normal, but a certain latitude is advisable in methods that involve manipulation and personal factors.

Table 1 has been divided into 2 parts; part 2 contains all the cases that showed discrepancies. Only three of the seven cases (Nos. 29, 30 and 32) showed an abnormal ratio at all times, and in the last two only single observations were made. The cases in the two parts of the table can not be differentiated clinically. In both there are diabetics, nephritics and miscellaneous cases. In none were cardiac or pulmonary lesions observed; in no case was there dyspnea without a true diminution of blood alkalinity. No mechanical factors interfering with respiration were discovered. In almost all cases, moreover, the ratio was disturbed temporarily and rapidly without evidence or reason to predicate a coincident anatomical disturbance. In four of the seven cases the discrepancy occurred after rapid changes in blood reaction. It seems reasonable, therefore, to consider the discrepancies in these cases as due to disturbances in the central mechanism rather than to an interference with the gas exchange in the lungs. The material is too limited to allow more accurate localization of the seat of the disturbance. We are continuing work along these lines.

The contrast between these cases and those in table 2 and 3 is very striking. Table 2 is entirely made up of cardiac cases in various stages of decompensation; table 3 contains the results obtained in three cases with very advanced pulmonary lesions that presumably diminished their respiratory capacity greatly. The common factors in all are a definite dyspnea, while at rest, unassociated with a change in the fixed acid of the blood, but with a definite diminution of the alveolar CO_2 tension as determined by the Fridericia method. (That this is not a fault of this method alone is demonstrated by the fact that Beddard

and Pembrey (4) with the Haldane method and Peabody (5), Porges, Leimdörfer and Markovici (6) with the Plesch method also found a lowered alveolar CO_2 in dyspneic cardiac cases.)

All but two of the cases (Nos. 45 and 49) showed an alveolar plasma ratio below 0.85. In one of these (No. 49) a ratio of 0.86 was found, the other showed little or no dyspnea. All the others showed definite dyspnea while at rest, as long as the discrepancy between the alveolar and plasma values persisted. In only two was there a definite diminution of blood alkalinity and in these two (Nos. 38 and 51) the acidosis did not determine the dyspnea. In all but three (Nos. 41, 43 and 45) the alveolar figures taken alone would have indicated an acidosis that did not exist. Always, with a return to compensation, the ratio returned to normal.

It is, of course, possible that the cause of the disturbed ratio in these cases is the same as that producing the discrepancy in the cases of table 1, but there is an essential difference in the two groups of cases other than the observed chemical distinction. In all these cardiac and pulmonary cases there is a physical or anatomical disturbance of the respiratory mechanism that does not occur in the miscellaneous group of table 1.

Peabody (5) finds that in patients with cardiac dyspnea the "vital capacity" of the lungs is diminished. This we also found in the seven cases of this series that we tested, one of them, No. 55, of table 3. All three patients of table 3, moreover, presented clinical and pathological evidence of a diminished pulmonary capacity. These facts in themselves indicate an anatomical defect in the respiratory mechanism. Besides, Peabody finds an increase in the "minute-volume" of air breathed, in the same cases.

Under normal conditions an increase in the "minute-volume" will produce a dilution of the alveolar air and therefore a diminished carbon dioxide tension unless the carbon dioxide output is greatly increased as after violent exercise. (Peabody has found the carbon dioxide output in cardiac dyspnea normal). This relation only obtains if there is no absolute nor relative increase in the "dead space," which is too uncertain to permit discussion.

Siebeck (7), after a careful study of the respiratory mechanism in cardiac insufficiency, came to the conclusion that all determinations of the alveolar CO_2 were useless in this condition. According to him the alveolar aeration in cardiac dyspnea is very imperfect, in consequence of which the expiratory air contains an excess of unchanged inspiratory

air. At first sight this would seem to offer an explanation of our observations and at the same time destroy their value. We believe this conclusion unwarranted. In the first place, Siebeck was considering the alveolar CO_2 tension as a measure of blood reaction. As such, our own results corroborate his perfectly; we agree that it is useless. But we are trying to employ it as a measure of the functional impairment of the respiratory mechanism and we believe that it is safe to neglect anatomical lines and to say that the last portion of a forced expiration represents the only air available to the subject under investigation for the exchange of gases between the blood and the outside atmosphere and that, therefore, for the purposes of studying the physiology of the respiratory mechanism it may be considered in the same category as alveolar air whether it comes from the alveoli or not.

Certain it is that in cardiac dyspnea a greater respiratory exchange is necessary to produce the usual output of CO_2. Conversely, this output cannot be effected without an unusually great respiratory exchange, and if the response of the respiratory mechanism is inadequate, there will be a damming back of carbon dioxide into the blood. This, in turn, will stimulate the respiratory center to greater efforts and the pulmonary ventilation will increase sufficiently to restore proper conditions.

In fact we are forced to the conclusion that the dyspnea of cardiac disease is due to a defect in the normal facilities for the exchange of gases between the blood and the air in the lungs with a damming back of CO_2 into the blood stream. This CO_2 retention produces an increased acidity, which in turn stimulates the respiratory center. The result is that the pulmonary ventilation is augmented until there is sufficient difference between the carbon dioxide tension in the blood and in the lungs to allow the normal output in spite of obstruction. It is merely a matter of relation between rate of flow and pressure. Siebeck merely changes the position of this pressure difference from the absolute point of contact between the blood and the alveolar air, to the more distal point between the alveolar air and whatever air is obtained in the end of a forced expiration.

It is not unreasonable to suppose that the enormous respiratory response produced in cardiacs by a totally inadequate amount of physical exertion may be best explained by their inability to compensate for an increased carbon dioxide tension with the same ease as would a normal person. We intend to make further studies of the whole respiratory mechanism with a view to determining the changes in the alveolar air during rebreathing experiments and to attempt similar experiments on other cases with a diminished alveolar CO_2 tension.

At first sight there would seem to be no difficulty in determining the presence of a carbon dioxide retention directly. However, all attempts have failed, though the idea is not new and the most ingenious methods have been employed. We have added to the list one more attempt and one more failure.

The publication of the method, in the face of its results seems useless. These repeated failures would seem to cast the shadow of doubt on the whole theory of carbon dioxide acidosis as the cause of cardiac dyspnea. The direct determination of the carbon dioxide saturation of the blood is, however, attended with such great technical difficulties that it could be expected to show only comparatively gross changes, while if there is a CO_2 acidosis or retention in cardiac dyspnea it must be extremely slight, in all but the most severe cases. The work of Poulton (8) on the H-ion concentration of the blood has demonstrated that if it is studied under the conditions of CO_2 tension that obtain in the body, no change of reaction can be detected by the most delicate known methods, except in moribund persons. This does not mean that there is no change in reaction. Experimental and clinical work force the conclusion that changes in H-ion concentration offer the natural stimulus for the respiratory center. It only means that the controlling mechanism is so delicately balanced that the most minute disturbance induces a quantitative response that so nearly restores the natural reaction that the departure from normal is too minute for our most refined means of measurement.

Thus, it seems to us, it must be in cardiac cases with a carbon dioxide acidosis. The response must be so accurately measured that the difference in CO_2 tension between the air in the lungs and that in the blood is just enough to maintain a normal blood reaction and the increased CO_2 concentration of the blood will be so infinitely small as to defy detection, except when the whole mechanism fails and the patient is in extremis.

The interference with the respiratory exchange induced by the pulmonary injury, although in itself apparently sufficient to account for the carbon dioxide acidosis, need not be the only factor at work. It is quite possible that stasis of the circulation may also play a part. In any case the immediate stimulant and the respiratory response must be the same, though the more remote effects should be different. In the latter case there should be a real accumulation of an excess of carbon dioxide in the venous blood and the tissues, while in the arterial blood the CO_2 might be even lower than normal. For this reason unless this

retention is detected by direct chemical methods, venous stasis as an active factor in the production of cardiac dyspnea must be considered as doubtful. The evidence thus far accumulated strongly favors pulmonary injury as the dominant influence.

Finally, if the whole hypothesis of a carbon dioxide acidosis is disproved the comparison of the alveolar carbon dioxide and the plasma carbonates will still retain a certain value. One thing seems clearly established: The alveolar carbon dioxide is lowered because of some anatomical or functional change in the lungs. The discrepancy between the alveolar and plasma carbon dioxide observed in cardiac and pulmonary cases is then an indication of the extent of this injury.

CONCLUSIONS

1. A comparison of alveolar carbon dioxide with the Van Slyke carbon dioxide pipette reading offers a simple method of studying the condition of the respiratory mechanism in man.

2. A comparison of the figures obtained shows that the ratio of alveolar to plasma CO_2 falls, in most cases, between 0.85 and 1.15.

3. Variations greater than this may mean an abnormal reaction of the respiratory center or an interference with the natural exchange of gases between the blood and the outside air.

4. In cardiac cases with severe decompensation the alveolar/plasma ratio usually lies below 0.85 and rises when compensation becomes established. It is suggested that this is due to an impaired gaseous exchange between the blood and the outside air with the production of a carbon dioxide acidosis. This is probably due to a diminished respiratory capacity with incomplete alveolar ventilation, but general venous stasis may play a part.

5. The discrepancy was obtained in three cases in whom there was reason to suppose that a very marked diminution of the respiratory capacity existed, demonstrating that this factor may produce the required picture.

6. Many severely decompensated patients also show a slight diminution of the plasma carbonates, suggesting a mild acidosis due to an increase of fixed acid.

7. An attempt was made to prove an increased CO_2 saturation of the blood by a direct method in those cases with mechanical defects in the respiratory mechanism, but failed. We have shown that this failure need not be considered as a serious criticism of the theory advanced.

8. A study of the alveolar carbon dioxide is usually an accurate

method for determining the reaction of the blood, but may be misleading in the presence of a disturbed respiratory center, impaired aeration of the blood or a diminished pulmonary ventilation. By comparison with the Van Slyke carbonate determination the changes in the reaction of the blood due to both fixed acid and carbonic acid may be determined.

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THE REACTIONS OF KITTENS AFTER DECEREBRATION

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I. INTRODUCTION

Ever since Sherrington's original description (12) of the prolonged contraction of extensor muscles supervening upon the removal of the cerebrum and thalmencephalon, the reactions of decerebrate animals have been quite extensively studied. Not only have the postural relations of the condition and its significance in the study of tonus (Sherrington (18)) been investigated but several descriptions of the general conduct of these animals have been published (Sherrington (12), Graham Brown (9), Weed (20)). As it is with these general phenomena following decerebration that this communication will deal, a brief summary of some of the peculiar reactions will be here given.

Decerebration properly, if Sherrington's usage of the term be followed, means the removal of cerebral hemispheres and basal ganglia. A cut through the brain stem anterior to the superior corpora quadrigemina or to the anterior border of the inferior corpora quadrigemina is usually made. The line of the bony tentorium is followed toward the base of the skull, so that the resultant section slopes from above downwards and forwards, including, in consequence, more cephalic structures in the basal portion than in the tegmental regions. Some slight variation in the line of the transection appears in the various reports but in general the residual fraction of the nervous system is identical. More careful and complete anatomical descriptions of the experimental ablations would undoubtedly aid greatly in the solution of some of the conflicting observations.

If such a decerebration be performed in an adult animal, there occurs as the anesthesia becomes diminished, a condition of extensor rigidity in neck, shoulders, hips, fore-legs and hind-legs. This contraction of the extensor muscles first affects the elbow joints with rapid spread to knee, shoulder and hip. The ankle joint in cats is usually involved in the extension, while the wrist is almost invariably free from the rigidity.

This condition of extensor contraction endures for hours, if the animal continues to breathe spontaneously and if the body-temperature is maintained. Sherrington (13 and 14) considers the condition to be essentially the reflex posture of standing; the muscles affected when the animal is upright are those which in that position act against the force of gravity.

Several hypotheses regarding the essential reflex-arcs involved in the production of decerebrate rigidity have been ventured (Sherrington (12), Thiele (19), Weed (20)). It has, from this and other work, become fairly established that the occurrence of this rigidity is dependent upon the integrity of the afferent nerves from the portion of the animal affected, and that these impulses ascend the cord in the lateral column. The exclusion of the pyramids from an essential part in the production of this extensor stiffness is also on a firm basis. The cerebellum surely possesses tracts used in the ordinary maintenance of the rigidity and plays an important, if not essential, rôle in its occurrence. The cerebellar cortex likewise presents an interesting area related to the inhibitory pathway for this enduring contraction.

The reactions of animals of different ages after decerebration have been the subject of this work. The plan has been to subject fetuses and newly-born animals to this experimental procedure, in the hope that in the developing animals, there might be obtained differing types of reaction which would afford valuable comparisons when considered in relation to the morphological development of the nervous system. Cats have been employed, due not only to the ease of obtaining kittens but also to the fact that many of the reflexes of this animal have been studied by Sherrington and his school. Thus, a developmental type of physiological reaction has been sought in this investigation. The findings in regard to the reactions of kittens after decerebration will be detailed in this paper; the morphological studies of these neuraxes will be reported in another place. For it is felt that by combined morphological and physiological observations in developing animals some progress may be made in the ultimate solution of the problems of the central nervous system.

II. METHOD OF EXPERIMENTATION

The kittens used in these observations were for the most part born in the laboratory, so that the exact ages, even to the hour, were known. Several of the litters were composed of three to five kittens, permitting many comparative observations.

These kittens were subjected to practically similar cerebral ablations. Ether was first administered with great care; it was early observed that, in cases in which the anesthesia was poorly taken, the reflexes never subsequently reached the same degree of activity. As soon as the animals were completely anesthetized, both carotid arteries were exposed and tied. These ligations were followed quickly by opening the skull on one side in the temporal region, controlling the hemorrhage from the diploetic vessels and cutting through the dura. With a blunt spatula the brain-stem was transected usually superior to or through the superior portion of the anterior colliculi. This transecting cut followed somewhat the plane of the bony tentorium, but this structure because of its meagre development in the younger kittens, did not serve as efficient a guide as in the adult preparations. In rare instances the operative procedure was varied somewhat but in general it was planned to create as nearly constant lesions as possible. Hemorrhage from the cut surface of the midbrain was controlled by pressure on the vertebrals or by simply sponging with dry cotton pledgets. Throughout the operation the kittens were kept warm, a very important factor in the success of the experiment.

Immediately following the decerebration, the anesthesia was stopped, for by this removal of the cerebrum the animals were permanently deprived of consciousness. The kittens were then placed in an incubator maintained at 38°, and in this way the maintenance of the decerebrated animal's body-temperature was secured. This warm-box was so arranged that fresh air was carried in to the animal; it was so constructed also that all the subsequent procedures could be performed within it. In order to provide a constant posture and to allow freedom of movement for the animals' legs, the kittens were suspended by temporal muscles and by tail, the feet being a few centimeters from the base of the warm-box. This method of suspension was employed throughout.

It was planned in these experiments to make use of electrical stimulation for the eliciting of the responses desired, but it was quickly ascertained that merely by touching or pinching parts of the animals the varied reflexes could be obtained. The advantage of this method, while admittedly crude, lay in the fact that the operative procedures were reduced to a minimum and the excitation by pinching or touching was thoroughly effective in initiating the interesting prolonged progressive movements with which this paper will especially deal. In addition to these methods of excitation, smooth surfaces or planes were frequently used to afford support for the feet of the suspended kittens;

these surfaces, when in approximation, likewise seemed especially efficacious in inaugurating the rhythmic beats.

III. GENERAL REACTIONS OF DECEREBRATE KITTENS

In this series of observations, forty kittens, varying in age from one hour to fifty-seven days, were subjected to practically identical experimental ablations, with removal of that portion of the central nervous system above the superior colliculi, or above the inferior border of the superior colliculi. In this way, a typical decerebration was performed.

These decerebrate kittens were, in general, much more responsive than the ordinary adult preparations. Very quickly after the neuraxial transection, as the ether was passing off, the animals became more and more active. Certain rather typical reflex-reactions have been obtained from all of these kittens; those few animals which showed these reactions but slowly or incompletely were in such poor physical condition following the anesthesia or the decerebration that no firm reliance could be placed on the negative.

One of the first reactions which could be demonstrated in all of the decerebrated kittens was the withdrawal of the leg on pinching the foot or foot-pads. Such a pulling away of the leg can be demonstrated in adult decerebrate cats only on very severe trauma to the foot. But in these kittens, as soon as they were free from the anesthesia, very rapid and extensive withdrawals of the legs could be secured on pinching of the foot by the fingers. The youngest of the kittens were, in greater part, by far the most active in this regard; frequently a mere touch to the under surface of the footpad would suffice as a stimulus for the withdrawal of the leg. As animals of greater age were used, it was noted that this reaction required greater excitation, but even in the oldest of these kittens the threshold was still quite low as compared to that of the adult.

Again, as illustrated by the reactions of these decerebrate kittens to trauma applied to the tail, these animals were much more responsive than the adult. In the more active of the decerebrate kittens (i.e., the younger in general) a slight pinch of the tail between the fingers caused a bilateral extensor thrust of the hind-legs—a quick, purposeful and well-executed response to a noxious excitation. In the less active of the decerebrate kittens, these bilateral extensor thrusts were single, (one in response to each stimulation) but the more active kittens fre-

quently exhibited several rhythmic thrusts of both hind-legs—typical leaping movements.

These decerebrate kittens all exhibited typical "scratch" reflexes. These rhythmic beats of the hind-legs could be elicited by any of the appropriate excitations, but the best response seemed to follow localized stroking of the base of the ear. In such a case the ipsilateral hind-leg would show rhythmic scratch beats throughout the period of stimulation and lasting, in certain experiments, for a few beats after the cessation of the stimulus.

In order to ascertain if the nervous mechanism for sensory impulses reached a functional activity first on the dorsal or on the ventral body-walls, tests were made on most of the animals. All of the preparations, however, responded equally well to both dorsal and ventral stimulation. These reactions were obtained by pinching, or by merely stroking the dorsal or ventral body-wall with a sharp needle. In the case of the more active kittens, the dorsal stroking was instantly followed by a marked ventral bowing of the back and occasional withdrawals or extensions of the legs. The ventral stroking resulted in a dorsal bowing of the vertebral column in the opposite direction from that occasioned by stroking the animal's back. In the less active preparations, it was found necessary to pinch the skin over the back or abdomen to elicit responses; these reactions in general were not outspoken, but they consisted usually in slight twisting movements or slight bendings of the back.

The general reactions detailed above are those which were tested on each of the decerebrate kittens studied. Other more special responses were obtained in certain of the animals; these will be discussed in other sections of this paper whenever they seem to possess more than an individual significance. It must be emphasized that the state of these kittens varied greatly after decerebration; many were very active indeed while others seemed far less active in their reflex-responses. All however, were far more responsive to the excitations used than are adult decerebrate cats. As a response to many noxious stimuli, the kittens frequently uttered hoarse cries, similar to those noted by Woodworth and Sherrington (21).

In addition to the individual variations in the reflex-responses, it must be understood that these decerebrate kittens presented different activities at different times during the observations. The period of experimentation was seemingly divided into three phases, in the first the anesthesia was passing off; in the second, the reflex-activity was

at its height; and in the third phase, the animal was declining physically. These periods of experimentation in the decerebrate kittens lasted varying lengths of time; the first phase was usually completed from thirty to fifty minutes after the neuraxial transection, but it was apparently shortened markedly in the more active animals. In the usual preparation, the second phase, or period of maximal activity, lasted from one hour to two hours (at times somewhat longer) and it was during this period that the critical responses were found. The third phase often developed rapidly and at times was of only a few minutes' duration, or it occasionally represented a long period of respiratory difficulties, with death finally ensuing from respiratory paralysis. Consequently, the reactions of many of these decerebrate cats must be considered in relation to the phase of the experiment at which the reflex was attempted. Thus a "scratch" reflex might not be obtained in the early stages of an experiment but during the second phase, at which the reflex-activities of the preparation were at their height, appropriate excitation resulted invariably in the typical beats. In these observations, therefore, care was had to record the reactions with regard to the time of occurrence and to regard as typical only those responses which were obtained during the second phase of the observation.

IV. THE OCCURRENCE OF RHYTHMIC MOVEMENTS

In the foregoing section of this paper, it has been pointed out that there existed a marked difference in the general activity of the individual decerebrate kittens. While such differences in reflex-responses were observed in the general reactions of the animals, an increased activity was manifested to a much more striking degree in the occurrence of definite rhythmic movements of progression in many of these animals.

a. Prolonged progressive movements

If these decerebrate kittens were subjected to noxious stimuli (i.e., stimuli which in the intact cat would cause pain) such as pinching of the tail, they practically all responded by making characteristic walking movements of all four legs. Such progressive movements have been noted as occurring in the adult decerebrate cat (Woodworth and Sherrington (21), Sherrington (13)), but they are of but very short duration and require for their elicitation almost maximal stimulation.

In this series, however, the progressive movements were very readily brought out in the more active kittens; in the less active kittens, repeated excitations of a rather mild degree were sufficient for their inauguration. Apart from this quantitative difference in the necessary stimulation, the more active decerebrate kittens exhibited the phenomenon of prolonged rhythmic progression as distinguished from the short-enduring type of walking movements. This differentiation of the kittens into two classes on this basis is arbitrary, but it seems more than warranted both on the temporal aspect of the progressive movements and also on the degree and character of the excitation required for the responses. The classification seems justified also for descriptive purposes and because of the reactional similarities between these decerebrate kittens and adult cats from which the cerebral hemispheres alone have been removed.

Out of the forty kittens in this series subjected to practically identical decerebrations, twelve are to be included in the first class, as they exhibited prolonged progressive movements of all four feet. These rhythmic beats did not occur immediately after the decerebration; they were elicited only after the anesthesia had well passed away and when the animal was in the period of highest reflex-activity. As soon as the kitten showed signs of declining physical condition, the progressive rhythm was replaced by asphyxial beats of a different character.

In these twelve kittens in which prolonged progression was noted, various types of excitation sufficed to inaugurate the rhythmic beats. The usual method of accomplishing this result consisted of pinching the tail of the animal between the fingers. Immediately the rhythmic beats would be inaugurated. Other noxious (or better "pseudaffective") stimuli also sufficed—pinching ear, foot-pad or skin. There seemed to be no essential difference in the efficacy of these various noxious stimuli; provided any one of the methods started the movements, all seemed equally effective. Thus a single slight pinch to the tail would be followed, in these twelve kittens, by as prolonged a progression as would be occasioned by other noxious stimuli. But in these exceedingly active kittens, the inauguration of the progressive beats could be equally well brought about by other agencies than mere noxious impulses. One of the most successful of these means consisted merely in lifting against the suspended feet of the kitten the smooth surface of a pan or glass plate. As soon as this support was afforded the animal, typical progressive movements of all four legs were started. And on such a surface, actual progression was accomplished by the decerebrate

kittens. Other sensory stimuli also, in these active animals, were found effective in initiating the rhythmic beats of all four legs; a mere jar or vibration transmitted to the supporting standards and communicated through threads to the animal very frequently started the walking movements. Most surprising of all sensory excitations, are the responses following auditory stimuli. That a decerebrate animal possesses intact acoustic mechanisms has been well known anatomically, but only recently have Forbes and Sherrington (2) reported a few cases of response of adult decerebrate cats to loud noises. Hence it seems of interest that three of these active decerebrate kittens should start the prolonged progressive movements of all four legs in response to auditory stimuli; loud, shrill whistling and clapping the hands were the effective noises.

Considered as a whole, almost any sensory stimuli which reached the remaining portion of the central nervous system, sufficed as the excitatory agent in occasioning the rhythmic progressive movements in this first group of more active kittens. But among these twelve animals, there were varying degrees of activity, as demonstrated by the differing responses to identical minimal excitations. All of the twelve, however, reacted to the slightest tactile or noxious stimuli.

The progressive movements exhibited by these twelve kittens, after decerebration, were wholly similar to the walking or running movements of adult cats. All four feet were involved in the beats, the succession being alternate like that of the "trotting" rather than that of the "pacing" horse. In other words, one fore-foot and the opposite hind-foot were brought forward while the other feet were retracted. In only one rather extraordinary animal, was there a unilateral balance in the progressive rhythm; in this case, the legs on one side moved together. Examined closely, the drawing of the legs backward seemed to be, in the suspended animal, a more vigorous and outspoken movement than the forward stepping. This apparently may be explained on the basis that normal progression is dependent on vigorous backward thrusts with rather easy forward returns.

These movements of the four legs were all rhythmic beats. The rate of this rhythm varied considerably in the different animals and also somewhat in the same animal. This variation from animal to animal may be accounted for on the basis of individual reflex-activity, while the variations in the same animal apparently have their explanation in the changing physical condition of the animal. In the more rapid cases, the progressive beats occurred at intervals of about one-half

second, while in the more slowly beating animals, the rhythm was about one beat of the leg in two seconds. These rates given were obtained by counting the rhythmic progressive beats of the one leg of the suspended animal, with the legs hanging freely in the air. This rate of rhythmic beating is similar to that observed by Graham Brown (6).

When support was given to the legs of one of these active decerebrate kittens, the progressive movements remained rhythmic and purposeful, but the rate of the beating was slowed somewhat. The animals tended to move along the flat surface, progressing forward until the supporting cords were taut. The slowing of the rate of these walking or running movements became more noticeable if the smooth surface held beneath the feet was inclined so that the animals were forced to climb uphill. In this case also, with the slowed rhythm, the kittens' paws slipped backward over the surface. If the inclination of the surface be not too great, the animal will move upward and forward to the limit of the suspending threads. Such reactions of these active decerebrate kittens when support was afforded, suggested that actual progression might be accomplished by the animals if they were given an opportunity to walk. This was done in the case of the three most active kittens. Each of these, when placed upon the floor, raised its body from the floor and started to walk actively and perfectly. When placed alongside of other animals from the same litters, the decerebrate animals were able to move slightly more rapidly than the normal kittens. This was particularly well shown by one decerebrate kitten of only one day in age.

Probably the most important feature of these progressive movements in the more active decerebrate kittens deals with the length of time that the rhythmic beats persisted. As has already been pointed out, there was no real difference between the progressive movements of these more active kittens and the less active, except in the length of time the movements were continued. Thus, in these twelve more active decerebrate kittens, the progressive movements endured for more than thirty seconds; many of these rhythmic beats were continued until exhaustion took place. This often meant a continuation of movement for over one hour after cessation of the initial stimulus. In other observations, the rhythmic movements persisted only as long as a smooth surface supported the feet. The majority of the progressive movements ceased, in the animals whose legs were swinging freely in the air, in from one minute to two and one-half minutes after the cessation of the exciting stimulus. In these kittens, the rate of beat became

gradually slower and slower as the time of continuance grew longer, until finally complete cessation occurred.

It must be further explained that the time of continuance of these movements varied greatly in the same individual animal. For the most active animal, when once the progressive movements were inaugurated, might continue these rhythmic motions until exhaustion occurred. In such an animal, similar long-continued movements could be subsequently elicited, but for the most part, they would never be continued for more than one or two minutes. Thus it would seem that one great exhausting effort to maintain these progressive movements was sufficient to do away with the possibility of future similar long-continued rhythmic beats.

While these progressive movements were being made by an animal, various sensory excitations were tried in order to ascertain their influence upon the rate or rhythm of the beats. Noxious stimuli (such as pinching tail, skin or ear), applied to these decerebrate kittens during the progressive movements, usually caused a temporary cessation of the beats during the period of excitation; the rhythmic movements were resumed thereafter with somewhat increased vigor. Strong pinching of the base of the ear, in the course of these rhythmic movements, changed frequently the character of the beats of the hind-legs. These were usually brought forward, both to the same side as that traumatized, in scratch-like beats of the same rhythm as was originally present in the progressive motions; after a few moments the hind-legs returned to their original position and resumed the progressive rhythm. Pinching the foot during the typical walking movements usually caused only the withdrawal of the leg affected, but at times the whole rhythm was disturbed and was resumed only when the noxious stimulation had ceased.

It was found rather difficult, and at times impossible, to stop these prolonged progressive beats if once they were well started. Many of the animals could be quieted by holding the legs and making movement impossible. In these cases, the animals became quiet after making several unsuccessful attempts to move the legs. This procedure, and another one of holding the body tightly in the palm of the hand, while successful at times, rarely succeeded in stopping the more outspoken progressive beats. In many, every method tried failed until the animal was exhausted. The administration of an anesthetic was not attempted in these cases; it seems most likely that this would have stopped the progression.

The ages of these twelve decerebrate kittens which showed prolonged progressive movements, ranged from one hour to sixteen days. Of the twelve, four were twenty-four hours or less in age at the time of the decerebration. Eight of the twelve were five days or less in age, leaving only four, or one-third of the total number, over five days in age. These four older kittens were respectively nine, twelve, fourteen and sixteen days old. These twelve more active kittens varied in length from 150 to 220 mm. Thus, in general, the younger kittens, particularly those of five days or under, possessed the greatest tendency toward reflex-activity, particularly in the maintenance of long-continued progressive movements.

In two of these actively walking kittens, the cerebellum was entirely removed at the time of maximal rhythmic progression. In the first animal, a complete cerebellar ablation was performed with only the slightest injury to the underlying medulla. Respiration was not interrupted by the operative manipulations and the animal seemed in excellent condition. The second case was equally successful. Both of these kittens, which previously had been walking actively, changed their whole progressive tendency after the cerebellar removal. No longer could rhythmic beats of any prolonged duration be occasioned by the greatest excitation. One only of these cats made a few rhythmic beats of the hind-legs when the tail was repeatedly and maximally pinched. It was very apparent that this cerebellar ablation had destroyed the tendency toward prolonged progression and also toward progression of all four feet. But it must be granted that such acute observations following the removal of the cerebellum are hardly decisive.

b. Short enduring progressive movements

Separated from the twelve more active decerebrate kittens are the others of the series—twenty-eight in number. These are the animals in which, after identical decerebrations, the general reactions and especially the progressive movements have been less outspoken. As has already been pointed out, the division of the kittens into two groups on the basis of the time of continuance of the progressive movements is wholly arbitrary. These kittens of this second group practically all made progressive movements of all four legs, but in none were the rhythmic beats continued for more than thirty seconds.

The characteristic methods of eliciting these rather short, progressive movements of all four legs differed in no essential from those em-

ployed in the more active kittens, except in the degree of excitation required. A few of these kittens resembled in their reflex-activities the less active of the first group of kittens, but such a correspondence was to be expected in an arbitrary classification of the reacting animals. By far the great majority of this second group were much less active in their rhythmic responses, than were those of the first division. This was particularly well shown in the degree of noxious excitation required to inaugurate and maintain a short progressive rhythm. For repeated strong pinching of the tail or base of the ear was usually necessary for the elicitation of even a few progressive beats; these likewise stopped immediately or very soon after the cessation of the stimulus. The rhythm of the beats was frequently the same as that of the rate of repetition of the stimulus; a single beat, hence, was the response to a single excitation. The slight tactile stimulations, used in the twelve active kittens, were wholly insufficient in this second group, to inaugurate any progressive movements. In this regard, these less active kittens resembled more the adult decerebrate preparations in which almost maximal noxious impulses are required for the four-footed rhythm. Single excitations by pinching or otherwise in these animals rarely resulted in any progressive movements; a single beat of each of the four legs in regular alternate succession might be recorded, but for continued progression, continued and repeated stimuli were usually necessary.

In physiological characteristics, the progressive movements of these animals, though short-enduring and usually requiring repeated excitation, were quite similar to the movements made by the twelve more active kittens. The order of movement of the four legs was wholly the same; the rate of the rhythm was usually quite slow. But if the excitation were repeated regularly, alternate beating motions resulted in regular sequence. It seemed wholly just to consider these movements in the less active animals as being typically progressive movements, differing only in degree from those made by the more active animals. In no case was there any difficulty in stopping those progressive beats in these less active animals, for usually the cessation of excitation was the signal for the cessation of movement. In but very few cases were the movements continued for more than about fifteen seconds after the excitation.

These short-enduring progressive movements were made by thirty-one decerebrate kittens out of the forty kittens subjected to practically identical extirpations. Of this total number, twelve showed also pro-

longed progressive movements and hence have been classified in the first group. The nineteen remaining which gave typical short-enduring rhythmic beats of all four legs varied in age from six hours to fifty-seven days; in length, the variation was from 150 to 265 mm. Of the nineteen, only four were less than ten days in age; six were between the tenth and twentieth days in age. The remaining nine animals were more than twenty days old at the time of the experimentation.

It seems only fair in such statistical studies of the reactions of animals to take some account of the individual condition of the animals whose reactions fail to coincide with those of the remaining majority. Thus it seems correct to ascertain, if possible, the reasons why nine of the forty kittens used in this work failed to make progressive movements. From an analysis of the protocols of these kittens, it is found that two of the animals were in very poor physical condition at the beginning of the experiment. One of these (No. 47) had a bronchial infection and lived but a few minutes after decerebration; the reactions of this animal were very sluggish and incomplete. The other kitten (No. 52), though in poor shape, gave, on repeated caudal pinching, rhythmic bilateral thrusts of the hind-legs but no progressive beats of all four legs. Another of the decerebrate kittens (No. 21) which failed to make progressive movements, had been previously subjected to the removal of the cerebral hemispheres; after the second operation of decerebration, no progressive beats (which had been typical) were obtained. Two other kittens of the series showed rhythmic progression only in front or hind-legs:—No. 25 showing these rhythmic beats only in the front-legs, while No. 36 gave similar movements of the hind-legs. One other decerebrate kitten (No. 31) gave, on appropriate excitation, typical progressive movements of the front-legs, but the hind-legs were carried forward, both to one side, in typical rhythmic scratch movements. Another kitten (No. 27) showed no true progression but did give typical asphyxial running movements of all four legs. Until these occurred, the animal was singularly inactive to every stimulus. The other two kittens in this group which showed no true four-footed progression (No. 6 and No. 18) gave no indications of rhythmic tendencies but were apparently otherwise active reflexly.

Thus, on analysis, of the nine abnormal kittens, only two showed no rhythmic tendency nor partial progressive movements, except those excluded by poor physical condition at the time of experimentation. It would seem, therefore, that on appropriate excitation rhythmic progressive movements may be elicited in practically all decerebrate prep-

arations, but the younger kittens show a greater tendency toward active and prolonged progression than do the older preparations.

Other rhythmic reactions

Of the other reactions of these decerebrate kittens, the scratch reflex should be mentioned as of rhythmic character; this rather typical response has already been recorded in a foregoing section. The rather extraordinary double-scratch reaction noted above as occurring in an animal which did not show rhythmic progression was found also in other animals. The usual exciting cause for such a rhythmic activity was a vigorous single pinch at the base of the ear, or repeated vigorous pinches. In these cases, both hind-legs were brought forward on one side toward the traumatized ear, and then showed alternate beating movements. In the more active animals, these bilateral rhythmic scratch movements were maintained, at times, for a considerable period in response to a single vigorous stimulation, but in the less active animals, each successive stimulation was followed by a single alternating beat. Such bilateral scratch movements often replace, in the more active animals, the true four footed progressive movements, as the typical response after pinching the base of the ear. The animals, however, which never made movements of true progression, but reacted with this rhythmic scratch (both legs pulled toward one side), have not been classed with those showing rhythmic progressive movements.

Instead of the decerebrate kittens invariably exhibiting a true alternate progression of all four feet, a peculiar galloping movement of all the legs may occur. This phenomenon has been noted in four of the animals recorded in this paper. This galloping of the kitten resulted from pinching of the tail and was usually merely a temporary phase in the reactions of the animal. Thus, such galloping might occur before or after the period of true progression, but it rarely was found during this period. Quite a number of the other kittens showed at times galloping movements of the hind-legs with alternate progressive beats of the front-legs. The true galloping movements are apparently similar to those noted by Graham Brown (3 and 7) in narcosis-progression.

In the last stages of these decerebrate kittens, when respiratory difficulties had become marked, many rhythmic responses or spontaneous rhythmic movements were noted. Most of these were somewhat convulsive in type and were truly asphyxial (as far as could be determined) in nature. They consisted of walking, running and galloping move-

ments of all four feet, of a very rapid rhythm and with usually a convulsive termination. At other times, definite convulsions occurred, followed by scratch-beats with a single hind-leg or both hind-legs, carried forward to one side or both. Twisting movements of the whole body have been observed in rarer instances. These movements occurred either in the terminal asphyxial conditions of the decerebrate animal, or in cases of intracranial hemorrhage of marked degree (in this latter case, removal of the intracranial pressure and hemostasis caused cessation of movement).

V. EXTENSOR RIGIDITIES IN DECEREBRATE KITTENS

In an adult cat subjected to decerebration, a condition of extensor stiffness or rigidity occurs as soon as the anesthesia has passed away. This marked rigidity may first be felt in the elbow joint of the cat and then rather quickly spreads to the knee. Subsequently the shoulder, hip and ankle become affected. This contraction endures as long as the animal remains in good physical condition; the degree of stiffness, however, may be modified in many ways. This rigidity may be tested by estimating the amount of pressure of the fingers required to overcome the contraction of the extensors of the joint; in this way a rough but fairly accurate estimation of the rigidity may be had. Throughout these experiments, the degree of the extensor contraction has been determined in this way,—overcoming the resistance to motion of the joint by pressure with the fingers.

The reactions of these decerebrate kittens differed from adult preparations in respect to the development of an invariable extensor rigidity. Out of the forty kittens in this series which were subjected to practically identical cerebral ablations, only twenty-four showed any extensor rigidity; in this group of twenty-four animals, there were several animals in which the occurrence of rigidity was considered doubtful. Thus, sixteen of the kittens out of forty, or 40 per cent showed no evidence as determined by pressure of the fingers, of an extensor stiffness. In adult animals the occurrence of such a rigidity would be invariable, provided the technical procedures were satisfactory.

Analyzing the kittens in which an extensor rigidity was noticed, it was found that only four of this group of twenty-four animals were under ten days in age. Of the four, the rigidity was considered as doubtful in three and positive in the fourth kitten—one of nine days in age. Thus four-fifths of the kittens in this series showing extensor

rigidities were over ten days in age. And only eight of the remaining kittens were between ten and twenty days in age. Hence, in this small series of forty kittens, 50 per cent of the animals showing decerebrate rigidity were over twenty days in age. And investigated still more, it was shown that not one of the decerebrate kittens over twenty days in age failed to exhibit a typical extensor rigidity. Thus there was indicated a distinct relationship between the age of the kittens and the invariable occurrence of a decerebrate rigidity.

But in addition to this suggestive relationship between the age of the kittens and the rigidities, there are other features of the onset of these muscular reactions which require comment. Thus, of the twenty-four kittens out of the forty which showed extensor stiffnesses, only one-half (or twelve) had appreciable rigidity in the hind-legs (especially knee) in addition to a definite stiffness in the elbow. Of the twelve, two are recorded as doubtfully positive. The other twelve decerebrate kittens showed the extensor rigidity only in the fore-legs as determined by examination of the resistance to movement of the joint. Hence it seems necessary to assume that in the developing kitten a mechanism for the production and maintenance of a rigidity in the fore-legs is acquired before a similar one for the hind-legs. This acquirement of the rigidity in the fore-legs first falls in line with the well-known sequence of the development and also of the degree of the stiffness in the adult preparation. Sherrington (13) thus comments upon this (p. 300): "In the dog and cat, just as spinal shock is more severe in the fore-limbs than in the hind, so decerebrate rigidity is more marked in the fore than in the hind limbs."

When the existence of an extensor rigidity in all four legs of a decerebrate kitten was considered in relation to the age of the animal, a very striking correspondence was made out in the records of this series. None of these experimental animals, in which a rigidity was present in the hind-legs as well as in the fore-legs, was under nineteen days in age, and in this youngest case the rigidity of the hind-legs was considered somewhat doubtful. The majority of the animals which showed this typically adult type of distribution of the rigidity were over thirty days in age.

With this suggestive age-factor in the occurrence of rigidities in these decerebrate kittens, it became of interest to ascertain the relationship of the rigidity to occurrence of prolonged progressive movements in these animals. The twelve decerebrate kittens which showed these long-enduring rhythmic beats varied in age from one hour to sixteen

days. Of these animals, five showed an extensor stiffness, but in three of these cases the rigidity, as determined by finger pressure, was considered as being extremely doubtful. All five of these animals exhibited the rigidity only in the fore-legs with no indications at all in the hind-legs. In addition to this feature of the occurrence of the rigidity, there was recorded a definite relationship of the rigidity to the anesthesia.

This relationship of the occurrence of the extensor rigidity in decerebrate animals to the anesthesia is a well known phenomenon in the adult preparations (Sherrington (12)). In the routine observation no rigidity can be made out until the anesthesia is partially eliminated. This usually takes from five to ten minutes. And from such a beginning, the rigidity becomes augmented more and more until at the end of an hour or so, it may be considered to have attained its maximum. In the twelve more active kittens, showing prolonged movements of progression, only five had any rigidity at all, and these five showed it merely in the fore-legs. But in addition to this, the rigidities in these animals showed another peculiarity in relation to the anesthesia. After decerebration, these kittens were suspended in a warm-box. Within five to ten minutes, as soon as a certain amount of the ether had passed off, a slight extensor stiffness could be made out in the elbows of these five active kittens. In three of these animals the rigidity remained questionable throughout, but in two it was quite definite. It did not spread from the elbow to any other joint, but for the few minutes of its existence, it remained in the elbow. Meanwhile the general reflexes of the animals were becoming more and more active; simply pressing the foot gently between the fingers caused an active withdrawal of the whole leg away from the noxious stimulation. Finally any touch of the foot-pads sufficed to cause a withdrawal of the legs and, as the reflexes became very active, the rigidity apparently disappeared. Certainly as soon as these decerebrate kittens began to show prolonged progressive movements, the decerebrate rigidity, even in the two wholly positive animals, was wholly abolished. The extensor stiffness, then, disappeared as the tendency toward rhythmic beating became more marked, and in no case did it reappear during the period of active progression. In a few preparations, an extensor rigidity was exhibited after the period of greatest reflex-activity; these were always the older kittens in which a rigidity of all four legs was found.

The problems connected with the abolition of the rigidity in the fore-legs of a few of the more active kittens (those showing prolonged pro-

gressive movements) are quite interesting. In the first place, the rigidity, extensor in type, occurred in the period of comparative depression of reflexes, following the anesthesia. As soon as the reflexes had reached an active stage, the methods of determining by finger pressure the degree of stiffness caused an active, purposeful withdrawal of the legs. This naturally made it impossible to verify the actual existence of an extensor rigidity in a joint at the time of testing. In the still more active animals, such slight excitation as the mere attempted determination of a rigidity was followed by prolonged progressive movements. The actual determination of a rigidity in these cases naturally was impossible.

Posturally, a few of these more active decerebrate kittens at times seemed to possess an extensor rigidity during the periods of quiet. It must be granted that such a posture suggesting an extensor rigidity in an active kitten was quite rare as compared to the customary positions assumed by the animals. Most frequently, these decerebrate preparations exhibited, when subjected to routine suspension, slight flexor-positions at the elbow of one or other of the fore-legs and a possible alternate correspondence of flexion in the hind-legs. At other times a posture indicative of almost complete flaccidity is present; this condition, or one with some apparent tonus, was probably the most characteristic. It was, in consequence, impossible to consider an extensor rigidity indicated by the postural reactions of these decerebrate kittens; rather did the postures argue strongly against such a view.

It might be conceived that the extensor rigidity persisted throughout the rhythmic progressive beats, that these were stiff-legged movements, and that the necessary alternate flexions and extensions occurred only at the shoulders and hips. This view derived no support from the observation of the progressive movements. These were graceful, well-executed rhythmic movements, plastic and in no way stiff or rigid. Definite alternating movements of all the necessary joints could be made out in the more slowly executed movements.

From these observations and considerations, it seems necessary to conclude that, during the occurrence of progressive movements in these decerebrate kittens, the extensor rigidity which may have been characteristic before, was replaced by the tendency toward rhythmic beating. But it must be noted that in only five out of the group of twelve kittens showing prolonged progressive movements, was there any extensor stiffness demonstrable at any time; in only two of these five cases was the rigidity definite and unquestionable. In the less active

decerebrate kittens the extensor rigidity, which might be abolished temporarily by the short-enduring progressive movements, usually returned again. In the more active preparations, the rigidity, when present, occurred in the early part of the experimentation during the period of reflex-depression due to the anesthesia. This suggested strongly a similar extensor stiffness which is noted frequently in animals, just recovering from anesthesia. In many ways this post-anesthetic stiffness resembles a true decerebrate rigidity.

In all essential characters, the decerebrate rigidity shown by these decerebrate kittens, when present, is similar to that of the adult preparations. In the younger, more active kittens, it is very difficult to demonstrate the similarity to the adult rigidities but in the older kittens the points of likeness are easily made out. Whenever referred to in the text of this paper, the adult characteristics of the rigidity must be considered; any difference noted in the kittens has been recorded.

VI. DISCUSSION OF RESULTS

In the foregoing sections, the reactions of kittens after removal of the cerebral hemispheres and basal ganglia have been described. It has been shown that, in this series of forty kittens subjected to practically identical experimental procedures, considerable variation in reflex response occurred in the different animals. Certain typical reactions (as the scratch reflex, withdrawal of legs on noxious stimulation of feet, and a jumping thrust of both hind-legs on pinching tail) were common to all the kittens. It is proposed to discuss here the dissimilarities in the reactions of the kittens and to attempt correlations between the diverging responses.

The greatest differences in reactions in these forty decerebrate kittens were found in the type of progressive movements and in the development or absence of a true extensor rigidity. The study of the occurrence of these two reactions in their relation to each other suggests somewhat strongly a reciprocal consideration. Such reciprocal relationship is based on the evidence given below; it is not absolute but rather suggestive.

These decerebrate kittens were divided arbitrarily into two groups, dependent upon the length of time that the rhythmic progressive movements were continued after the cessation of the excitation. On this basis, twelve kittens were found to compose the first group of animals (those in which the progressive movements continued for more than thirty seconds). These twelve kittens ranged in length from 150 to

220 mm.; in age, they varied from one hour to sixteen days. Eight, or two-thirds, were five days or under in age; the other four kittens were hardly as active. Compared with this group, the ages of the kittens which showed only short-enduring movements of progression were striking. There were nineteen typical animals in this group; the other kittens in the whole series of forty, for the most part, exhibited rhythmic movements of some sort but not typically those of four-footed progression. Of the nineteen animals grouped in the second class, the age-variation was from six hours to fifty-seven days; the length-measurements were from 150 to 265 mm. Of the nineteen, only four were less than ten days in age.

Contrasted with the first group of kittens, which gave after decerebration prolonged movements of progression, this second group shows a rather marked age-difference. Of the first group of twelve kittens, two-thirds were five days or under in age while only four out of the nineteen in the second group were under ten days in age. It would seem therefore, that as the kittens advanced in age, the tendency to prolonged progressive movements after decerebration decreased markedly. The results are in no way absolute, but they indicate strongly an increasing tendency toward the disappearance of the very active type of reaction with the increasing age of the kittens. The animals must, however, be considered as individuals in their reflex-activities; individual differences probably account for the occurrence of the different types of reaction in kittens of the same age.

The same comparisons of the ages of the kittens may be made in regard to the occurrence of a definite type of extensor rigidity. Of the forty kittens in the series of decerebrate animals, twenty-four showed an extensor rigidity. Only four of the twenty-four animals were under ten days in age, and of these four animals the rigidity was considered as doubtful in three. Hence, it seems quite established that with the increasing age of the kittens subjected to decerebration the tendency toward the development of a true decerebrate rigidity becomes greater and greater. Likewise, the rigidity in the fore-legs of these animals develops first and appears more likely in the younger animals than a stiffness involving all four legs.

Thus, on the basis of age, and also possibly on the basis of length of the animals, the tendency of the younger kittens after decerebration is to exhibit prolonged movements of true progression while in the older animals an extensor rigidity and short-enduring progression are more typical. The relation between the prolonged progressive movements and the extensor rigidity is in many ways suggestively reciprocal.

But a few of the animals which exhibited prolonged progressive movements also had a temporary extensor rigidity, which was abolished by the onset of the rhythmic beats. Individual variation, however, seems to be a very marked feature of the reflex-activity of these kittens and it seems to be the chief factor in the overlapping of the age-limits for the different reactions.

By using kittens of the same litter, some very interesting results were obtained. It has been stated above that kittens of the same age did not necessarily give the same reactions. But when kittens of approximately the same age are from the same litter, the tendency toward similarity of result becomes much greater. This is shown in the following table:

Reactions of decerebrate kittens—Litter E

AGE	LENGTH	GENERAL REACTIONS	PROLONGED PROGRESSION	RIGIDITY FORE-LEGS	RIGIDITY HIND-LEGS
	mm.				
6 hours.....	150	+	+	0	0
1 day.....	150	+	+	0	0
3 days.....	160	+	+	0	0

This similarity in reaction of kittens of the same litter and of approximately the same age is not invariable but was, in this series, found more frequently than was the dissimilarity. Such a finding would be expected, as many of the individual variations disappear in the animals of the same litter. As the animals of a litter grow older, variations in the reactions of the individuals subjected to experimental procedures at differing ages should be expected. Thus, in any one litter, the animals used when still very young should show the characteristic reflex-activities of animals of that age and the older animals the characteristics of their age. This is well brought out in the table which follows; it shows also the apparent reciprocal relationship between the prolonged progressive movements and the extensor rigidity:

Reactions of decerebrate kittens—Litter F

AGE	LENGTH	GENERAL REACTIONS	PROLONGED PROGRESSION	RIGIDITY FORE-LEGS	RIGIDITY HIND-LEGS
days	*mm.*				
2	150	+	+	0	0
9	175	+	0	+	0
44	220	+	0	+	+
51	245	+	0	+	+

There remains merely the cataloguing of the different reactions of kittens of approximately the same ages. The likelihood of this dissimilarity in animals of the same litter is much less than in those from different litters. In the table given below, five animals from different litters are recorded; the animals showing prolonged progressive movements are the oldest observed in this series. The table represents, then, the transition between the period of prolonged progression and that of the short-enduring type. And in this, the occurrence of the extensor rigidity in the front-legs is becoming more certain. Following is the table:

Decerebrate kittens from different litters

AGE	LENGTH	PROLONGED PROGRESSION	RIGIDITY FORE-LEGS	RIGIDITY HIND-LEGS
days	*mm.*			
12	195	+	0	0
13	170	0	+	0
14	220	+	+	0
15	185	0	+	0
16	190	+	+	0

It must be emphasized that all of the reactions recorded in the foregoing pages are those of acute experimentation. The reflex-activities of the kittens are those shown shortly after the experimental ablations. And in this connection, the peculiar activities of the twelve kittens which were characterized by the prolonged progressive movements certainly suggest the similar rhythmic movements of an adult animal from which the cerebral hemispheres have been removed. Such decorticated adult cats, on suspension similar to that employed for these kittens, exhibit typical rhythmic progressive movements of all four legs as soon as the anesthesia has disappeared. These, like the beats in the very active kittens, may be inaugurated by noxious stimuli in the less active animals, but in the more active animals, as in certain of the kittens, almost any tactile excitation is sufficient. When placed on the floor, most of these adult decorticated animals will show progressive movements, purposeful in every way.

The occurrence of the definite progressive movements in most of the forty kittens subjected to decerebration is an interesting phenomenon in view of recent work concerning such rhythmic activities. In the early descriptions of decerebrate animals, particularly cats, as given by Sherrington (12), and Lowenthal and Horsley (11), no mention of

the occurrence of rhythmic movements in these animals is made. Subsequently Woodworth and Sherrington (21), using decerebrate cats for the purpose of ascertaining the spinal pathway for the "pseudaffective reflex" (a stimulus producing pain in the intact animal), observed at times following the excitation (p. 235) "diagonal alternating movements of the limbs as in progression (sometimes producing progression)." In commenting further on the general reactions of these animals, they state (p. 235): "In some cases the movements were vigorous and prompt but they never amounted to an effective action of attack or escape. A characteristic feature of the ineffectiveness was their brief duration. The movement even when most vigorous and prompt, died away rapidly, to be succeeded in some cases by a few weaker repetitions, each in succession weaker and more transient than the last." Sherrington (13) in his Silliman lectures in 1906, again mentioned the occurrence of rhythmic movements in decerebrate animals (p. 252) in further discussion of the pseudaffective reflex.

Graham Brown (9) has given (p. 147) a description of the condition of the monkey after "comparatively high decerebration (that is, when the neuraxis is divided across slightly anterior to the anterior colliculi) or even when the division is through the anterior colliculi." He found that the animal was not perfectly immobile but that the winking movements of the eyelids and occasional movements of the eyes were observed. The animal often slowly changed its position with the occurrence of a slow postural flexion and extension in the fore-legs. No definite rhythmic progressive movements were recorded in his description of the animal's condition.

From the results obtained by Woodworth and Sherrington in their study of the "pseudaffective reflex," it seems established that adult decerebrate cats under appropriate noxious stimulation will give rhythmic movements of progression. These movements, rather rare, to judge from their report, are ineffective and of very brief duration. In the course of these experiments on kittens, a certain number of adult cats were subjected to the same ablations. These developed a typical extensor rigidity and showed the customary reactions of such animals. But when subjected to repeated noxious stimulations (strong pinching of the tail with instruments) very short-enduring rhythmic movements of all four legs were obtained. These were diagonal alternate movements, similar to those movements of progression described for the decerebrate kittens. The progressive beats in these adult preparations, however, were never prolonged and were apparently entirely similar to those movements recorded by Woodworth and Sherrington.

The interest in such progressive movements becomes much greater when considered in connection with the more recent conceptions of rhythmic alternation in the movement of antagonistic muscles or of limbs. The first steps in the solution of the rhythmic reaction were made by Sherrington (14) in 1910, when he suggested that the rhythm of the scratch reflex was conditioned by the interference between a maintained activity produced by a continuous skin stimulus and an antagonistic discontinuous activity from impulses in the moving limb itself. Later in the same year, Sherrington (15), further suggested that the stepping-reflex of the limbs might be due to an analogous rhythmic inhibition of a maintained activity by proprioceptive impulses from the moving limbs. In this same paper, he records the occurrence of these stepping movements in a de-afferented hind-leg as well as in the three normal legs.

In 1911, Graham Brown (4) studied the progressive movements evoked by rapid division of the spinal cord in the lower thoracic region. These movements occurred when the recording muscles were de-afferented and when all the other muscles of both hind-legs were de-afferented. This indicated that the phenomenon of progression is conditioned centrally and not by a peripheral self-regulating mechanism. Later in the same year, Graham Brown (5) likened the phenomenon of "rhythmic rebound" to rhythmic progression, for the rebound was shown to be dependent upon a balance between antagonistic activities in the centers.

Graham Brown (3, 7) also studied "narcosis progression" in the rabbit and cat; these movements are those of walking, running and galloping and occur usually in the stage of light narcosis. During subsequent experiments (4), the spinal cord was severed during the "narcosis progression;" if the cutting be done during light narcosis, rhythmic movements are usually abolished for a short phase, but return; if the cord be severed during deep narcosis, there is usually no interruption to the rhythm. Progressive movements following stimulation of the spinal cord have also been described by Graham Brown (3). This investigator at the same time reported rhythmic phenomena (immediate and terminal) which were evoked by compound stimulation and occasionally by single peripheral stimulation.

Shortly thereafter, Forbes and Graham Brown independently obtained rhythmic reactions during compound stimulations. Forbes (1) found that during simultaneous stimulation of two afferent nerves, which when stimulated singly provoked antagonistic responses in the

knee extensor, rhythmic movements occur. He also evoked a rhythmic response in the same muscle from stimulation of a single nerve. Graham Brown (6) likewise found that when two antagonistic stimuli are balanced against each other, rhythmic responses may be obtained in the decerebrate or low-spinal cat or in de-afferented preparations. The rate of movement in the experiments presented was one or two beats per second—a rate much slower than that recorded by Forbes; a distinct resemblance of these movements to those of progression was noted. Rhythmic reactions were also obtained by stimulation of single nerves in both the low-spinal and decerebrate preparations. It seemed that this rhythm was not conditioned by the efferent neurones but by the balance of two activities in antagonistic neurones.

Subsequently Graham Brown (8) described in detail rhythmic responses to single stimuli. These, he found, were rare in the spinal preparation except immediately after section of the spinal cord. But in the decerebrate animal, rhythmic movements resembling those of scratching or of progression, were not uncommon both in the intact and de-afferented subject. Although the stimuli used were apparently simple, the rhythmic responses seemed to occur only during antagonized activation of the centers.

Sherrington (16, 17) has investigated further the rhythmic responses in the flexors and extensors of the two knees. These rhythmic movements occurred during balanced antagonistic stimulation and were regarded as instances of reflex-stepping. Since then the phenomena of such rhythmic responses has been carefully studied by Sherrington and Graham Brown. From their work it is now possible to conclude that "rhythmic progression is conditioned by an equal balance of two antagonistic central activities" (Graham Brown). The further question regarding the possible conditioning of the phenomenon of progression by peripheral stimuli, or the possible consideration of progression as not being fundamentally reflex has been brought forward by Graham Brown (10).

Considered from the standpoint of these more recent investigations of rhythmic movements and of progression, the movements of the decerebrate kittens here are of interest. The walking movements of the decerebrate preparation described by Woodworth and Sherrington (21) are apparently similar to the movements observed in this study. But obviously the rhythmic movements obtained by these workers were of very short duration and ineffectual. The rhythmic beats of the kittens were, at least in part, not of this character; they were effective,

purposeful movements of escape in which all four legs took part. Especially in the twelve more active kittens, the inaugurating stimulus was very slight and differed markedly from the condition in the adult.

The nature of the excitatory stimulus is of great interest when related to the necessary conditioning of the rhythm by balancing two central antagonistic forces. In the less active animals, the stimulations were essentially noxious, but in the more active animals, the slightest excitation sufficed. Perhaps the resultant rhythm is similar to the rhythmic beats observed by Forbes and Graham Brown in response to single excitations. Certainly the rate of the progressive rhythm coincides closely with that described by Graham Brown. It seems best, therefore, to consider these decerebrate kittens as being able to exhibit progressive movements in response to excitations of a single stimulus which may be of possibly different character.

SUMMARY

Out of forty kittens subjected to decerebration with removal of cerebral hemispheres and basal ganglia above the anterior colliculi, twelve showed on appropriate excitation prolonged progressive movements. Eight of these twelve kittens were five days or under in age. The oldest kitten to show these long-enduring rhythmic beats was sixteen days in age. The other kittens of the series for the most part, gave only short-enduring progressive movements; the separation of these animals from the twelve is arbitrary but is based on differences in reflex-activity. In the majority of the twelve more active kittens, no decerebrate rigidity could be made out; the rigidity seemed present only in the older, less active kittens. An inexact reciprocal relationship between the occurrence of prolonged progressive movements in decerebrate kittens and an extensor rigidity is indicated.

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THE EXCITATION OF MICROSCOPIC AREAS: A NON-POLARIZABLE CAPILLARY ELECTRODE

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In the study of the response of tissues presenting an aggregate of contractile elements, it has been necessary for the most part to interpret the activity of the unit in the light of data furnished by the whole. This is especially true of skeletal muscle, where the observer is hampered in the functional isolation of fibers through the mechanical influence exerted by each upon its neighbors.

Whereas the diffuse stimulation of functionally independent cells, such as melanophores (1), may yield information strictly relative to the cell under consideration, the most convincing results in the field of the muscle fiber permit only of inference from the behavior of reduced aggregates (2), (3), (4). It would seem important that the study of fiber-activity be carried beyond the realm of even the clearest deduction into that of direct observation. Any vestige of doubt attaching to the validity of the all-or-none principle, or to the adequacy of our conceptions of the genesis of tetanus, the factors of fatigue, etc., cannot but delay approach to the ultimate problem, that of the energy transformation within and about the sarcomere. It is evident that such direct observation can be attained only through a means of excitation that shall affect a contractile area of no greater diameter than that of the element under scrutiny.

In a series of experiments made in this laboratory during the last three years, designed primarily to amplify the evidence—so masterfully

established by the work of Keith Lucas—for the all-or-none principle in skeletal muscle, a form of electrode has been evolved which renders the delicately controlled excitation of exceedingly minute superficial areas possible over long periods of time, without injury to the tissue.

Credit is due to Mr. John P. Eisenberger, undergraduate assistant in physiology, for aid in the construction of the apparatus, and for essential contributions to the method of preparing the capillary pore.

CONDITIONS FULFILLED IN THE ELECTRODE

In order to apply a controlled electrical stimulus to one of an adjacent number of contractile units, such as the superficial fibers of a muscle, the following requirements are to be met:

(1) The device should be unipolar in action.

(2) The active electrode must be of less diameter than one functional unit, yet must not involve excessive resistance.

(3) Contact between electrodes and tissue must cause no mechanical or other injury, and must be uniform in tension.

(4) The electrodes must be of the non-polarizable type.

The author has made considerable use of microscopically pointed metallic electrodes, in many cases with useful results; but in no instance can all of the above conditions be fulfilled. Platinum may with some difficulty, copper with great ease, be sharpened to the requisite tenuity. Moreover a light, uniform contact may be attained by embedding the point in a globule of glass or sealing-wax, and permitting it to rest upon the surface of the preparation by gravity. It is extremely difficult, however, to keep such an electrode clean without marring the point; and its consecutive use is short lived, owing doubtless to electrolytic and polarization effects.

In the electrode here described, the active pole is formed by a physiological solution (NaCl or Ringer's) contained in a glass tube, the converging lumen of which ends in a pore which is of distinctly less diameter than the frog's sartorius fiber. This pore is thus at the apex of a conical liquid conductor of relatively low resistance, bringing into contact with the tissue a minutely circumscribed area of the solution.

The indifferent pole is that portion of a like solution which lies in contact with the tissue at the opening of an annular orifice formed by the junction of two concentric glass tubes, the inner of which, described above, contains the fluid of the active electrode and ends below in the pore (fig. 1).

Both electrodal orifices lie in nearly the same plane; they are separated by a surface of polished glass, which may be kept at uniform tension against the surface of the preparation by an appropriate adjustment.

The fluid of each pole is led through glass tubing to a bath of like solution, in which lies a porous cup (conveniently a porcelain "boot-electrode") containing saturated zinc-sulphate solution. An amalgamated zinc rod, lying in this, forms one terminal of the stimulating circuit (fig. 3).

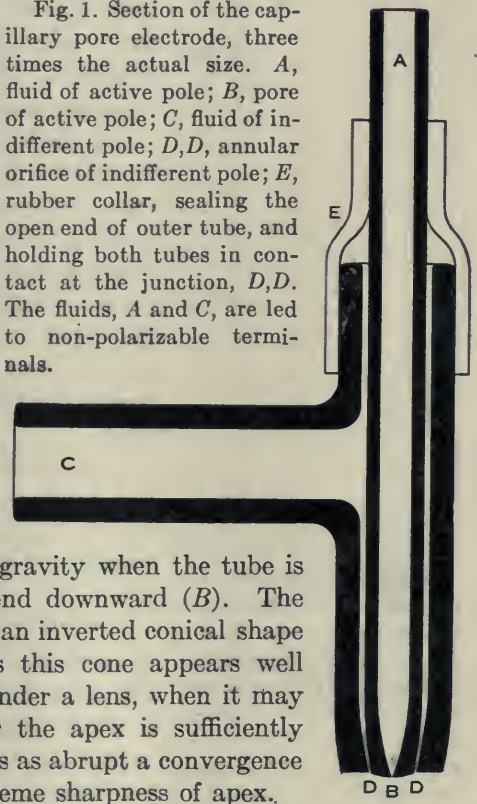

Fig. 1. Section of the capillary pore electrode, three times the actual size. *A*, fluid of active pole; *B*, pore of active pole; *C*, fluid of indifferent pole; *D,D*, annular orifice of indifferent pole; *E*, rubber collar, sealing the open end of outer tube, and holding both tubes in contact at the junction, *D,D*. The fluids, *A* and *C*, are led to non-polarizable terminals.

PREPARATION OF THE ELECTRODE

The active electrode tube is made from glass tubing of about 3.5 mm. outside, and 2 mm. inside diameter. One end is heated in the flame of the blast lamp until closed by fusion (fig. 2, *A*). Heating is then continued until the viscous end of the tube elongates slightly under the influence of gravity when the tube is held vertically with the fused end downward (*B*). The lumen will now tend to assume an inverted conical shape at its lower end. As soon as this cone appears well marked it should be examined under a lens, when it may readily be determined whether the apex is sufficiently sharp. The form to be sought is as abrupt a convergence as possible, consistent with extreme sharpness of apex.

The closed end of the tube is now ground flat on a carborundum wheel, until the apex of the lumen is within a millimeter of the surface. Grinding is continued on fine carborundum cloth until the apex is nearly reached (*C*), when crocus cloth is substituted as an abrasive. The polishing is completed on a well-worn area of the crocus cloth. Here the greatest caution is necessary to avoid undue encroachment upon the apex. Frequent examination with a powerful lens is essential, and the appearance of the slightest speck in the center of the sur-

face should call for especially critical scrutiny. A bright point of light appearing when the tube is held for examination with the open end toward the source of illumination, indicates the attainment of the apex (fig. 4). The orifice may, however, have become plugged as soon as produced, in which case it is necessary to resort to the method of clearance described later (page 165). The process of polishing is laborious and time-consuming, but due care yields a pore of extremely small size, quite circular in form, opening abruptly upon a slightly convex surface unmarred by scratches. The tube may now be cut to the requisite length.

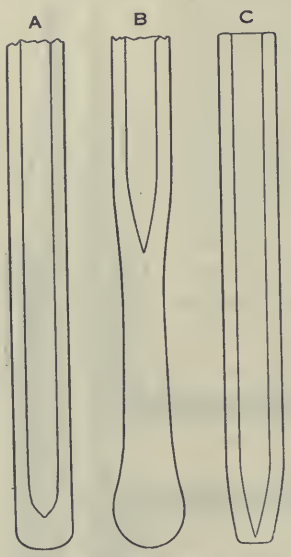

Fig. 2. Stages in the preparation of the capillary pore. *A*, tube closed by fusion; *B*, appearance of the conical extremity of the lumen when slightly elongated by gravity; *C*, after grinding. For the completed tube, see figure 1.

For the indifferent electrode a small T-tube is selected, into the main piece of which the tube just completed will glide snugly without binding. One end of the main piece is constricted slightly by heat, and ground flat. A ground joint is now made between the inner and outer tubes, with the aid of fine emery, so that the periphery of the pore-bearing surface is flush with the encircling rim (fig. 1, *D*, *B*, *D*).

When the inner tube is fixed rigidly in position by means of a coupling of rubber tubing (fig. 1, *E*), the electrode is in position for use when filled and connected.

ARRANGEMENT OF ACCESSORY PARTS

As shown in figure 3, the introduction of the electrode into the circuit involves simply a continuation of the inner tube and the branch of the T-tube, each to a bath of the physiological solution employed in the experiment. The vertical arms of the connecting tubes, just before entering the liquid, are supported by soft rubber clips attached to a horizontal rod common to both. This rod is actuated by rack and pinion for horizontal, and by worm and gear for vertical adjustment. This device (omitted from the figure) permits of delicate and uniform approximation of the electrode to the surface of the tissue. Each porous "boot" is held against the side of the glass container by a rubber band. The zinc rods, lying in the zinc-sulphate solution, have the usual connection with the stimulating circuit.

The live preparation—in most cases a frog of medium size—is entirely enclosed in a moist chamber consisting of a glass or composition dish with ground edges, covered by a plate of glass pierced with a circular orifice to admit the end of the electrode.

The large mechanical stage, carrying the moist chamber with the entire electrodal system and its adjustments, is made by extending the carriage of a stage of ordinary size by means of a glass plate, supporting the overhanging weight with a steel ball-bearing. This permits of

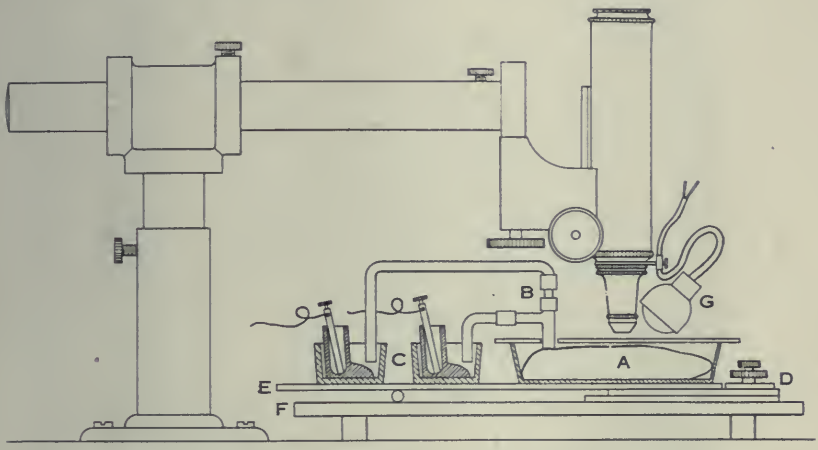

Fig. 3. Semi-diagrammatic elevation and section, showing the arrangement of accessory apparatus. The device for adjusting the electrode is omitted for clearness. *A*, live preparation, contained in the moist chamber, covered by a glass plate perforated to receive the electrode; *B*, the electrode, each fluid pole continuous with its bath, *C*, in which lies the porous "boot" containing $ZnSO_4$-Zn terminal; *D*, mechanical stage, extended by the glass carriage, *E*, which is supported upon a ball-bearing moving over the heavy plate-glass base, *F*; *G*, electric bulb, illuminating the surface of the preparation.

sufficiently frictionless movement on the part of the stage extension over the fixed base. As the contraction of single muscle fibers are best observed at a distance from the point stimulated, this arrangement enables the contracting element to be readily found, and adjusted with great nicety in the microscopic field. The figure illustrates a very rigid stand for the microscope, made from a set of ordinary steam connections. It should have horizontal swivel and forward and back adjustments, all delicate control being cared for by the mechanical stage. It is merely a convenient substitute for the expensive large dissecting stand, and on comparison has been found equally reliable for this work.

Illumination is afforded by a small electric bulb, attached by means of stout but readily flexible wires to the tube of the microscope. It should furnish an amount of heat just sufficient to maintain the temperature of the inner surface of the cover-glass above the dew-point.

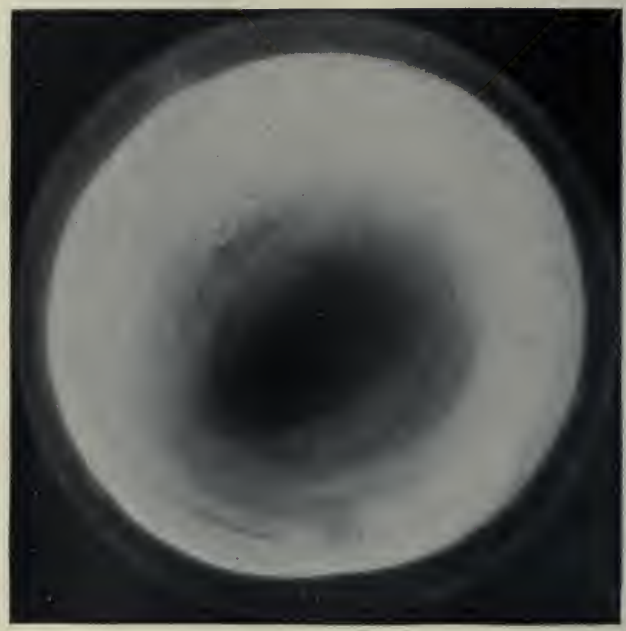

Fig. 4. Face of the active electrode, magnified 30 diameters and photographed by transmitted light. The pore, 8 μ in actual diameter, appears as a bright point in the center.

PROCEDURE IN THE USE OF THE APPARATUS

Since the preparation, so far in practice, is fed by an intact circulation, it has been found advisable to bare the tissue and enclose the pithed animal in the moist chamber at the outset, with the lamp adjusted and lighted. The delay will thus permit temperature and vasomotor conditions to attain equilibrium. Meanwhile the saline baths and porous cups are filled, the zinc terminals connected, and finally the electrode examined, coupled and fixed in place.

Three precautions are especially important:

(1) The pore of the active electrode must be clear.

(2) The electrodal system of tubes must be protected from siphonage while in position.

(3) Short-circuiting must be guarded against.

The pore in present use is 8μ in diameter—about that of the human erythrocyte—and consequently subject to frequent occlusion. The pore-bearing tube should be kept in filtered distilled water when not in use. Fortunately, the difficulty of maintenance is vastly less than in the case of a true capillary tube, which is impracticable for this purpose, owing to the excessive electrical resistance and liability to penetrate the tissue, as well as to inevitably serious plugging. In most cases, all that is necessary to free the pore of an occluding particle is to centrifuge the tube, previously filled, *in the direction of its open end*, by a sharp downward swing of the hand, as in replacing the mercury column of a clinical thermometer. The particle is usually carried into the abruptly diverging lumen, whence it may be washed out with a capillary pipette.

The connecting tubes may be guarded from siphoning out the contents of the electrode by stuffing them in a few places with absorbent cotton. A few air-bells may safely be ignored, provided there is continuity of fluid about them. These tubes, with the T-part of the electrode, may be kept permanently in the physiological solution, protected from evaporation.

The free end of the active electrode (fig. 1, *A*), when in use, is coupled to its connecting tube leading to the saline bath. Obviously, any continuity of electrolyte extending over the tube at the level *A*, to the fluid within the collar, *E*, must short-circuit the current. Spontaneous drying usually remedies this; but it is advisable to see that all superfluous solution is removed from the outside of this joint, and the possibility of leakage excluded, before proceeding to an experiment.

In adjusting the two parts of the electrode for use the rubber collar, *E*, is first raised slightly. Both parts being filled, the inner tube is thrust into the rubber collar under an ample bath of the solution. Both are then removed together from the bath, and the inner tube carried further downward until it seats itself firmly in the ground joint. The rubber collar is now slid downward slightly, providing a uniform elastic tension sufficient to hold the inner tube securely in its ground seat.

The connecting tubes are inserted in the clips, the electrode attached and contact with the preparation made by means of the adjustments already described. Under the conditions, the tension upon the tissue

is not a rigid one, owing to the yielding of the rubber connections and the flexibility of the light arm supporting the clips. It is subject, moreover, to delicate variation at will, and, once adjusted, is uniform.

DISCUSSION OF PRINCIPLES INVOLVED

An examination of the possible field of excitation (fig. 1, *D*, *B*, *D*), reveals an annular orifice surrounding a minute central pore, the two being electrically united by a film of liquid occupying the region of approximation with the tissue. It may be assumed that current density is relatively high at any tissue surface opposite the pore, diminishing with great rapidity as the annular orifice of the indifferent pole is approached. Current should penetrate little if at all the interior of the tissue, owing to the high resistance there to be encountered as compared with that of the electrolyte employed. Deep stimulation, therefore, is avoided, as evidenced by the fact that at no time is an experiment disturbed by nerve response in uncurarized preparations.

Local excitation of contractile elements beneath the face of the electrode must therefore be limited to a superficial region surrounding the pore, determined in area by (a) the strength of current, (b) the threshold of the elements impinged upon.

Since the mass of conducting electrolyte, and inversely its excitation-value, varies rapidly between the center and the periphery of the system, being based upon the extremes of a pore, 8 μ in diameter,[1] and an orifice, π (2550) μ in periphery (leading from the ample crevices of an annular ground-glass joint), it is unlikely that more than two elements can be excited when the stimulus at the pore-region is just liminal. In the case of the sartorius fibers, which exhibit a surface breadth of approximately 20 to 50 μ, it seems certain that a delicate adjustment of current will in the majority of instances cause an excitation of but one fiber, provided its surface be sufficiently near the pore.

The construction of the active electrode tube renders this close approximation of pore and tissue possible (necessarily allowing for the interposition of delicate fascia), owing to the convexity of the face of the electrode, produced, and in fact hardly to be avoided, by the method of grinding. The firmer contact with the tissue at the region of the pore than elsewhere would, indeed, further emphasize the conductor-difference between *B* and *D*, *D* (fig. 1).

[1] A pore of less than half this diameter has recently been produced, and it is probable that further experience will enable still smaller sizes to be made.

The ease with which perfusion may be accomplished within the lumen of the active electrode tube suggests its possible adaptation to sharply localized chemical stimulation or other chemical influence, with or without the use of the electric current.

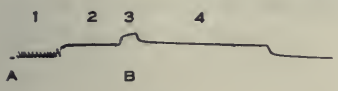

FIG. 5

FIG. 6

FIG. 7

Fig. 5. Photomicrographic record, traced by the light-reflex from a globule of mercury resting upon the active area of a sartorius with intact circulation. The plate was moved by clockwork. The stimulus, an alternating magneto current, underwent continuous increment of strength (but not of frequency) from *A* to *B;* then was maintained at full strength for the remainder of the record. Note, *1*, fiber-rhythm, often characteristic of response in the threshold region; *2*, minimal step; *3*, primary submaximal step, with contracture (treppe?); and return to *4*, minimal step, subject to contracture addition. *Continuous increment of excitation yields discontinuous increment of response. Continuous constant excitation yields discontinuous decrement of response.*

Fig. 6. Tracing obtained as in figure 5. The stimulus, in full strength at *A* suffered continuous decrement to *B*. Note the two submaximal steps and the minimal step. *Continuous decrement of excitation yields discontinuous decrement of response.*

Fig. 7. Minimal tetanus, obtained as in figure 5. Between *A* and *B* the stimulus underwent continuous increment and continuous decrement in immediate succession. The fact that the curve of relaxation is accurately superimposable upon the corresponding minimal drop in figure 5 suggests that decrement of stimulus in one case, and fatigue in the other, have eliminated the same contractile entity. This statement is made on the basis of a comparison of direct photographic enlargements five times the size of the above figures.

These curves are all from the same microsopic field and index-point, without alteration in the position of the electrode. The tracings, presenting an original magnification of 13 diameters, are enlarged by one-half and reversed to read from left to right. One and one-half millimeter on the abscissa corresponds to one second in time. The frequency of excitation was approximately 50 per second. Four hours after the adjustment of the preparation, circulation and irritability were apparently unimpaired.

An extended series of observations, shortly to be published, has indicated that the above considerations hold good in actual practice. Muscle fibers excited with tetanic stimuli glide singly or in small groups among their fellows, dragging them, passive, into positions of altered tension. The method permits readily of quantitative record—photographic, semi-mechanical, or by direct observation over the microme-

ter scale. The actual minimal response apparently reveals itself; and in clear relation to the submaximal effects elicited by increase of the area of adequate excitation. Figures 5, 6 and 7, explained by their legends, typify results regarded by the author as supporting the contention that the all-or-none law of Bowditch and of Lucas is applicable in the gradients of both tetanus and fatigue as a principle of physiological quanta.

SUMMARY

1. The desirability of a method for the individual stimulation of closely associated functionating elements is emphasized, in view of the discrepancy which is possible between unit and aggregate behavior.

2. A form of electrode is described, having an active pole 8 μ in diameter, uninjurious to delicate tissues, and non-polarizable. The active pole occupies a minute pore opening from the fused and ground extremity of a glass tube; the indifferent pole occupies the annular orifice formed by the junction of this tube with an outer tube bearing a T-arm. Each tube is filled with a physiological solution led to a porous cup containing a $ZnSO_4$-Zn terminal.

3. The method of preparing the electrode, its arrangement for use with a mechanical stage, and details relative to the use of the apparatus are described.

4. The distribution of current between the face of the electrode and the preparation is such that an exceedingly minute area may be excited without overstepping the threshold of adjacent contractile elements.

5. The adaptation of the active electrode tube to chemical stimulation is suggested.

6. Photomicrographic tracings are appended, illustrative of the method and supporting, in the author's estimation, a principle of physiological quanta for gradients in tetanus and fatigue.

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THE ACCURACY OF MOVEMENT IN THE ABSENCE OF EXCITATION FROM THE MOVING ORGAN

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From the Johns Hopkins University and the Government Hospital for the Insane

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For psychology the problem of motor activity has been largely one of the perception of movement. Discussion has centered about the questions of the receptors which are excited differentially by changes in extent and force of movement, about the psycho-physics of the constant error, the influence of the emotions upon the perception of movement, the relation of the "will impulse" to the perception of movement; with the result that the equally important questions of the nervous mechanism of initiation, continuation and cessation of adaptive movements have been dealt with only incidentally as throwing light upon this perception. Whether such an attitude on the part of investigators is the result of the general concept of psychology which would restrict its scope to the study of sensation, or is a consequence of the relative ease with which the problems of sensory and motor physiology may be attacked, the result has been an almost total neglect of the neurological problems presented by skilled movements.

In many cases the perception and control of movement may be one and the same phenomenon, but certain experimental data and facts of every day experience indicate a certain degree of independence of the accuracy of movement from any stimulation resulting from the movement itself. Such is the accuracy of automatic movements as seen in the production of steps of uniform length, or, more strikingly, the swift movements of the musician, many of which are made more quickly than the briefest cortical reaction time. These movements are frequently regulated in extent and force with the utmost nicety, under conditions where any reflex control from excitations arising in the moving hand seems excluded. Again, the evidence obtained by Bowditch and Southard, Loeb, Woodworth and others, while by no means conclusive, indicates a partial independence of the motor control from afferent processes originating in the moving organ. The reëstablishment of walking movements in the dog after section of the dorsal roots gives further evidence for the same view.

Clearly the ideal opportunity for the study of these relations would be given by a case of complete sensory anesthesia in man with no more impairment of motor functions than would necessarily arise from the anesthesia. A partial condition of this character is occasionally reported in tabes dorsalis and the complete absence of afferent impulses in rare cases of spinal lesion. Through the courtesy of the staff of the Government Hospital for the Insane, I have had opportunity to examine a number of patients showing various degrees of anesthesia and to carry out the experiments reported in the following pages with one who showed a condition particularly adapted to answer some of the questions centered about the control of movement.[1]

The present paper is devoted to a study of the sensory and motor condition of this patient. He is a young man of more than average intelligence who has, as a result of a gun-shot injury to the spinal cord, a partial anesthesia of both legs with motor paralysis of the muscles below the knees. The anesthesia of the left leg is much more extensive than that of the right. Rough preliminary tests indicated a sensitivity to movement of all joints except the left knee and ankle, and the paralysis of the muscles controlling the latter made it necessary to restrict the study to the movements at the knee. The experiments were arranged for the investigation first, of excitations from movements at this joint; second, of the accuracy of control of such movements as failed to excite afferent impulses from the limb.

PHYSICAL CONDITION OF THE SUBJECT

Neurological examination shows the left leg, with the greater part of the left groin and gluteal region, to be insensitive to light touch, temperature, and to protopathic stimulation. The extent of the area of cutaneous anesthesia is shown in figure 1. Except for the rather large areas in the gluteal region and the somewhat smaller areas on the calf (shown in white in figure 1) there is complete loss of cutaneous sensitivity of this leg. The loss of sensitivity to deep pressure is much less extensive, complete anesthesia to deep pressure involving only

[1] I wish to express my obligation in particular to Superintendent W. A. White, who has given me every opportunity for investigation, and to Dr. S. I. Franz, who first brought the patient to my attention and who has given important suggestions concerning the control of the experiments. Finally my greatest debt is to the subject of the present study who has worked tirelessly, and who has himself suggested improvements in technique which were essential for the success of the experiments.

the foot and anterior surface of the lower leg (cross-striated area in figure 1), but in the regions retaining sensitivity this is reduced much below normal. The minimum amount of pressure that can be detected in the region of cutaneous anesthesia is 900 grams on an area one-

FIG. 1

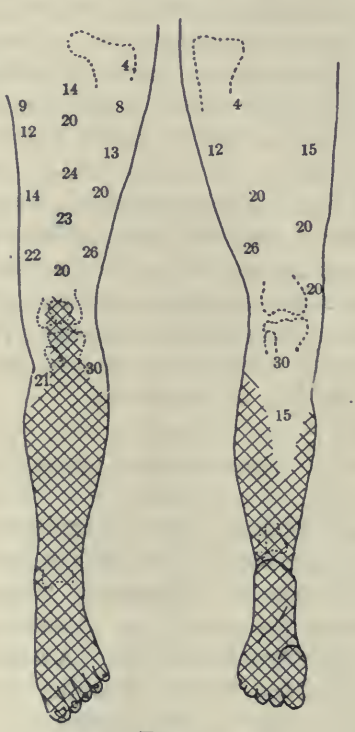

FIG. 2

Fig. 1. The extent of anesthesia of the left leg of the subject studied. White areas, cutaneous sensitivity; light shaded areas, cutaneous anesthesia with deep sensitivity to pressure; dark shaded areas, complete anesthesia to all stimulation.

Fig. 2. Threshold of sensitivity to pressure applied over an area ½ inch in diameter. Shaded area completely insensitive. The figures give the sensitivity of other regions in 100 gram units.

half inch in diameter in the region of the groin and this increases to 2000 grams over the greater part of the thigh and calf. The thresholds to deep pressure over the anesthetic area are shown in figure 2. Except in the gluteal region, in the groin and on the calf, localization

of deep pressure is very inexact, the average error of localization being about eight inches. No amount of pressure that I was able to apply excited deep pain.

There is a slight sensitivity to vibration (50 per second) in the femur. A small area of the patella also seems sensitive to this type of stimulation, but its sensitivity is probably due to the transmission of the vibration to the femur. There is no sensitivity to this type of stimulation in the region below the knee.

The cutaneous and tendon reflexes of the leg are completely abolished but a slight degree of tonicity is retained by the muscles of the thigh. We do not know the exact extent of the lesion and no detailed clinical history of the operation to remove the bullet is available, but the clinical picture indicates an extensive destruction of the dorsal bundles in the second or third lumbar segment of the cord with invasion of the dorsal horns or injury to the afferent roots in the sacral region sufficient to abolish the tendon reflexes. The determination of the exact extent of the lesion is not essential to the present work since the conclusions depend wholly upon the experimental determination of the extent of anesthesia to movement.

SENSITIVITY TO POSITION AND TO PASSIVE MOVEMENTS

Preliminary tests had indicated that in spite of the sensitivity to pressure retained by the subject, his sensitivity to flexion and extension of the knee was abolished. Before a study of the accuracy of active movements could be undertaken it was necessary to establish this point beyond question. A number of experiments was therefore carried out with a view to revealing any sensitivity to movement which might play a part in determining the accuracy of adaptive movements. In all the tests on movement the subject was seated on a soft cushion in a rather high chair with his left thigh supported by a padded rod, 3 inches behind the knee, so that his foot could swing freely from the knee through an arc of about 130 degrees from complete extension to the point where the heel came in contact with the rungs of the chair. The foot was attached by a cord to the carriage of a modified form of the Münsterberg movement apparatus, so that the rate and extent of movement could be recorded. The subject was blindfolded during all the tests and precautions were taken to eliminate any auditory stimulation from the recording apparatus which might serve as a clue to the extent of movement. The distance moved was recorded in centimeters

read directly from the recording device. A slight inaccuracy is introduced here, since the measurement was in a straight line while the movement was about the perimeter of a circle. In the records one centimeter is equal to approximately 1.28 degrees rotation about the knee.

Detection of passive movements. The experimenter grasped the subject's foot in his right hand and, holding his left hand on the subject's knee to detect unintentional movements of the thigh, flexed and extended the knee passively through angles varying from 10 to 130 degrees. The subject was asked to describe the distance and direction of movement whenever he felt that any movement had been made. The rate of the passive movements was varied from about 2 cm. to 100 cm. per second.

When the passive movements were made at a rate of less than 20 cm. per second, the subject was unable to detect any movement, however great its extent, and throughout a series of fifty such movements gave no reaction except when the movements resulted in hyperextension of the knee. More rapid movements were detected in about 50 per cent of the trials but reports of their direction and extent seemed to be wholly a matter of chance.

Fifty passive movements of the foot at a rate of more than 20 cm. per second were made under conditions where auditory cues were eliminated. The subject was asked to give the direction of movement and position of the foot whenever he felt that it was moving. Twenty-four of the movements brought no reaction. The remaining 26 were detected as movement; 4 were described as of uncertain direction, 22 brought definite statements of direction of movement which were, however, quite inaccurate. Seven movements of extension and 4 of flexion were described correctly. Five movements of extension were described as flexion and 6 movements of flexion were described as extension. The description of the position of the foot was equally inaccurate. The subject usually denied any knowledge of its position and in the instances where he ventured a definite statement he was as frequently wrong as right. Thus, in 3 out of 6 cases flexion at 70 degrees was described as complete extension and in 2 of 5 cases hyperextension was described as flexion.

When the knee was hyperextended the subject invariably stated that the knee was being moved. His knee was alternately hyperextended and flexed a number of times and he was asked to describe the movements. Only those of extension were detected and these the subject alternately stated to be extensions and flexions. Flexing move-

ments brought no reaction. From this it seems that the reactions to hyperextension were to the strain on the joint or ligaments and not to the movement as such.

An attempt was made to locate the source of stimulation by which rapid movements were detected. Slight flexion of the hip (elevation of the knee through a distance of 2 cm.) was for the first three trials interpreted as extension of the knee, thereafter as a movement of uncertain direction. Upward pressure against the patella was once interpreted as an extension of the knee, twice as a movement of the knee of uncertain direction, and remained undetected in more than 20 cases. Heavy pressure above the knee was once described as a movement of extension but later failed to elicit any response. The patient was instructed to contract the antagonistic muscles of the thigh so as to increase the tension upon the knee joint and fix the leg at the thigh. When this was done no passive movements were detected, unless they produced flexion of the hip. The quick movements which brought reactions were frequently described as "jerks" and I could not be certain that any of the movements that were detected did not involve a certain amount of strain upon the muscles of the trunk.

These tests suggest the absence of any afferent excitations from the leg during slow passive movements of the knee which may stimulate verbal reactions to the direction or extent of the movements. From them it seems probable, also, that the recognition of quick passive movements is not based directly upon excitations from the moving parts but only indirectly upon the spread of the shock of movement to adjacent parts of the body. Whatever may be the basis of the reactions to rapid passive movements the stimuli involved are not differentiated enough to allow the subject to determine the direction or extent of the movement.

Maintenance of position. The preceding tests required chiefly reaction to movement with, possibly, very little stress upon local signs from the position of the leg. A further series of tests was made with the emphasis upon position.

The subject's knee was hyperextended and he was asked to maintain that position as long as he could against the force of gravity. The quadriceps became tense and the leg was kept extended for about ten seconds, then dropped to vertical with a series of spasmodic twitches. The subject, when questioned, stated that he was still holding the leg extended and one minute later said "Now I am letting it down," the leg, in the mean time, hanging lax. The test was repeated a number of

times and gave similar results in every case. There was momentary maintenance of position, relaxation without recognition of the movement, and later an illusion of relaxation.

Other positions than that of hyperextension could not be maintained at all. When the subject's foot was placed passively in any other position and he was asked to hold it there, the leg dropped back to the vertical as soon as the support was removed, jerked back and forth spasmodically for a few seconds, and then came to rest in the vertical position, the subject asserting, without certainty, that he was holding the leg still.

Reaction to position. With the weight of the subject's foot wholly supported by the experimenter, the knee was flexed, starting from 105 degrees, until the recording carriage moved 10 cm. The subject was told that his foot would be moved back to the starting position and was asked to indicate verbally when it reached that position. The movements from the starting position were made as a uniform rate; the rate and direction of the "returning movements" were varied. The subject was warned in each case that the displacement of the foot was completed and that the experimenter was returning it to the starting point. In five series different procedures were employed: (1) The foot was moved back very slowly toward the initial position; (2) It was returned to the initial position at the same rate at which it had been displaced; (3) It was moved back very quickly; (4) The displacing movement was continued so as to bring the foot farther from the starting point; (5) The foot was held motionless after being displaced.

The positions stated by the subject to be the one from which the foot was originally displaced are given in table 1. Slow return was overestimated, quick return underestimated, and lack of movement or movement in the wrong direction was not detected. The subject stated that he was depending upon the time interval after the warning that the foot was being returned to its original position, and the relation of the positions identified as the starting point to the rate of passive movement confirms his statement. The results of the tests show clearly that there were no afferent excitations capable of arousing a reaction to position.

Duplication of passive movements. The data presented thus far indicate that any excitations from the position or from slow passive movements of the leg are subliminal for the language mechanism of the subject. It seemed possible that the excitations from passive movements

might still be above the threshold for the reflex control of active movement and to test this experiments were carried out in which the subject was asked to duplicate the extent and direction of a passive movement by making an active movement.

The position of the subject described above was used. Distances of 2 and 10 cm. were selected as pattern movements, and the subject's foot was moved backward or forward through these distances at a rate of less than 10 cm. per second. The subject stated that he could not tell when the pattern movement was made, so he was warned each time

TABLE 1

Reactions to position of the leg. A constant position of the knee (105 degrees extension) was adopted as a "starting point." The subject's foot was displaced passively 10 cm. from this and he was asked to indicate when it had been returned by a second movement. The figures, each based on the average of five trials, give the distances and the directions traversed by the foot in the second movement before the subject indicated that the starting position had been reached.

FOOT DISPLACED		EXTENT OF SECOND MOVEMENT		ERROR IN IDENTIFICATION OF POSITION	RATE OF SECOND MOVEMENT
	cm.	*cm.*		*cm.*	
Backward	10	1.4	Forward	− 8.6	Very slow
Backward	10	12.2	Forward	+ 2.2	Same as that of displacement
Backward	10	22.5	Forward	+12.5	Quick
Backward	10	0.0	Forward	−10.0	No movement
Backward	10	25.5	Backward	−35.5	Quick
Forward	10	2.1	Backward	− 7.9	Very slow
Forward	10	8.3	Backward	− 1.7	Same as that of displacement
Forward	10	16.7	Backward	+ 6.7	Quick
Forward	10	0.0	Backward	−10.0	No movement
Forward	10	8.5	Forward	−18.5	Quick

his foot was moved and was asked to continue the movement through the same distance and in the same direction as the passive movement.

Owing to the fact that subject could not hold his foot steadily in any position against the pull of gravity it was necessary to counterbalance the weight of the foot immediately before and at the expiration of his movements. Since the weight to be supported changes greatly with the degree of extension of the knee, no practicable method of supporting the weight could be employed in the limited time available exept that of partial support by the experimenter's hand. This introduces into the distances moved an element of error from the personal equation of the experimenter. On page 181 is recorded an experiment in which the same technique is used where the subject attempted to duplicate his own active

movements. In the forward movement no resistance except that of gravity was offered to the movement; in the backward movements the experimenter tried to oppose a constant resistance equal to that necessary to hold the foot in position before the beginning of the movement. The slight difference in the variability of the movements made under the two conditions of resistance indicated that the error introduced by the personal equation of the experimenter is not great enough to affect the results seriously.

The relation of the direction of the subject's movements to the direction of the pattern set is shown in table 2. The direction was interpreted wrongly in almost 50 per cent of the trials, which indicates that the direction of the subject's movements was wholly a matter of chance. The lengths of the movements varied from 0 to 27 cm. (the maximum

TABLE 2

Record of attempts to duplicate the direction and distance of passive movements. The length of the passive movements in each series was kept constant but the directions were varied in irregular order.

PASSIVE MOVEMENT	ACTIVE MOVEMENT
Backward flexion 2 cm..... 15 trials	Flexion..... 6 trials Extension..... 9 trials
Extension 2 cm..... 21 trials	Flexion..... 7 trials Extension..... 14 trials
Flexion 10 cm..... 18 trials	Flexion..... 7 trials Extension..... 11 trials
Extension 10 cm..... 20 trials	Flexion..... 8 trials Extension..... 12 trials

Total, direction correct 39 trials
Total, direction incorrect 35 trials

movement permitted by the position of the subject's foot). The variations in two series of tests are shown in figure 3, other tests giving similar results. There is no correlation either in direction or extent between the active movements and the passive pattern which they were intended to reproduce.

The subject's failure to give any precise or constant reaction to the position or movement of his knee seems to prove that the anesthesia of the leg is sufficiently extensive to exclude any reflex control of the accuracy of movement based upon cortical excitations arising from the moving limb. The one remaining possibility of excitation from the limb calls for the postulation of receptors in the muscles which are stimulated by active contraction and not by passive tension. To

test this the relative lengths of the voluntary movements which the subject estimated as equal when different amounts of resistance were opposed to the movement were measured.

AFFERENT EXCITATION FROM THE CONTRACTING MUSCLES

Rough tests were first carried out with different amounts of resistance opposed to the subject's active movements. He was asked to flex his knee so that his foot was drawn back 3 inches from a given position (120 degrees extension) in which his leg was held by the experimenter and to indicate verbally when he had moved his foot through this distance. The latter precaution was taken to test the relation between the duration of the motor innervation and any excitation of the language mechanism which might exist.

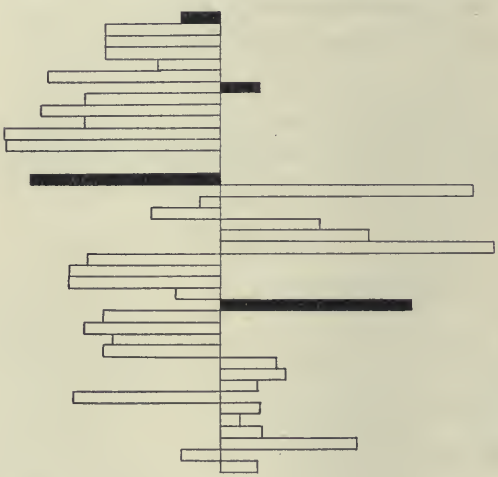

Fig. 3. Record of attempts to duplicate by active movements a pattern set by passive movement of the leg. The direction and extent of the passive movements are shown by the solid rectangles; the active movements of the subject are shown by those in outline. The pattern was repeated before each active movement.

In the first trials the foot was allowed to move at a rate of only about 1 cm. per second. The average distance moved and reported as 3 inches under this condition was 1.65 cm. with a range from 0.9 to 2.5 cm. (ten trials). In the next ten trials the pull against the subject's movements was increased to such an extent that the knee was extended slightly during his attempts to flex it. In these tests an average forward movement of 1.04 cm., with a range of 0.5 to 1.5 cm. was reported as a backward movement of 3 inches. During these tests the subject reported that he felt resistance but had increased the force of his movements until he had compensated for it completely. The mechanism by which the resistance was detected was not determined. It may have been a residual joint or tendon sensitivity which

was too slight to be stimulated by unresisted movements, or to the deep muscle sensitivity to pressure, or to certain stimulation from strain on the muscles of the trunk which could not be altogether eliminated. The subject indicated his knee as the source of stimulation, but was very uncertain. Whatever the locus of excitation, the stimulus was not specific enough to give a clue to the extent of movement of the foot. In a third series of tests the active movement of the subject was accelerated by the experimenter, the subject being asked, as before, to stop the movement and indicate when his foot had moved 3 inches. The average distance moved in ten trials was 28.94 cm. with a range from 26.0 to 30.5 cm. This was practically the maximum extent of movement allowed by the position of the subject.

TABLE 3

The average length of voluntary movements (flexion of knee) stated by the subject to be equal in extent, when the amount of resistance opposed to the movement was varied

RESISTANCE		AVERAGE DISTANCE MOVED. (TEN TRIALS)	
grams		*cm.*	
1111	Accelerating	26.0	Limit of movement
0		17.4	
266	Retarding	18.4	
644	Retarding	12.4	
866	Retarding	9.6	
1133	Retarding	8.7	
1380	Retarding	8.3	
Foot clamped		0.0	
Knee forcibly extended		2 to 10	Extension

The same tests were repeated with a better control of the amount of resistance offered to the movement, and with virtually the same results. The subject's foot was supported at 120 degrees extension by a spring exerting 100 grams for each 4.5 cm. extension and he was asked to draw his foot back three inches. The initial tension on the spring was varied from 266 to 1380 grams. In other tests the foot was held motionless, in others the knee was extended during the attempt at flexion, and finally the spring was set to flex the knee. As far as possible the subject was kept in ignorance of the procedure. He was asked to indicate verbally when he had carried out the instructions. The results of the tests are shown in table 3. The distances

moved vary inversely with the resistance encountered but not in direct ratio. The foregoing tests seem to show that there are no excitations from the actively moving limb which are specific enough to give a clue to the extent of the movement.

A certain amount of adjustment to the resistance is indicated by the fact that the extent of movement is not inversely proportional to the resistance. The work done in extending the heavier springs (computed as distance times weight lifted) is greater than that performed with the lighter springs. Such a method of considering the data is misleading, however, for it considers that no work is done unless external resistance is opposed to the movement, whereas a certain amount of work is done in the contraction of free muscle, in the stretching of the antagonistic muscles, and in overcoming the resistance at the joint. It is impossible to estimate the amount of force expended in this way, but if we assume that the work done in moving the leg without external resistance is the equivalent of lifting 50 grams for the distance moved, the total amount of work done in moving against each of the resistances recorded in the table is practically the same. There is no justification for assigning this particular value to unresisted movement, but there is also no certain evidence that there was any compensation for the different resistances encountered.

There remains the subject's statement that he felt resistance and made adequate allowance for it by giving a harder pull. How much of the apparent increase in work done was due to this cannot be determined without more data than are available at the present time upon the internal resistance to movement of muscle and joint.

Such imperfect compensation for resistance as the subject may have made is irrelevant to the problem in hand since the subject failed to distinguish the extent of movement. No difference between a flexion of 26 cm. and an extension of as much as 10 cm. was detected except in the amount of resistance encountered. The recognition of resistance was evidently not based upon any excitation which could give evidence upon the direction and extent of movement.

We may conclude that we are dealing with an anesthesia to passive and active movements of the knee which is practically complete for a rate of movement of less than 20 cm. per second within an arc of 45 degrees in each direction from the right angle. With this established it is possible to test the accuracy of voluntary movements within these limits with the certainty that the intensity and duration of the innervation involved in them are not reflexly controlled by afferent excitations from the moving limb.

THE ACCURACY OF ACTIVE MOVEMENTS

Direction of movement. In the preceding tests where active movements were requested the subject made no errors in the direction of his movements. Only twenty additional trials were given in a formal test of accuracy in the direction of active movements, in all of which the movement was made correctly. In many hundreds of voluntary movements, however, I have never seen the subject make a mistake in direction, except when he misunderstood instructions. It seems certain that the voluntary excitation of a specific group of muscles is possible in the absence of afferent excitation from it.

Extent of movement. Two somewhat different methods were used for testing the accuracy in control of extent of voluntary movements. In the first experiments the subject was asked to move his foot through a given distance (an estimated inch, 2 inches, etc.) while the experimenter gave a slight nearly constant support to the backward moving foot in order to control the inability of the relaxed quadriceps to support it against the pull of gravity. The inaccuracy of this method has been considered (page 176). The resistance was applied only to the backward movements, probably accounting for the fact that they are slightly shorter than the corresponding forward ones (tables 4 and 6), and does not influence the extent of the forward movements, which are equally accurate. When a movement was made its extent was recorded and the foot was brought back passively to its initial position, usually about 110 degrees extension. None of the subject's active movements exceeded the rate of 20 cm. per second so that the controls for slow rates of passive movement apply to all the active movements studied.

In the first experiment the subject was asked to make ten attempts to move his foot through distances which he judged to be $\frac{1}{2}$, 1, 2 and 3 inches. The averages for the different distances are given in table 4. The movements were all longer than the distance asked for but there was practically no overlapping between the movements estimated as different. The pattern set by the first voluntary movement was duplicated rather accurately in later movements.

In these tests the ten trials for each distance and direction were given successively and it seemed possible that this might contribute something to the accuracy of the movement through the establishment of a rhythm of motor excitation. A series of tests was made therefore in each of which movements through distances of from $\frac{1}{2}$ to 6 inches were

TABLE 4

Average distances, each based on ten trials, through which the subject moved his foot when asked to move through a distance which he judged to be that given at the left. Inches were used because he was not familiar with the metric scale

ATTEMPT TO MOVE FOOT		AVERAGE DISTANCE MOVED	
inches		*cm.*	
½	Forward	2.88 ± 0.37	Forward
1	Forward	3.86 ± 0.14	Forward
2	Forward	5.16 ± 0.50	Forward
3	Forward	13.42 ± 0.87	Forward
2	Forward (later test)	7.00 ± 0.27	Forward
½	Backward	1.46 ± 0.25	Backward
1	Backward	3.40 ± 0.24	Backward
2	Backward	7.34 ± 0.68	Backward
3	Backward	11.17 ± 0.57	Backward

made successively. The results of five such tests are given in table 5. In only one of the five tests, which began with the shortest and progressed to the longest movement, was a movement shorter than the one preceding it. This case is marked in italics in the table. The subject was not told that he had made an error yet an apparent compensation appeared in the next movement, which is the longest made for that distance.

A third series of the same general character was carried out in which all the movements of a given estimated distance were made successively but the different distances to be estimated were taken in irregular order so that an estimate of the absolute distance moved rather than a

TABLE 5

Same as table 4 except that the subject attempted to estimate distances from ½ to 6 inches successively

SUBJECT ASKED TO MOVE		DISTANCE MOVED					AVERAGE
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
inches		*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
½	Forward	3.0	5.0	3.6	2.5	5.0	3.82
1	Forward	4.2	10.2	9.0	8.2	7.1	7.74
2	Forward	7.0	13.0	12.2	12.3	11.0	11.10
3	Forward	12.1	17.0	14.5	15.8	13.2	14.52
4	Forward	13.2	*13.0*	16.5	18.0	17.2	15.58
5	Forward	16.8	22.0	20.4	21.9	18.6	19.94
6	Forward	22.9	24.0	23.3	23.0	22.0	23.04

comparison of the length of successive movements was required. The order in which they were taken up was ½, 6, 1, 3 and 2 inches. The averages for the different distances with their standard deviations are given in table 6.

TABLE 6

Same as table 4 except that the different distances were estimated in irregular order

DISTANCE TO BE ESTIMATED		AVERAGE DISTANCE MOVED	STANDARD DEVIATION
inches		*cm.*	*cm.*
½	Forward	2.5 ± 0.11	0.499
1	Forward	6.5 ± 0.44	2.271
2	Forward	11.2 ± 0.71	4.357*
3	Forward	16.3 ± 1.32	6.187*
6	Forward	22.1 ± 0.56	2.633
½	Backward	2.9 ± 0.13	0.589
1	Backward	4.8 ± 0.20	1.016
2	Backward	9.3 ± 0.38	1.823
3	Backward	15.9 ± 0.41	1.929
6	Backward	20.6 ± 0.36	1.782

* The high standard deviations here are the result of one trial in each group in which the subject reported that the movement had been made when his foot did not move. It is possible that the foot caught against the floor in these cases but as this could not be verified they were included.

In all these tests there is a surprising accuracy in the extent of the voluntary movements. Too few trials were given at each distance to lend much significance to the coefficients of variation for the attempts to estimate given distances, but in every series of tests the average distances moved are roughly proportional to the distances which the subject was asked to estimate. The pattern set by voluntary movement could be duplicated with a fair degree of accuracy and the intensity of innervation could be graduated in a series of distinctly different steps.

Comparison of accuracy of movement with that of a normal subject. For the determination of the variability of movements when the subject was asked to copy a pattern set by his own active movements and for a comparison of this with the normal variability, it was necessary to eliminate any influence which the experimenter might exert in supporting the subject's foot. This was done by supporting the foot by a light spring so that the knee was partially extended. The subject was then asked to draw his foot back through a given distance and then

allow it to swing forward freely. The carriage of the recording apparatus was arranged to stop at the limit of the backward movement.

Series of twenty or more trials were obtained for estimated distances of 1, 2 and 3 inches. The average extent of movement for these three distances with the standard coefficient of variations are given in table 7, and the distribution of variations is shown in figure 4. For comparison, the results of a similar experiment on an apparently normal individual are included in the table and figure. This subject, a physician,

TABLE 7

Variation in the extent of movements estimated as equal by the anesthetic subject and by a normal individual

	DISTANCE ESTIMATED	AVERAGE EXTENT OF MOVEMENT	STANDARD COEFFICIENT OF VARIATION	ERROR FROM DISTANCE ESTIMATED	NUMBER OF TRIALS
	inches	*cm.*		*cm.*	
Anesthetic subject.....	1	4.62 ± 0.08	0.254	+ 2.12	84
	2	14.40 ± 0.26	0.122	+ 9.40	21
	3	23.98 ± 0.44	0.167	+16.48	38
Normal subject..	1	2.04 ± 0.09	0.476	− 0.46	50
	2	11.24 ± 0.21	0.193	+ 6.00	50
	3	22.30 ± 0.16	0.076	+14.80	50

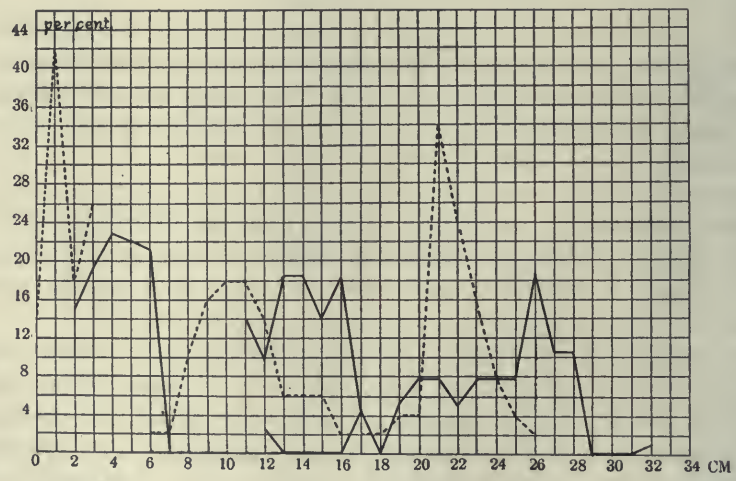

Fig. 4. Distribution of variations in the length of voluntary movements in the anesthetic and in a normal subject. The ordinates represent the percentage of the movements in each series which were of the lengths given on the abscissae. ——— Anesthetic subject.- - - - Normal subject.

was selected at random, the instructions and method of supporting the foot and recording the extent of movement were the same as those employed with the anesthetic subject so that the results with the two subjects may be taken as closely comparable. There is a surprising similarity in the results obtained with the anesthetic and with the normal subject. The movements of both showed wide errors from the distances which they were asked to estimate and the normal subject was not greatly superior to the anesthetic in this respect. In the variability of the movements estimated as equal there was no constant superiority of either subject. The normal individual gave more uniform movements for the longer distance but varied more in estimation of the shorter ones.

We may conclude that the anesthetic subject's control of his movements is not significantly less accurate than that of the normal individual, and it is not clear that for the simple movement studied the afferent impulses from the moving limb contributed anything to the accuracy of movement in the normal subject. The chief mechanism for the control of movement is located in some other body segment than that of the moving organ.

THE RELATION OF RATE TO ACCURACY OF MOVEMENT

Earlier studies of movement, particularly those of Loeb and Dellabarre have indicated that the duration of movement may serve as a clue to its extent, in place of the changing pattern of stimulation from the moving limb. It seemed possible that the subject of the present experiments was depending upon the duration of movement by maintaining a constant motor discharge during time intervals corresponding to the distances through which he was asked to move. In the following tests the rate of movement was recorded with the distance.

The subject's foot was suspended with the knee extended to 110 degrees by a spring having a coefficient of 100 grams for each 4.5 cm. extension and he was asked to draw his foot back through distances and at a rate suggested by the experimenter. The results of the tests are summarized in table 8. From this it will be seen first, that the duration of movement is not proportional to the distance when the subject is allowed to choose his own rates but that the rate of long movements is less than of the short ones (tests A, B and C); second, that the movements may be made of equal extent, although the rate is quite different (tests D and F), third, that, except in test A, the variability in the

time of movement is considerably greater than that of its extent; and fourth, that the variation in both extent and time of movement decreases with increasing rate. The experiments thus show a degree of independence in the rate and extent of movement which precludes the possibility that the extent of movement is determined merely by the control of the duration of the excitation of motor pathways. They indicate, on the contrary, that there is a control of the intensity of motor discharge which is independent both of the duration of excitation and of the effects of the discharge upon the effectors. The increase in accuracy with increased speed is in accord with the results obtained by Woodworth (6) in his study of the accuracy of automatic move-

TABLE 8

Variation of the rate of movement compared with variation in the extent of movement. In tests A, B and C the subject was allowed to select his own rate of movement; in test D he was asked to move quickly, in E, still more rapidly, and in F, to jerk his foot back as quickly as possible. The averages are each based on ten trials

TEST	AVERAGE DISTANCE	STANDARD COEFFICIENT OF VARIATION	AVERAGE TIME	STANDARD COEFFICIENT OF VARIATION	DISTANCE REQUIRED
	cm.		*seconds*		*inches*
A	4.27	0.334	0.69	0.272	1
B	8.05	0.156	2.19	0.216	2
C	11.52	0.153	3.97	0.398	3
D	12.59	0.121	1.44	0.324	3
E	9.87	0.119	0.80	0.264	3
F	11.41	0.115	0.68	0.235	3
Average		0.166		0.285	

ments and confirms his assumption that rapidity of normal movement interferes with its accuracy only by reducing the influence of the "current control," of the excitations aroused by the moving organs.

The time records showed further that the slow movements were not the result of a single muscular contraction but consisted, in practically every case, of a series of from two to five successive contractions resulting in alternate acceleration and retardation of the movement. This furnishes additional evidence against a temporal control of movement and also raises the question whether an initial set is adequate to account for an accurate movement which is excited by a series of innervations, without some controlling mechanism which is active continuously during the course of the movements.

REACTIONS TO ERROR OF MOVEMENT

In occasional instances the subject stated that a given movement was longer than he had intended. Records of only eight such movements have been obtained but in every case the recorded movement was considerably greater than other movements of the series in which it occurred. The recognition of such movements is ascribed by Woodworth (6) to sensory elements arising from the movement. If, as seems established by the tests recorded, the subject of the present experiments was anesthetic to movements of the knee, the detection of error must be ascribed to some mechanism other than the receptor system of the moving organ. This demands a distinction not only between the initial set or intention of movement and the final adjustment due to sensory stimulation, but also the recognition of a third factor in the control of movement, the capacity for reaction to the intensity of innervation which is independent of both the initial set and the excitations from the moving organ. This suggests the old doctrine of the feeling of innervation, although an alternative hypothesis must be considered. This is outlined on page 193.

EFFECTS OF FATIGUE

In some of the earlier tests, after many repetitions of a given movement, the subject complained of feeling resistance to his movements and at the same time increased their length. It seemed that this might be the result of fatigue and a number of series of movements was therefore made to test this more thoroughly. The subject was required to repeat a movement of a given length from 20 to 85 times. Resistance to the movements was offered by a spring which drew the foot back to the starting point after each movement.

Table 9 shows the results of this test. In each case repetition of the movement led to a considerable increase in its extent. The progression in the length of movement in two series is shown in figure 5 which is based upon the average of successive groups of five trials. During the later trials of the long series the subject stated that he felt tired and that it seemed to require a greater effort to move his foot than had been necessary at first.

We can scarcely interpret such data at present. The progressive increase in the length of movements estimated as equal seems almost certainly the result of the frequent repetition of the movement. From the subject's statement it seems probable also that the increase resulted

from some feeling of resistance or of increased effort necessary for the movement, which led to an over compensation. The source of the stimulation leading to this compensation offers an interesting problem. It does not seem probable that with the extensive anesthesia to all other forms of stimulation there should still persist a normal sensitivity to chemical changes in the muscles which give rise to the feeling of fatigue. The alternative seems to be some cortical mechanism by which the

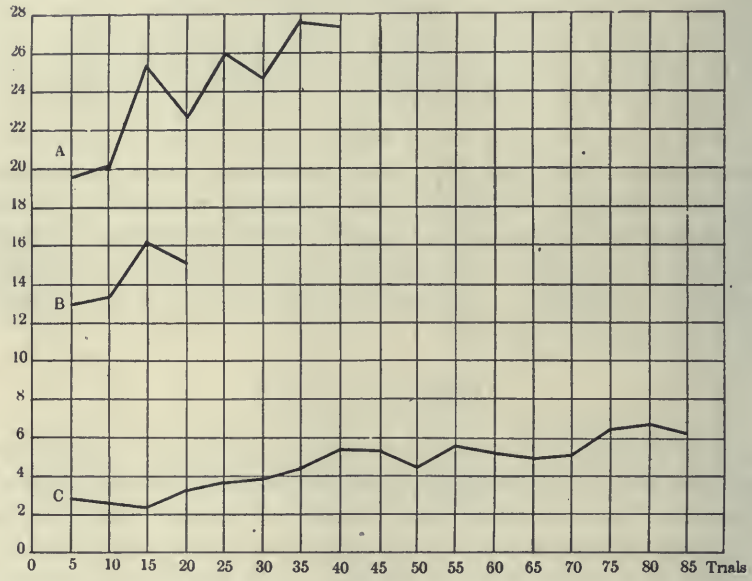

Fig. 5. The effects of fatigue upon the length of movements estimated as equal. The ordinates represent the average length in centimeters of successive movements taken in groups of five.

TABLE 9

The effects of fatigue upon the length of movements intended to be of equal extent. The subject pulled against a spring which exerted a resistance of 100 grams per 4.5 cm. extension

LENGTH OF MOVEMENT ATTEMPTED	AVERAGE EXTENT OF MOVEMENT		INCREASE	TOTAL NUMBER OF TRIALS
	First five trials	Last five trials		
inches	*cm.*	*cm.*	*per cent*	
1	2.9	6.4	120	85
2	13.0	15.1	16	20
3	19.6	27.4	39	40

increase in the threshold of excitability of the motor cells resulting from fatigue directly modifies the behavior of other action systems besides the one which is immediately involved in the movement.

THE INTERACTION OF DIFFERENT MUSCLES IN THE CONTROL OF MOVEMENT

Owing to the lack of adequate means for determining the degree of tension of the muscles of the subject's thigh, it was not possible to determine the relative functions of the flexors and extensors in controlling movement, but a few crude observations indicate that much of the normal complex interplay of the muscles is retained. In quick flexing movements a preliminary contraction of the quadriceps extensor is detectable although the inertia of the subject's foot prevents the appearance of a form of reaction movement similar to that first described by Smith (5) for finger movements. There is also, seemingly, an increase in the tension of the quadriceps as the limit of movement is approached.

When the subject is asked to contract both flexors and extensors, to "make his leg tense," an apparent fluctuation in the intensity of innervation results in oscillations of the foot, yet a given degree of extension is maintained much longer than when he is merely asked to hold his leg extended as in the experiment described on page 174. In the latter case only the extensors are in active contraction so it seems that the simultaneous excitation of both flexors and extensors permits of a steadier and longer motor discharge than is possible when only one set of muscles is innervated.

The complete loss of the tendon reflexes and the great reduction in the tone of the muscles makes a reciprocal innervation of the antagonistic muscles improbable and suggests that the interaction of antagonistic muscles in the control of movement is regulated by some part of the nervous mechanism cephalad to the spinal segment from which the muscles are innervated.

THE INFLUENCE OF TRAINING

The final question arises as to whether the condition of control of movement in this subject is comparable to that of a normal individual or whether some mechanism of control has been developed by practice which was not functional at the time of the lesion. The present observations were made five years after the spinal injury but the subject's

history scarcely supports the view that his control of movement has been reacquired by practice during this time. For the first year after injury he practiced daily walking with crutches for two hours but he made so little progress that he became discouraged and gave up all attempts to recover the lost functions, spending his time either in bed or in a wheel-chair. Except for this relatively brief practice, which aimed only at a visual control of movement, there is no history of any activity which could develop an accurate control of short, slow movements.

DISCUSSION

Every adaptive movement seems to involve three physiologically distinct processes when we attempt to analyze its neurological mechanism. These are, 1, the initiation of motor excitation resulting in muscular contraction; 2, its continuation by a series of disturbances propagated either in the central nervous system, or reflexly as a result of the motor discharge; 3, the cessation of excitation of the protagonists and excitation of the antagonists. The first of these has much in common with the simple reflex twitch and is not of moment in the problem of the control of accuracy of movement unless in some way the extent of movement may be determined by the initial excitation of the motor pathway. The continuation of the movement implies the production of a series of tetanic contractions arising from successive nerve impulses which have been excited by a single momentary stimulus. The duration of the tetanus varies with the extent of the contraction. Curiously enough, no more than vague suggestions have been advanced to account for this change from brief to long-continued excitation. The demonstration by Forbes and Gregg (2) that a single strong stimulus may induce the propagation of two or more waves of disturbance in the nerve may furnish the clue to the continuation of movement, but the increase in duration of excitation which they have shown is very slight and seems inadequate to account for movements continued for several seconds. Upon this the present work gives no data except the probable elimination of circular reflexes by which a contracting muscle might stimulate its own contraction; a possibility perhaps adequately disproved already by operative experiments.

The cessation of movement is no more explicable today than its continuation. To be useful, a movement must end with the attainment of a result which is specific for a given stimulus; it must reach a determined distance, exert a determined force, etc. In many cases the stim-

ulus to cessation or inhibition of movement evidently comes from exteroceptors and does not directly involve the receptors of the moving organs, but in the experimental duplication of a pattern set by active movement no extero-stimulation is present to determine the cessation of movement and its duration and extent must be determined wholly within the organism, either by excitation of proprioceptors or by processes carried out wholly within the central nervous system. Several alternative hypotheses to account for the cessation of movement in such cases have been formulated in such a way as to make experimental test possible.

The first of these appeals to the local sign, assuming that the extent of movement is determined by a change from one pattern of stimulation in the moving organ to another. It seems to demand a vast if not infinite series of specific reactions to different patterns of stimulation. (Ladd and Woodworth, (3) 408.)

A second assumes that the extent of movement is determined by the amount of excitation coming from the moving organ, the amount varying with the extent of movement. The hypothesis seems to demand the assumption of a priming or preliminary integration of efferent neurones before the initiation of movement.

Third, there may be purely intracortical control by some spreading of excitation, of whose nature we can form no concept at present.

Some evidence bearing upon the rôle of excitations from the moving organ in the control of accuracy has been obtained by other experimenters. Lack of space prevents any extensive summary of the literature and the thorough review of Woodworth (7) makes this unnecessary. Indications of the relative independence of motor discharge from direct control by circular reflexes come chiefly from three sources. (1) The discovery by Bowditch and Southard (1) that the reproduction by movement of a pattern distance set by visual stimulation is more accurate than that of one set by kinesthetic gives evidence of the importance of the preliminary set for accurate movement, although it throws no light upon the mechanism of the preparation for movement. (2) The observations of Loeb (4) upon the inequality of simultaneous movements of the two arms indicate that the movements of the two have a common source of control, the action of which is relatively independent of the extent of movement. (3) In his study of automatic movements Woodworth (6) found that the accuracy of movement increased in direct ratio to the speed. From this he concluded that such movements are controlled by the initial set.

The significance of the results of the present study depends upon the validity of the evidence obtained for anesthesia to slow movements. The various tests recorded have shown that: (1) The subject is unable to determine the position of his leg (except occasionally when the knee is hyperextended); (2) he can not detect the extent, duration, direction, or even the presence or absence of passive movements of the knee, if such movements are made at a rate of less than 20 cm. per second; (3) he is unable to detect the movements and changes in degree of active contraction of the muscles of the thigh occurring during his attempts to hold the knee in a given state of contraction against the pull of gravity; (4) he is unable to determine whether or not his attempts to bend his knee have resulted in movement when various amounts of resistance are opposed to the movement. In the face of this evidence we can scarcely hold that he retains a sufficient sensitivity to movement to make possible a reflex control of the extent of movement or a distinction between the extent of actual movement and the intended extent, based upon any stimulation of the receptors of the leg. We are rather forced to the conclusion that the phenomena observed are independent of afferent excitation from the moving organ.

The experiments have shown that the subject is able to control the extent of his movements with almost normal accuracy, to vary the speed and extent of movement independently, and to make rhythmic alterations of flexion and extension. The evidence for anesthesia makes it necessary to assume that all these activities may be carried out in the absence of excitation from the moving organ. The mechanism of control must be sought either in the central nervous system or in some other body segment. Data on the accuracy of movement at different rates show that in the present case its extent is not determined solely by its duration. This makes it necessary to assume some regulation of the intensity of motor discharge which is independent of its duration. Is this determined immediately by the incoming stimulus to movement resulting in a "set"[2] by which a given intensity of motor excitation is aroused explosively without further possibility of control, or is there such a spreading of the motor impulse that some control of its intensity is possible during the discharge? A certain amount of evidence bearing upon this question has been obtained. It

[2] It would be profitless to discuss here the nature or existence of attitude or set. Whether it be a priming of reflex pathways, the assumption of an altered muscular tonus, or what not, it seems distinguishable from the condition in which control of movement occurs after the initiation of the motor impulse.

comes from three sources. First, very slow movements were not made by the subject as a steady contraction of the muscles but by a series of impulses following each other at intervals of one-tenth second or more. Second, the subject was able to detect the excessively long movements of a series and to state that they were longer than he had intended to make them Third, he complained of fatigue at the same time that he showed objective signs of some disturbance in the normal conditions of movement, while chemical sensitivity to fatigue products in the muscles of the anesthetic leg seems improbable. These points are not at all firmly established by the data at hand but all indicate that there is a spreading of the motor excitation which plays a part in the control of movement and may perhaps lead to some phenomenon such as that described as the feeling of innervation. The hypothetical explanation of such a condition which is most open to experimental test is that assuming a spread of the motor impulse to other action systems with reflex control from them. It may, perhaps, be tested by a study of the possibility of controlled movements within intervals less than the minimum cortical reaction time. Experiments to this end are now in progress. As evidence for the importance of the initial set, on the contrary, there is the fact, emphasized earlier by Woodworth (6) for automatic movements, that the accuracy of movement increases directly with the rate. The evidence at hand is not adequate to rule out either alternative.

SUMMARY

Active movements of the left knee were studied in a subject having a complete anesthesia to movements of this joint. Evidence bearing upon the nature of the control of movement in such a condition suggests the following conclusions.

1. Accurate movement of a single joint is possible in the absence of all excitation from the moving organs. The interaction of the various muscles concerned in the movement is not obviously different from that found in normal subjects.

2. In contrast to reflexly controlled movements, the accuracy of movements under such conditions is in direct ratio to their rate, that is, within the limit tested, the quicker the movement the more accurately it is made.

3. The control of accuracy of the movements is relatively independent of their duration; movements of different length do not result from a uniform excitation continued for varying time intervals but from variations in the intensity of motor discharge.

4. It is probable that a control of the intensity of motor discharge after its initiation is possible in the absence of excitation from the organs activated.

5. The normal phenomena of fatigue occur when it is highly probable that the chemical sensitivity of the fatigued muscles is reduced.

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THE CARDIO-SKELETAL QUOTIENT

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It has long been known that the strength of faradic stimulus necessary to provoke response in heart tissue is greater than that required to elicit activity in skeletal muscle. The exact relationship existing between these two tissues with respect to strength of stimulus necessary to arouse contraction has not hitherto been definitely established. Doubtless this has been due to unreliable means of measuring faradic stimuli. In the investigation of another problem in this laboratory it became necessary to make a preliminary study of the relationship existing between the threshold of the frog's ventricle and the threshold of its gastrocnemius muscle.

In this investigation the thresholds were determined by means of Martin's method of quantitative faradic stimulation (1). β units were determined in all cases. Particular care was used in selecting healthy frogs for the experiments. Each frog was pithed and weighed. The heart was exposed by a wide free incision into the anterior body wall. The pericardium was slit throughout its length and the frenum was cut. A looped silk ligature was next thrown around the heart. Posteriorly it was in contact with the white crescent, anteriorly it was tied so that the aortae were included in it. In some preliminary experiments it was found that the ordinary Stannius ligature usually stopped the heart but that it would not remain stopped long enough to determine the threshold, or would start beating the moment the threshold had been reached. If in tying the ligature the aortae were included, the heart remained quiescent for a time sufficient for the determination of the threshold. The electrodes used were the ordinary platinum electrodes of the Harvard Apparatus Company. The points were sharpened and adjusted 2 mm. apart. After the heart ligature was tied the frog was placed under the recording apparatus. The tip of the ventricle was caught in a clip which was connected with a light heart lever writing on a slowly revolving smoked drum. Then the electrodes were thrust

directly into the ventricle about 2 mm. from the auriculo-ventricular groove. The stimulation caused by placing the electrodes was usually followed by several rhythmical contractions of the ventricle. It was noted that in the first few minutes after placing the electrodes the threshold varied considerably, but that after about fifteen minutes the response became quite uniform and remained so for an hour and frequently longer. As soon as the heart showed uniformity of response the threshold was quickly determined. The usual routine was followed of finding first the threshold with no added resistance in the secondary circuit and then finding the thresholds with 10,000, 20,000 and 30,000 ohms in the secondary circuit. The resistance of the tissue was then measured by the Kolrausch method. The heart was not moistened during the experiment since it was noted that the irritability apparently shifted rapidly with each application of solution. When necessary to prevent rapid drying, a moistened filter paper was suspended so as to surround the heart without touching it and thereby serve as a moist chamber. Usually the experiment was completed within three-fourths of an hour and during this time the response was quite constant. One heart which was not moistened after it was connected with the recording apparatus maintained a uniform irritability over a period of two hours. The experiments were all performed at a temperature of 20°C. After the heart thresholds were determined, a slit was made in the skin over the gastrocnemius muscle and the same electrodes that were used in the heart were thrust directly into the middle of the belly of the muscle. A line joining the two points of the electrodes would be at right angles to the long axis of the muscle. By this method it was found that the muscle became uniform in its response in about the same time that the heart required to become uniform in response. The least visible response of the muscle was taken as the indication of the threshold. In some experiments in which the thresholds were recorded on a smoked drum it was found to correspond closely with the visible contraction observed in the intact muscle. Following the determination of thresholds with varying resistances in the secondary circuit, the tissue resistance was measured by the method previously mentioned.

Table 1 shows the results obtained. The average for the threshold of the ventricle is seen to be 191.0 Z units. The average of the β units is 98.3. In each instance the ratio of β to Z was determined. The average is 0.50. The average threshold for the gastrocnemius muscle is 33.3 Z units, 16.1 β units. It is of interest to note that the threshold

of the gastrocnemius in this series is greater than that reported by Martin in his series of eighteen experiments (2). This is due to the fact that the experiments were not performed under comparable conditions. Martin has already called particular attention (3) to the im-

TABLE 1

Showing thresholds of heart and gastrocnemius muscle and their relationship as expressed by the cardio-skeletal quotient

EXPERIMENT NO.	HEART			GASTROCNEMIUS			CARDIO-SKELETAL QUOTIENT	
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$	Z	β
4	110.0	50.6	0.46					
10	126.8	63.6	0.50	30.0	12.4	0.41	0.23	0.19
27	134.5	54.6	0.40	12.1	4.9	0.40	0.08	0.08
12	138.2	46.5	0.33	40.2	15.2	0.37	0.29	0.32
24	138.2	81.4	0.58	29.2	17.1	0.58	0.21	0.21
26	142.1	90.5	0.63	26.1	16.3	0.62	0.18	0.18
25	147.1	70.3	0.47	26.4	13.0	0.49	0.18	0.18
28	147.1	85.5	0.58	30.0	17.1	0.57	0.20	0.20
13	151.8	57.0	0.37	40.2	12.4	0.30	0.26	0.21
8	151.8	72.3	0.47	45.0	20.9	0.46	0.29	0.28
2	160.1	85.8	0.53					
6	161.1	69.0	0.42	26.1	10.1	0.38	0.16	0.14
30	166.5	99.0	0.59					
5	187.2	112.2	0.59	32.2	14.9	0.46	0.17	0.13
7	188.0	93.0	0.49	13.5	7.0	0.52	0.07	0.07
17	195.5	69.0	0.35	32.4	11.4	0.35	0.16	0.16
1	198.7	98.0	0.49					
15	212.5	111.9	0.52	22.4	12.4	0.55	0.10	0.11
22	220.5	102.7	0.46					
3	222.0	125.2	0.55	23.0	12.2	0.53	0.10	0.10
9	246.8	110.9	0.44	67.0	34.1	0.50	0.27	0.30
18	265.9	139.8	0.52					
20	276.5	198.5	0.71	61.8	38.1	0.61	0.22	0.19
29	332.8	172.5	0.51	30.4	16.4	0.53	0.09	0.09
14	355.5	197.9	0.55	45.8	21.5	0.46	0.12	0.10
Average	191.0	98.3	0.50	33.3	16.1	0.48	0.17	0.17

portance of using similar conditions if there is a desire to duplicate the experiments of another observer. In this series the electrodes were 2 mm. apart and placed in the middle of the muscle. This was done in order that they might be used in the heart and thus provide uni-

formity in the application of the stimulus to both tissues. In Martin's series an electrode was thrust into each end of the muscle, the kathode being nearest the origin. Martin used isolated muscles, in this series the muscle was left in situ. The average β unit in this series is 16.1, in Martin's series it is 8.1. Some experiments which will be discussed later were arranged in which the technique used by Martin was employed. The average β unit was then found to be 9.0.

Attention is called to the ratio of the gastrocnemius β to Z. It is 0.48. This is practically the same as Martin found in his series of eighteen experiments upon the same muscle. Martin found the ratio of β to Z to be 0.49, and the average variation from this ratio was 15 per cent. In this series the average variation is 15.6 per cent. In the heart series the ratio of β to Z is 0.50. The average variation from this is 14 per cent. The cardiac ratio $\frac{\beta}{Z}$ and the skeletal muscle ratio $\frac{\beta}{Z}$ are evidently of the same order of magnitude and from the evidence may be considered identical.

In order to determine the relationship existing between the heart Z units (HZ), and the gastrocnemius Z units (GZ), also the heart β units (Hβ) and the gastrocnemius β units (Gβ), the ratio of GZ to HZ and the ratio of Gβ to Hβ were determined in each instance. The average of the ratio $\frac{GZ}{HZ}$ was found to be the same as the ratio $\frac{G\beta}{H\beta}$. It is 0.17. In the individual experiments it is noted that the ratios were usually equal or nearly so. This equality in the ratios GZ: HZ and Gβ: Hβ was so constant that it was thought to be indicative of accuracy in the determination of the thresholds. It seemed desirable to use some expression to signify the relationship existing between the threshold of the heart and the threshold of the gastrocnemius so the term Cardio-skeletal Quotient is suggested for that purpose. It will be used throughout this discussion to signify the ratio of the threshold of the gastrocnemius muscle to the threshold of the ventricle. Thus there is a cardio-skeletal quotient for the Z units, indicated by $\frac{GZ}{HZ}$, and a cardio-skeletal quotient for β units, indicated by $\frac{G\beta}{H\beta}$. Theoretically $\frac{GZ}{HZ}$ should equal $\frac{G\beta}{H\beta}$ and in the present series this was found to be true. The average quotient in each case is 0.17.

The cardio-skeletal quotient may serve as a means of making corrections where obviously only one determination is in error out of the four usually made. Thus if the cardio-skeletal Z quotient is 0.21, then in accordance with the evidence obtained in this series the cardio-skeletal β quotient should also equal 0.21. If in making the determinations it is found that the cardio-skeletal β quotient is widely divergent from the cardio-skeletal Z quotient, it is possible by referring to the averages of the series to determine whether the error is in the Z units or in the β units. If the error is in the β units it may be ascertained, by reference to the averages in the series, whether the error is in the heart β units or the gastrocnemius β units. Finding that the error is in the heart β units the equation $\frac{GZ}{HZ} = \frac{G\beta}{H\beta}$ may be used to determine the real value of $H\beta$. An example will serve to make the point clear. In experiment 24 in table 1, the $H\beta$ was determined by the above equation. As originally determined the units and ratios were as follows: HZ 138.2, $H\beta$ 33.0, $\frac{\beta}{Z}$ 0.23; GZ 29.2, $G\beta$ 17.1, $\frac{\beta}{Z}$ 0.58; $\frac{GZ}{HZ}$ 0.21, $\frac{G\beta}{H\beta}$ 0.51. Referring in the table to the average cardio-skeletal Z quotient and the average cardio-skeletal β quotient it will be seen that in this experiment the cardio-skeletal Z quotient is approximately that magnitude which is normally present. The cardio-skeletal β quotient however is plainly an error. Apparently there was a mistake in calculating the β units. The $G\beta$ is 17.1. Reference again to the averages of the series reveals a close correspondence in the figures. The gastrocnemius ratio $\frac{\beta}{Z}$ is seen to be 0.58. This while differing from the average by about 20 per cent may be considered within the range of error. Inspection of the heart β units and the heart ratio $\frac{\beta}{Z}$ reveals the source of the error. The heart β is 33.0. This is a variation from the average as shown by the series of 66 per cent. The heart ratio $\frac{\beta}{Z}$ is 0.23. This is 54 per cent divergent from the average as shown by the series. Obviously therefore the error is in the β units of the heart. By use of the equation $\frac{GZ}{HZ} = \frac{G\beta}{X}$, in which X is the $H\beta$ sought, it is found that the $H\beta$ is 81.4. This correction necessarily implies that the heart ratio of β to Z is always the same as the gastrocnemius ratio of β to Z. That such is the case is indicated by the table. This method of correction applies necessarily to β units.

The evidence presented here indicates that the cardio-skeletal quotient is a fairly constant quantity, the average variation being a little under 30 per cent. This apparent constancy of the cardio-skeletal quotient suggested the possibility of its utilization as a means of studying the effects of various agents upon the heart, using as an index of the effect an alteration in the average cardio-skeletal quotient. In order to test the validity of the quotient, it was decided to make some practical applications of it. Use was made of the gastrocnemius experiments in table 1. Taking each experiment the gastrocnemius Z units and β units were divided by the cardio-skeletal quotient 0.17. The results were taken to represent respectively the heart Z units and the heart β units. The following averages are the results: Z units 196.1, β units 95.1, $\frac{\beta}{Z}$ 0.48. Reference to the actual determinations in table 1 shows the very close agreement of the averages. Individual experiments revealed in many instances wide variations from the actual determinations. This method clearly would not apply to one experiment but to a series.

Another test of the validity of the quotient was made. In table 1 it will be noted that there are six experiments in which the units for the gastrocnemius were not determined or were discarded because of faulty technique. In this case the corresponding heart units were multiplied by the cardio-skeletal quotient 0.17 to determine the theoretical values of the gastrocnemius muscles. Table 2 shows the results obtained. Comparison with table 1 shows again a very close agreement between the

TABLE 2

Theoretical determinations of gastrocnemius thresholds (omitted from table 1) from the corresponding heart thresholds by means of the cardio-skeletal quotient 0.17

EXPERIMENT NO.	HEART			GASTROCNEMIUS		
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$
4	110.0	50.6	0.46	18.7	8.6	0.46
2	160.1	85.8	0.53	27.2	14.5	0.53
30	166.5	99.0	0.59	28.3	16.8	0.59
1	198.7	98.0	0.49	33.7	16.6	0.46
22	220.5	102.7	0.46	37.4	17.4	0.46
18	265.9	139.8	0.52	45.2	23.7	0.52
Average.....	186.9	95.9	0.50	31.8	16.2	0.50

average theoretical determinations in these six experiments and the average actual determinations in nineteen experiments of the series.

Evidently the cardio-skeletal quotient is a reliable guide for the determination of threshold units where the conditions of experimental procedure are comparable. In case the methods are not uniform they may be reduced to uniformity in some instances and the cardio-skeletal quotient still be applied. Thus in the series of eighteen experiments upon the frog's gastrocnemius reported by Martin the average Z unit and β unit were 50 per cent lower than the same units reported in the present series. But attention has already been called to the different technique used by Martin. In his series the average Z units was 17.1, the average β units was 8.1, the average $\frac{\beta}{Z}$ 0.49. In the technique employed in this series it required approximately six times the strength of stimulus to make the heart contract that it required to make the gastrocnemius contract. Using the technique employed by Martin it would require twelve times the strength of stimulus to make the heart contract that it required to make the gastrocnemius contract. Since by the latter method results are obtained that are 50 per cent lower than the same units obtained in the present series there would also be a lowering of the cardio-skeletal quotient by 50 per cent. Difference in the manner of application of the stimulus led to a corresponding diminution in the strength of stimulus necessary to arouse the activity of the tissue. Accordingly it was assumed that the cardio-skeletal quotient in Martin's series was 50 per cent less than the same quotient in the present series or 0.085. By use of this quotient theoretical determinations of the heart units in Martin's series of gastrocnemius thresholds were made. Thus each gastrocnemius Z unit and β unit was divided by the assumed cardio-skeletal quotient 0.085 and the results taken to represent respectively the heart Z unit and β unit. The results were as follows: average Z unit 195.0, average β unit 95.3, average $\frac{\beta}{Z}$ 0.49. Comparison with the general averages in table 1 shows a striking closeness of the results.

In order to determine if the method of obtaining thresholds in the present series accounted for the difference in the same values as revealed by Martin's series, a few experiments were arranged in which the technique was the same as Martin's. The results are shown in table 3. That the assumption in regard to lowering of the cardio-skeletal quotient because of difference in technique is tenable, is clearly shown by

the results. The heart units are of the same magnitude as those of the present series, but the gastrocnemius units are of the same order of magnitude that Martin found. The ratio $\frac{\beta}{Z}$ is somewhat higher than those of the series. This could not be accounted for except that the frogs had just begun dying rapidly and some unusual disturbance may have been present which slightly raised the ratio of β to Z. The most interesting fact is the close agreement of the determined cardio-skeletal quotient with the assumed one for Martin's experiments: 0.085 and 0.09. In table 4 is presented a summary of the results obtained where actual determinations were made, also where theoretical determinations were computed.

TABLE 3

Showing thresholds of heart and gastrocnemius muscle using Martin's technique for the gastrocnemius determinations. Relationship expressed by the cardio-skeletal quotient

EXPERIMENT	HEART			GASTROCNEMIUS			CARDIO-SKELETAL QUOTIENT	
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$	Z	β
A	147.0	71.6	0.48	14.9	8.3	0.56	0.10	0.11
C	152.7	83.9	0.58	16.7	9.5	0.57	0.10	0.11
B	164.5	80.5	0.48	15.7	8.6	0.54	0.09	0.10
E	191.3	99.1	0.51	17.5	9.4	0.53	0.09	0.09
G	197.8	113.7	0.51	18.0	9.6	0.53	0.09	0.08
D	204.2	116.4	0.56	16.9	9.0	0.53	0.08	0.08
F	206.4	117.7	0.58	17.7	9.2	0.51	0.08	0.07
Average.	180.5	97.5	0.53	16.7	9.0	0.53	0.09	0.09

The curious effect of removing the heart entirely from the body was shown in one experiment. It was found impossible to stop the heart by means of the ligature so it was isolated by cutting through the auricles. It stopped promptly. Upon stimulating it was found to require 2496 Z units to obtain a response. The resistance of the ventricular tissue included between the stimulating electrodes and the gastrocnemius tissue included in the same area was the same. The average of each was 1500 ohms.

The evidence presented in this series of experiments seems to justify the conclusion that the cardio-skeletal quotient is a body constant. Also this quotient may be utilized in studying the effects of various

TABLE 4

Summary of average results obtained by actual and theoretical determinations

PROCEDURE	HEART				GASTROCNEMIUS				CARDIO-SKELETAL QUOTIENT		
	No. averaged	Z	β	$\frac{\beta}{Z}$	No. averaged	Z	β	$\frac{\beta}{Z}$	No. averaged	Z	β
Actual determinations.	25	191.0	98.3	0.50	19	33.3	16.1	0.48	19	0.17	0.17
Theoretical determinations of heart units in table 1, by means of the gastrocnemius experiments and the cardio-skeletal quotient.	19	196.1	95.1	0.48							
Theoretical determinations of gastrocnemius thresholds (omitted in Table 1) by means of corresponding heart experiments and the cardio-skeletal quotient.					6	31.8	16.2	0.50			
Theoretical determinations of heart thresholds from Martin's series of gastrocnemius experiments, by means of cardio-skeletal quotient 0.085. Figures in italics are Martin's.	18	195.0	95.3	0.49	18	*17.1*	*8.1*	*0.49*	18	0.085	0.085
Actual determinations using Martin's technique with the gastrocnemius muscle.	7	180.5	97.5	0.53	7	16.7	9.0	0.53	7	0.09	0.09

agents upon the heart, and conversely it may be used to study the effects of various agents on the gastrocnemius muscle. It may also be used as a means of correcting β units when there is a series to obtain averages from.

SUMMARY

1. The threshold stimulus of the frog's ventricle as shown by this series is 191.0 Z units, 98.3 β units, and the ratio $\frac{\beta}{Z}$ is 0.50.

2. The threshold of the frog's gastrocnemius by the method used in this series is 33.3 Z units, 16.1 β units, and the ratio $\frac{\beta}{Z}$ is 0.48.

3. The cardio-skeletal quotient is defined as the ratio of the gastrocnemius threshold to the threshold of the ventricle. By the method employed in this series it is 0.17.

4. Utilization of the cardio-skeletal quotient as a means of studying conditions that affect the heart is indicated.

5. By the method described in this series it is shown that it requires six times as strong a stimulus to make the frog's heart contract as it does to make the frog's gastrocnemius contract.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLI. The Alleged Influence of the Removal of the Salivary Glands on the Secretion of Gastric Juice

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There are many factors involved in the secretion of gastric juice. Besides the reflex or psychic factor, we have the saliva itself, (Pavlov (1)), the secretagogues of the food (Schiff, Pavlov, Bayliss and Starling), and of the pyloric mucosa (Edkins). Tarulli and Pascucci (2), report gastric secretagogues in the spleen. In addition to these Keeton and Koch (3) tested various organs for the presence of secretagogues ("gastrin") and found some positive, while others, such as the submaxillary glands, gave negative results.

The present work was carried out to determine whether or not such a hormone exists in the salivary glands (and consequently affects the secretion of gastric juice by way of the blood), as has been affirmed by some but denied by others.

Since the secretion of saliva is the initial process of the digestive act, one might expect some results on digestion, on health and on the secretion of gastric juice from extirpation of the salivary glands; but in an animal like the dog, which bolts its food, and in which the ptyalin is absent, (Carlson (4)), with the secretion of the sublingual glands strongly alkaline, and with no evidence of an adaptation of the character of the saliva to diet (Garrey (5)), the effect of the removal of the salivary glands on gastric secretion is brought into question.

Hemmeter, working on dogs, reached the conclusion that a hormone is present in the salivary glands, and that the absence of the salivary glands leads to diminished gastric secretion and peptic digestion. He did not discuss the effect of their removal on the acid secretion of the gastric juice. This depressed gastric secretion, he states, can be brought back almost to normal by feeding extracts of the salivary glands. In

his work published in Science (6), he states that he used the Pavlov pouch, while in the conclusions from his work as published in the Transactions of the American Gastro-Enterol. Assoc. (7) and Biochem. Zeitschr. (8), he states that the work was done by use of the simple gastric fistula. He adds that "in some cases there is a secretion after removal of the glands" and concludes that this is due to one of the three following factors:

(a) That the lobules of the parotid are not completely removed; (b) that the psychic secretion was not completely eliminated; (c) a possible abnormal secretion.

In the dog we found that the removal of the parotid is not, after all, such a difficult task, because it is smaller than the submaxillary and because its relation to the latter and its location over the ear always give one a good clue to its location, while the space in which it lies can be increased in size by extending the head. To eliminate the psychic secretion would appear to indicate a diminished and possibly prolonged gastric secretion, and therefore lead to an incorrect interpretation of the results. The possible presence of an abnormal secretion would somehow have to be controlled by the salivary glands.

Hemmeter's conclusions are as follows:

(1) In dogs with simple gastric fistula the extirpation of all of the salivary glands produces a marked diminution in the gastric secretion. This is also evident in the analysis of test meals drawn by the test tube from animals with intact stomachs. It is necessary to prevent psychic secretion in order to bring about the phenomenon described; (2) even in animals with intact vagi it may sometimes happen that the removal of all the salivary glands causes a decided impairment of gastric secretion, so that a causative relation between the loss of the salivary glands and the reduced proteolytic and milk coagulating power of the gastric juice appears certain, even in these cases; (3) in nine salivary dogs in whom the gastric secretion has been decidedly diminished, it is not restored to the normal by the feeding of food that has been well masticated and insalivated by other normal dogs; (4) when the gastric secretion is diminished a temporary restoration may be brought about by intravenous or peritoneal injection of extracts made from the salivary glands of normal dogs; (5) this temporary restoration of gastric secretion takes place even when the stomach is isolated from the central nervous system; (6) the presence of an exciting gastric secretion hormone formed in the salivary glands. Salivary gland extract fed directly with the food or placed into the stomach directly is not capable of exciting gastric secretion. Ground up fresh salivary glands cause approximately the same gastric secretion as an equivalent amount of ground beef in these animals.

Loevenhart and Hooker (9), working on dogs with simple gastric fistulae, tried to determine the presence or absence of a salivary hormone

by feeding extracts of the salivary glands. They assumed that the presence of such a hormone would cause an increased secretion of gastric juice in normal dogs, and since they did not obtain evidence of such increased secretion they concluded that a gastric secretory hormone is not present in the salivary glands.

Hemmeter (10) later took exception to their method and conclusions, emphasizing his findings that extracts of the salivary glands raise a depressed gastric secretion almost to normal, and that their experiments on dogs with normal gastric secretion could not be used to disprove his conclusions.

EXPERIMENTAL PROCEDURE

The method employed is based on the use of the Pavlov pouch, the secretion of which may be considered a true index of the course of the secretion in the main stomach. After allowing seven to ten days for complete recovery from the Pavlov pouch operation, the gastric juice was collected from the pouch for six to eight hours each day. The rate of gastric secretion was determined by measuring the juice secreted at intervals of one hour, beginning one hour before feeding a standard meal of lean meat. In this manner a normal secretion curve was obtained over a period of seven to ten days. For each hour the rate, peptic digestion and acidity were determined and curves plotted. After the determination of the normal secretion curve the three pairs of salivary glands were removed in one operation. This was preferred to two separate operations because we observed that a second anesthesia was prone to induce infection of the respiratory tract (distemper).

After considerable experimentation, the most satisfactory *modus operandi*, and the one finally adopted, consisted in making two incisions, one on each side, extending from the ear to the angle of the mandible, each being about $2\frac{1}{2}$ inches long.

In the dog the three glands on each side approximate each other very closely; the parotid lying over the ear, its medial portion in close relation to the submaxillary; the sublingual consisting of two parts, the aboral portion lying directly on the submaxillary gland and the oral portion further up along the duct of the submaxillary in the sublingual triangle.

The manner of collecting the gastric juice and determining the rate consisted in placing a perforated rubber tube in the pouch and collecting the secretion in a container somewhat similar to the one sketched by Keeton (11) for use on cats.

TABLE 1

Summary of observations on the gastric juice of two dogs before and after extirpation of the salivary glands. The averages are made up from 20 observations of each dog, 10 before and 10 after removal of the salivary glands

	DOG	BEFORE			AFTER		
		High	Low	Average	High	Low	Average
Rate of secretion in cubic centimeters.....	1	15.75	7.25	**10.33**	18.50	7.00	**10.00**
	2	26.25	11.50	**17.39**	23.50	12.25	**19.33**
Total acidity in per cent	1	0.0931	0.0639	**0.0761**	0.2263	0.0712	**0.1204**
	2	0.2327	0.0626	**0.1325**	0.2934	0.1384	**0.2381**
Free acidity in per cent	1	none	none	**none**	0.1733	none	**0.0536**
	2	0.1900	none	**0.0718**	0.2489	0.0791	**0.1819**
Pepsin concentration in millimeters (Mett).	1	18.25	14.75	**13.99**	20.75	11.50	**13.96**
	2	18.75	9.75	**13.61**	25.50	14.75	**17.21**

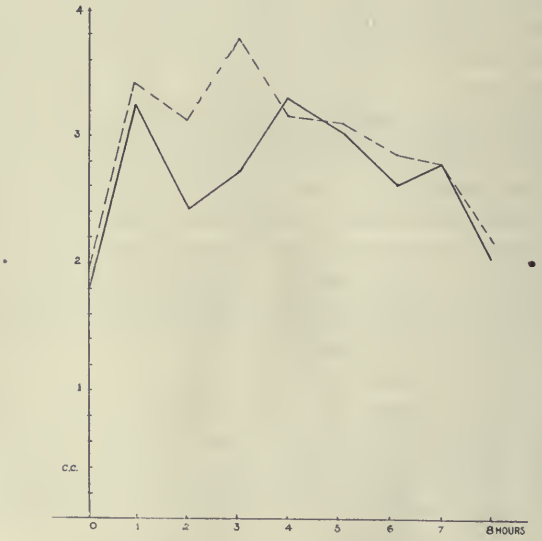

Fig. 1. Represents the rate of gastric secretion on a standard meal of meat as determined by 20 observations on a dog with a Pavlov stomach, the continuous line indicating the average of 10 observations before removal of the salivary glands, while the broken line indicates the average of 10 observations after removal of the salivary glands.

The peptic digestion for a period of twenty-four hours was determined according to Mett as modified by Cobb (12).

The free acidity was calculated by titrating 1 cc. of the gastric juice diluted with 20 cc. of distilled water with N/40th NaOH using dimethylamidoazobenzol as an indicator for the free acidity and phenolphthalein as an indicator for the total acidity.

Two vigorous dogs survived all the operative procedures, their wounds healed perfectly and their health did not seem to be at all impaired.

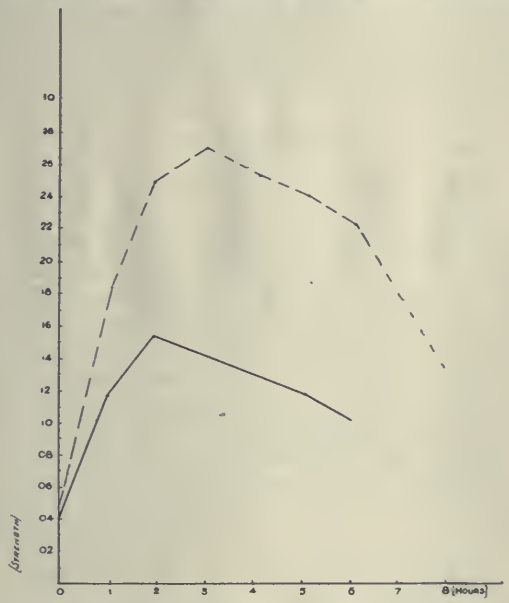

Fig. 2. Dog. 2. Represents the total acidity of the gastric juice as obtained by the average of 20 experiments. The continuous line indicates the total acidity as determined by 10 observations before the removal of the salivary glands. The broken line indicates the total acidity for a series of 10 observations after the removal of the salivary glands.

Their mouths did not appear as dry as would be expected after loss of all the salivary glands, which is probably due to the numerous mucous glands present in the oral cavity. The dogs soon learned to swallow their food and their taste did not appear to be altered.

RESULTS

The rate or quantity of secretion of gastric juice in both dogs was not altered by complete removal of the salivary glands (fig. 1).

The acidity of the gastric juice shows a greater variation, there being a decided increase after the removal of the salivary glands (figs. 2 and 3). In dog 1 before removal of the glands there was at no time any free acidity, while out of thirteen days following there was only one day in which free acid was absent. In dog 2 free acid was present both before and after removal of the glands, but the free acidity after removal of the salivary glands showed a marked increase. The maximum total acidity in both dogs occurred, on the average, half an hour to one hour

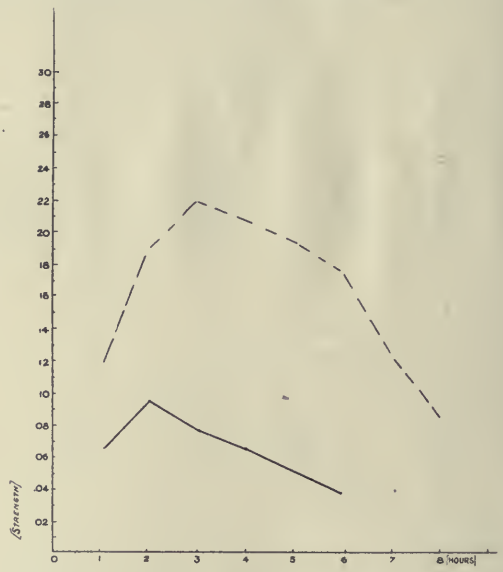

Fig. 3. Dog. 2. Represents the free acidity of the gastric juice as obtained by the average of 20 experiments. The continuous line indicates the free acidity as determined by 10 observations before the removal of the salivary glands. The broken line indicates the free acidity for 10 observations after the removal of the salivary glands.

later than in the control periods, and the subsequent fall in acidity was more gradual.

The peptic digestion was about the same both before and after the removal of the glands in dog 1, but in dog 2 there was a slight increase.

CONCLUSIONS

1. Our results contradict the theory of a hormone in the salivary glands stimulating the secretion of gastric juice. Extirpation of the

salivary glands in the dog does not decrease the gastric juice secretion (appetite and secretagogue juice).

2. Extirpation of the salivary glands causes a distinct rise in the acidity of the gastric juice. This increase in acidity is greater than can be accounted for by the slight increase in the rate of secretion. The slight increase in quantity may be due to the absence of the alkaline saliva.

3. After extirpation of the salivary glands, the maximum secretion rate after a meal appears slightly retarded. This may be due to the absence of the water of the saliva, and to decreased psychic secretion, owing to the dryness of the mouth and consequent impaired taste.

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THE COMPOSITION OF SALIVA IN RELATION TO THE INCIDENCE OF DENTAL CARIES[1]

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INTRODUCTION

It has been previously reported by the writer (4) that the ratio of the neutralizing powers, or the power to maintain neutrality, of normal resting saliva and of the activated saliva, obtained by the chewing of paraffine, bears a definite relationship to the incidence of dental caries. In persons with carious teeth this ratio, expressed as a percentage, exceeds 80 while in persons whose teeth are temporarily immune from (or, more correctly, resistant to) caries the ratio falls below 80. In other words, as the difference between normal resting saliva and activated diminishes so does the liability to the incidence of caries increase. Shepard and Gies, in discussing this relationship or "salivary factor" have maintained (5) that it is inconstant. They based their conclusions, however, upon data which included all the different types of stimuli indiscriminately without regard to the nervous impulses and reflexes produced by the sense of taste. In their experiments they used paraffine, sucrose, sodium chlorid, alcohol and certain combinations of these. It was subsequently stated by the writer (8) that comparisons of saliva, the samples of which have been collected under different conditions, are inadmissible since such procedure ignores entirely psychic influences. Recalculation of their figures confirms the findings originally reported (4).

In a second communication (6) the writer presented data which both substantiated and developed the above thesis. Reports were made of investigations conducted in some of the state institutions for the in-

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sane, and consisted of analyses of saliva from certain cases of dementia praecox and epilepsy. This work was likewise criticised by Gies (7) and answered in turn by the writer (9). No data have been presented which disprove any of the conclusions drawn in either paper (4 and 6) and it is in the further development of the consequences arising out of these conclusions that the following experiments were undertaken.

There are two main questions which fall under consideration in this connection, namely, first, the origin of the change of the neutralizing power of saliva which occurs in response to certain stimuli, and second, the significance of this change in relation to the incidence of dental caries. In other words, whether the alteration of the salivary factor is a contributing cause of dental caries, or conversely, an effect produced by dental caries, and lastly, whether there is a cause common to both altered factor and dental caries in which case the factor would become merely an incidental symptom.

In connection with the first of these problems, I have sought to throw light on the origin of the differences between the neutralizing powers of different samples of saliva: (a) By dialysis experiments in which the attempt has been made to determine the relative magnitudes of the parts played by the inorganic, or at least the diffusible substances, and the non-diffusible, and presumably organic, constituents of saliva. (b) By the determination of the amino nitrogen content of various samples of saliva after hydrolysis with a view to estimating more exactly the part played by protein in contributing to the difference in properties and composition between normal resting and activated salivas. In connection with the second problem I have sought to extend the observations of Pickerill upon the relationship of diet and habit to the incidence of dental caries and furthermore to determine the influence of the locality of the stimulus upon the neutralizing power of the secretion which is evoked.

Part 1

The origin of the differences in neutralizing powers displayed by normal resting and paraffine activated saliva

a. The relative magnitude of the parts played by diffusible and non-diffusible substances in determining the neutralizing power of saliva

The experiments were carried out as follows: Saliva was titrated and a second set of samples was dialyzed for a period of time; then the liquids both outside and inside the membrane were separately titrated.

The dialysis of the samples was made in these cases where a sufficient quantity of saliva could be obtained without conscious exertion on the part of the patient. Following the method of Clausen (10) and Porter (11) a solution of gun cotton in an ether alcohol mixture was made. From this solution collodion thimbles were fabricated and then placed in recently boiled distilled water until ready for use. From 2 to 5 cc. of the sample were measured directly into the thimble and then placed in a small prescription vial. Boiled distilled water was pipetted into the bottle until the level of the sample exactly coincided with that of the water. Decomposition of the sample was prevented by adding

TABLE 1

Comparison of undialyzed and dialyzed saliva

NUMBER	RESTING SALIVA								ACTIVATED SALIVA							
	Resting saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Activated saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed
	Cubic centimeters of HCl				Cubic centimeters of NaOH				Cubic centimeters of HCl				Cubic centimeters of NaOH			
E 4	6.60	3.00	2.00	5.00	13.75	9.20	4.20	13.40	24.15	16.00	2.65	18.65	4.75	2.85	1.65	4.50
E 1	9.00	6.20	5.40	11.60	9.50	8.25	0.20	8.45	17.75	14.00	0.95	14.95	5.50	4.40	0.69	5.09
E 6	19.50	12.30	1.28	13.58	6.50	6.00		6.00	48.30	46.50	3.40	49.90	1.20			
E 7	8.75	6.50	0.00	6.50	9.80	10.70		10.70	25.20	21.40	2.40	23.80	4.55	3.40	1.00	4.40
E 9	10.70	8.10	1.00	9.10	7.30	4.85	0.85	5.70	15.80	14.45	1.15	15.60	3.10	3.00		3.00
E10	14.00	9.25	3.15	12.40	5.00	4.90	0.75	5.65	16.35	13.70	2.00	15.70	4.80	2.10	2.25	4.35
E11	17.45	10.25	7.00	17.25	7.40	3.30	0.90	4.20	19.10	18.30	3.00	21.30	2.90	1.75	1.00	2.75
E12	7.25	4.00	3.15	7.15	11.75	8.10	2.70	10.80	19.90	16.70	2.20	18.90	4.85	3.10	0.95	4.05
E13	8.50	4.85	3.20	8.05	12.40	9.60	3.00	12.60	22.70	20.10	1.50	21.60	5.40	4.00	0.90	4.90
E14	16.40	9.70	5.80	15.50	8.90	4.80	3.90	8.70	35.40	23.10	3.40	26.50	1.70	1.00		1.00

one drop of chloroform and one drop of xylol. The bottle was tightly corked and placed in an air tight cabinet for twenty-four hours, at a temperature between 20° and 24°C. At the end of this time the liquid outside the membrane was titrated separately from that inside the membrane. Theoretically, if there were no loss of CO_2, from the sample, the sum of the two titration figures for either alkalinity or acidity should equal the original titration value. But a slight precipitation of phosphates, which is always evident, demonstrates that some loss of CO_2 has occurred. Data based on twelve, thirty-six and forty-eight hour dialyses showed a wider variation than those based on the twenty-

four hour limit and this later time, consequently, was chosen as a standard.

The results of this work are reported in tables 1 and 2. The analyses, although of questionable quantitative value demonstrate, qualitatively, that the greater percentage of alkalinity and acidity is found in that portion of the sample which has dialyzed through the membrane and is due, therefore, to inorganic constituents. With subject No. E4 the alkalinity of 10 cc. of the resting saliva was 6.60 cc. N/200 HCl. After dialysis the alkalinity outside of the membrane was 3.00 cc. and inside the membrane, 2.00 cc. The activated sample exhibited a

TABLE 2

Dialysable proportion of neutralizing power

NUMBER	CONDITION OF MOUTH	NORMAL RESTING SALIVA			PARAFFINE ACTIVATED SALIVA			
		Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Salivary factor
E 4	Immune	20.35	18.40	90.01	28.90	23.15	80.10	70.41
E 1	Immune	18.50	20.05	108.38	23.25	20.04	86.19	79.57
E 6	Immune	26.00	18.58	75.31	47.10	49.90	105.94	55.20
E 7	Immune	18.45	17.20	93.22	29.75	28.20	94.79	62.02
E 9	Carious	18.00	14.80	82.03	18.90	18.60	98.41	95.23
E10	Carious	19.00	18.05	95.00	20.15	20.05	99.50	94.29
E11	Carious	24.85	21.45	86.11	22.00	24.05	109.32	113.00
E12	Immune	19.00	17.95	94.47	24.75	22.95	92.77	76.76
E13	Immune	20.90	20.65	98.80	28.10	26.50	94.31	74.37
E14	Immune	25.30	23.20	91.70	37.10	27.50	74.12	68.20

marked difference for the titration figure of the undialyzed sample was 24.15 and for the dialyzed, 16.00 outside the membrane and only 2.65 inside the membrane. The acidities likewise show the same phenomena, the undialyzed normal resting saliva having a reading of 13.75 cc. N/200 NaOH and the dialyzed 9.20 ouside the thimble and 4.20 inside.

b. The amino-nitrogen yielded by hydrolysis of normal resting and activated saliva

In the utilization of the Van Slyke apparatus for the determination of the amino-nitrogen in the saliva the author has employed a method

which combines accuracy with simplicity. The wide application which this apparatus has found in blood analysis recommends it favorably to the problem at hand. The procedure outlined in Hawk (14) was followed with a few modifications. The technique of the analytical work was performed by Mr. S. A. Waksman and I take pleasure in acknowledging his service.

It was at first thought best to analyze the samples as soon as they were obtained from the patient but this procedure is open to objection on account of the fact that there is so small an amount of gas evolved in the reaction that accurate readings of the gas volume are exceedingly difficult. Since it has been the custom in the salivary work to secure the material between eight and eleven in the morning it was found inconvenient to make the determinations at the same time. To overcome these objections all the samples were hydrolyzed. Ten cubic centimeters of well mixed saliva were measured into a special digestion tube and 4 cc. of concentrated HCl were added. The tube employed was of soft glass about nine inches long and one inch wide. One end was sealed off and the other drawn out until the diameter was reduced to nearly $\frac{1}{4}$ inch. After the introduction of the sample and the addition of the acid the small end was sealed. These tubes closed at both ends were placed in a water bath and digested at 100°C. for four hours. Attempts to digest the samples by boiling over a flame and using a reflux condenser were unsatisfactory as bumping and loss of the material could not be controlled.

Having prepared the Van Slyke micro-apparatus in the usual manner 2 cc. of the well mixed hydrolyzed sample were carefully transferred to the measuring tube and run into the decomposing bulb. The bulb was shaken for ten minutes and the NO absorbed by the permanganate solution. The volume of nitrogen was read and the room temperature and barometer noted. Duplicate determinations were made and, in many instances, triplicates.

The results of the analyses are reported in tables 3 and 4 and represent the cubic centimeters of amino nitrogen at standard pressure and temperature yielded by 10 cc. of sample. This calculation was made so that the data would be comparable to those of the titration experiments. Inspection of the table shows that, in the greater percentage of those cases which were classed as immune from dental caries, the nitrogen content of the normal resting saliva is appreciably higher than that of the paraffine activated saliva. Those samples, however, taken

from the mouths in which caries existed did not exhibit the same difference. These facts substantiate the work done on the dialysis experiments. For it was shown that the increase of total neutralizing power in paraffine saliva from immune cases was due to a larger amount of inorganic constituents. These later tests demonstrate that a smaller percentage of organic bodies, represented by the amino nitrogen values, is contained in this same type of saliva and that therefore the difference in titration equivalents must be due to inorganic material.

TABLE 3

Caries

DATE	SERIAL NUMBER	NORMAL RESTING, CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE
September 18, 1916	G 2	2.20	3.00	
September 21, 1916	G 2	2.50	3.00	
September 25, 1916	G 8	3.70	3.80	
October 27, 1916	G28	3.70	3.60	
October 27, 1916	G29	3.70	3.60	
October 27, 1916	G31	4.30	4.30	
November 2, 1916	G36	4.10	3.60	
November 2, 1916	G37	4.20	3.80	
September 25, 1916	G 9	3.10	3.30	
October 5, 1916	G 9	3.00	3.00	
October 30, 1916	G 9	3.40	2.80	
September 29, 1916	G10	3.30	3.40	3.80
November 8, 1916	G10	4.15	4.27	
November 10, 1916	G10	3.77	3.63	
November 10, 1916	G18	2.80	2.95	
November 7, 1916	G18	3.10	3.20	
November 9, 1916	G18	4.10	4.00	
November 4, 1916	G38	3.90	3.80	
November 5, 1916	G42	4.20	4.10	
November 8, 1916	G44	4.40	4.50	
November 15, 1916	G 9	4.27	3.70	
November 20, 1916	G 9	5.80	3.57	
November 23, 1916	G10	4.02	4.02	
November 2, 1916	G35	4.10	4.00	
November 4, 1916	G43	5.90	4.87	3.90
November 21, 1916	G52	4.48	4.84	
November 21, 1916	G53	3.80	4.10	
November 23, 1916	G58	3.12	3.39	

TABLE 4

Immunity

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
September 16, 1916.....	G 1	2.40	2.30	2.20
September 19, 1916.....	G 1	3.20	2.90	
September 19, 1916.....	G 3	4.60	3.80	3.50
September 26, 1916.....	G 3	5.70	3.30	
September 21, 1916.....	G 4	3.00	2.60	
September 28, 1916.....	G 4	3.50	4.00	3.20
October 9, 1916.....	G 4	3.80	3.20	3.40
September 21, 1916.....	G 5	2.90	2.70	
September 21, 1916.....	G 5	3.20	3.00	
September 28, 1916.....	G 5	3.70	3.90	4.60
October 9, 1916	G 5	3.60	3.00	2.90
October 3, 1916.....	G 6	3.40	2.60	
October 3, 1916.....	G11	3.40	3.10	
October 9, 1916.....	G11	4.90	4.10	
October 3, 1916.....	G12	5.00	4.10	
October 9, 1916.....	G12	4.20	3.80	
October 9, 1916.....	G14	4.00	3.40	
October 9, 1916.....	G15	4.20	3.20	
October 13, 1916.....	G16	3.90	3.70	
October 13, 1916.....	G17	4.60	4.00	
October 20, 1916.....	G19	2.90	2.30	
October 20, 1916.....	G20	3.60	3.40	
October 20, 1916.....	G21	3.50	2.70	
October 20, 1916.....	G22	4.20	3.40	
October 20, 1916.....	G23	4.50	3.40	
October 23, 1916.....	G24	4.10	3.70	
October 25, 1916.....	G24	4.20	3.90	
October 27, 1916.....	G24	4.10	4.00	
October 25, 1916.....	G25	5.60	2.90	
October 25, 1916.....	G26	5.60	3.00	
October 28, 1916.....	G26	4.30	3.60	
October 28, 1916.....	G32	5.10	4.30	
October 28, 1916.....	G33	5.60	4.50	
October 28, 1916.....	G34	4.30	3.80	
November 2, 1916.....	G34	4.20	3.90	
November 18, 1916.....	G26	5.80	4.10	
November 11, 1916.....	G26	5.75	4.00	
November 4, 1916.....	G39	4.80	4.10	
November 4, 1916.....	G40	4.60	4.15	
November 4, 1916.....	G41	5.70	4.05	
November 11, 1916.....	G45	4.87	4.63	

TABLE 4—Continued

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
November 11, 1916.....	G46	4.80	4.00	
November 11, 1916.....	G47	6.24	4.37	
November 11, 1916.....	G48	4.97	3.97	
November 13, 1916.....	G48	4.63	4.05	
November 14, 1916.....	G48	4.70	4.13	
November 15, 1916.....	G48	4.90	4.06	
November 16, 1916.....	G48	5.04	4.20	
November 17, 1916.....	G48	3.51	3.30	
November 23, 1916.....	G25	5.25	3.10	
November 24, 1916.....	G25	5.44	3.19	
November 24, 1916.....	G26	3.80	3.20	
November 13, 1916.....	G26	5.80	4.10	
November 15, 1916.....	G26	5.75	4.00	
November 6, 1916.....	G49	4.97	4.14	
November 13, 1916.....	G49	4.87	4.14	
November 14, 1916.....	G50	5.50	4.06	
November 17, 1917.....	G50	5.21	3.99	
December 4, 1916.....	G55	5.93	3.67	
December 11, 1916.....	G55	5.70	3.67	

On averaging these tables, the following figures are obtained:

Immunity normal resting saliva.....	4.47 cc. amino N
Immunity paraffine activated saliva.....	3.63 cc. amino N
Difference.....	0.84
Equals.....	20.7 per cent of the mean
Caries normal resting saliva.....	3.89 cc. amino N
Caries paraffine activated saliva.....	3.72 cc. amino N
Difference.....	0.17
Equals.....	4.5 per cent of the mean

The difference in nitrogen content between the normal resting saliva in cases of immunity and that in caries is strikingly brought out by the above tabulation. In the determination of averages however there is always an error which must be measured before the data may be considered conclusive.

The determination of the standard deviation of the mean demonstrates, however, the reliability of the results presented. These calculations were evaluated from the formula given by Davenport (15) as follows:

$$\text{Standard deviation} = \sqrt{\frac{\text{sum of the squares of the deviation from the mean}}{\text{number of measurements,}}}$$

$$\text{Probable error of the mean} = 0{,}6745 \times \sqrt{\frac{\text{standard deviation}}{\text{number of measurements.}}}$$

The appended table thus summarizes the data:

	CARIES	IMMUNITY
Standard deviation normal resting saliva.....	0.645	0.913
Probable error.....	0.08	0.07
Equals.....	2.1 per cent of the mean	1.8 per cent
Standard deviation paraffine saliva.....	0.5564	0.569
Probable error.....	0.0735	0.0496
Equals.....	2.0 per cent of the mean	1.4 per cent

It is evident from these figures that the difference between the yields of amino nitrogen by normal resting and activated saliva in persons afflicted with dental caries is only twice the probable error of the mean; in other words, that there is either no difference, or at the most only a slight one between the average protein content of normal resting saliva and activated saliva secreted by such persons. In normal individuals, on the contrary, the difference between the amino nitrogen yields is no less than ten times the probable error of the determination and it is evident that in such persons there is a very definite divergence of composition between normal resting and activated saliva. The normal resting saliva of a person with caries approaches, in protein content, the activated saliva of a normal person, and stimulation by chewing paraffine results in little change either in protein content or in neutralizing power of the saliva secreted.

These relations are illustrated in the appended table showing the average neutralizing powers (that is the number of cubic centimeters of N/200 acid and alkali required to change 10 cc. of saliva from neutrality to phenolphthalein to neutrality to paranitrophenol) of normal resting and activated saliva in normal persons and in persons afflicted with caries. These averages are compiled from salivary analyses completed within the last two years and comprise data obtained from over one hundred individuals. It is evident that saliva from subjects with

carious teeth presents two distinct differences from saliva of immune subjects, namely, that the neutralizing power of the resting saliva is supernormal while that of the paraffine activated saliva is subnormal.

Average total neutralizing power

	NORMAL RESTING SALIVA	PARAFFINE ACTIVATED SALIVA	AVERAGE SALIVARY FACTOR
Immunity	23.693	38.324	61.82
Caries	30.096	30.952	97.24

The normal resting saliva of persons with carious teeth is, therefore, characterized by (1) a relatively high neutralizing power and therefore, presumably, (2) a high content of diffusible substances; (3) a low content of proteins.

TABLE 5

Comparison of values of amino nitrogen with the total neutralizing power

NUMBER	NORMAL RESTING SALIVA				PARAFFINE ACTIVATED SALIVA				
	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	Salivary factor
	cc.	*cc.*		*cc.*	*cc.*	*cc.*		*cc.*	
G24	17.10	5.00	22.10	4.10	38.50	1.00	39.50	3.70	55.95
G26	27.40	4.70	32.10	5.60	58.45	−1.50	56.95	3.15	56.40
G26	20.00	4.50	24.50	4.30	49.00	0.50	49.50	3.60	49.50
G27	15.00	12.90	27.90	8.00	41.10	1.75	42.85	4.90	65.10
G 9	27.75	2.55	30.30	3.40	36.55	1.50	38.05	2.80	79.60
G45	28.50	−1.60	26.90	4.87	57.60	−4.15	53.45	4.63	50.30
G48	34.75	2.15	36.90	4.63	47.55	2.50	45.05	4.05	81.90
G48	24.00	3.30	37.30	4.90	35.00	0.60	35.60	4.06	76.70
G49	16.10	5.70	21.80	4.97	35.50	1.25	36.75	4.14	59.30
G40	18.40	3.70	22.10	5.50	31.60	2.30	33.90	4.06	65.20
G52	33.00	8.00	41.00	4.48	40.65	1.00	41.65	4.84	98.45
G53	24.15	3.95	28.10	3.80	30.80	2.80	33.60	4.10	83.60
G58	7.70	8.00	15.70	3.12	32.45	1.30	33.75	3.39	46.50
G58	11.90	6.00	17.90	4.10	33.45	1.25	34.70	3.70	51.60
G59	24.55	2.75	27.30	3.19	26.70	0.90	27.60	3.00	98.90

The inter-relation between the amino nitrogen and the salivary factor is shown in table 5. Subjects 52 and 59 were classed as carious, the others as immune. This connection of the one to the other is rendered all the more striking when the results of the dialysis experiments

are kept in mind, for the data evaluated in table 1 pointed to the fact that the increase of the total neutralizing power is due primarily to an increased amount of inorganic substances. Conversely, the lowered amount of organic bodies in activated salivas coupled with their greater neutralizing power brings further evidence to substantiate this conclusion.

From these results it is evident that immune persons secrete in response to stimulation by chewing tasteless substances a saliva which has a greater neutralizing power than normal resting saliva and is furthermore differentiated from normal resting saliva by a considerably lower content of protein and higher content of inorganic salts. The alteration in the character of the saliva is not merely due to dilution, consequent upon more rapid secretion, but involves a marked change in the relative proportion of the constituents. Persons with carious teeth differ from normal persons in that their normal resting saliva approximates in composition and neutralizing power to the composition of the activated saliva, in other words the salivary glands of such persons behave as though they were constantly receiving stimulus analogous to that constituted by the act of chewing a tasteless substance. Such a stimulus might conceivably be provided by carious teeth themselves, or on the other hand, both conditions may be attributable to a common underlying cause.

Part 2

Relationship of dental caries and the composition of saliva to dietary conditions and the locality and nature of stimuli promoting secretion

a. The relation of diet to the incidence of caries

The alteration in the salivary factor may be due to either a direct or an indirect cause. If the former, then the presence of dental caries in an otherwise healthy mouth would initiate the change. If the latter, then the change is either incidental or comprises a portion of a vicious circle.

Among the indirect factors which may initiate an acute disturbance may be mentioned diet, a lesion or an infection, a chronic peripheral nervous affection, a central nervous affection or lastly a defect in the processes of repair and growth correlated with a defect in salivary function.

Diet comprises per se two factors, namely composition and taste.

The former has been the subject of much discussion and research and the conclusions reached by the many authorities appear to be rather negative in character. Data hereinafter reported will deal more particularly with this phase.

It has been shown (6) that the salivary factor is constant in certain types of insanity to the same extent as in the normal individual and from this fact the deduction may be made that differences in neutralizing power are not related to the central nervous system.

In reviewing the literature concerned with the problems of dental caries and its possible relation to habits of cleanliness, climatic conditions, diet and general health of an individual or of a race, one notes a lack of uniformity in the recorded observations. This may be attributed to the fact that the many different writers were influenced in their judgment by different standards of observation, so that teeth which superficial examination would designate as non-carious might disclose, upon a more thorough examination, exactly the opposite condition.

Pickerill (1) states that certain food investigations point to the fact that the modern dietary of the civilized world differs from the diet of the uncivilized world in that the former is less hard but more tough and requires, therefore, more triturating but less crushing. From this conclusion the idea is advanced that, since different sets of muscles are used in triturating than in crushing, the over-development of these triturating muscles (buccinator and pterygoids) is responsible for both the abnormally shaped as well as the undeveloped dental arch. This, in turn, accounts for the increasing number of malposed teeth which accompany the under-developed arch. Malposed teeth are very susceptible to dental caries and therefore the increase of this disease among the modern civilized nations is correlated to our changed habits in masticating. Pickerill notes further that of the races of the world, the meat eaters, or at least those whose food is largely protein in character, were quite as susceptible to caries as those whose diet was mainly vegetarian. This is contrary to the views expressed by both Mummery and Patrick. The argument advanced by Pickerill is that the immune races, which include according to some authorities the Asiatics, Africans, Polynesians, Australians, et cetera, owe their comparative freedom from caries to the fact that their diets were both varied and sapid. Their universal use of masticatories resulted in the prevention of stagnation within the oral cavity (p. 314) a fact which other investigations appears to support.

Dr. R. Thurnwald, in speaking to the author of his anthropological researches in New Guinea, mentioned that the inhabitants in that section of the world seem to be comparatively free from dental caries. Their diet is mainly vegetable consisting of yams, sago, rice and sugar cane, etc.; meat is rather an accessory and, with the exception of the rather scarce mango, there are no acid fruits. The custom of chewing the betel nut plays, unintentionally, an important part, no doubt, in their oral prophylaxis. For at the age of puberty this custom, often connected with one of the initiatory ceremonies, is commenced and continued throughout the life of the individual.

Contrary to the usual belief, this betel nut habit does not blacken the teeth. For that purpose there is employed a mixture of cocoanut oil and soot which is vigorously rubbed on the teeth.

The betel nut is not used alone but is combined with seeds or leaves of a peppery nature together with pulverized lime from calcined shells. These three substances are taken into the mouth one after the other and are masticated between meals. The old men are edentulous and prepare a paste by mixing the material before chewing it. The natives expectorate profusely after the use of this mixture and the saliva is colored blood red. This coloration might be ascribed to the bleeding or to a compound formed by the action of the lime on the betel nut. The teeth are lost between the ages of forty and fifty and are exfoliated comparatively rapidly once the process has commenced. This exfoliation is accompanied by swollen and bleeding gums.

Calculus is deposited upon the teeth in almost unbelievable quantity, and it is often the case with the adults to see the size of the lower incisors increased by these concretions to 300 or 400 per cent.

Underwood (2) after examinations of skulls from different collections makes the following comment:

> In the hot belt of the earth including India, Africa and Southern China, bathing and washing are natural habits because of the heat; rinsing the mouth after meals and the use of sticks, toothpower, ashes and salt for cleansing the mouth is almost universal among the natives; while the food is largely rice and no alcohol is used. In all of them caries is so rare that to all intents and purposes the natives may be regarded as immune.

He states further that the people of the arctic regions whose personal habits, at least in regard to the care of the mouth, leave much to be desired, and whose diet is quite different, likewise enjoy immunity. He considers the Australian native equally immune. These observations lead him to conclude that the use of artificial foods and the re-

placing of breast feeding exerts a direct influence in the "weakening of the tooth defences." Just what constitutes "tooth defence" is not mentioned.

The effect of certain drugs upon the teeth has been dealt with by Austen (3). The systemic conditions which are supposed to favor the development of caries are anemia, dyspepsia, pregnancy, acute rheumatism, enteric and other continued fevers. Various salts and compounds of mercury, lead, bismuth, silver and copper were used in the experiments. Although it was found that the drug was partly excreted into the oral mucosa, yet it is rather an open question whether this excretion at one time may be so long continued as to accelerate or even cause any deleterious effect upon the erupted teeth. Another point however which may well be considered, is that the frequent drugging of growing children promotes a disturbance in the nutrition of the ameloblast and of the odontoblast thereby bringing about structural changes in the enamel and dentin respectively. Histological examinations conducted along this line of experimentation would undoubtedly throw light upon certain phases of present therapeutic methods.

In table 6 are presented certain abstracts and notations upon the teeth and diet of a few races from different parts of the world. Definite information on the subject appears to be rather scattered for in many instances an author may detail the foods at great length, the manner of cooking and habits of eating but will overlook entirely the conditions of the masticatory apparatus. In so far as the relative amounts of protein to carbohydrate in the diet are concerned, the data appear to confirm Pickerill's (1) conclusion in this regard, namely, that the protein eating races are as susceptible to dental caries as those whose food is mainly carbohydrate. On the other hand they appear to negative the popular impression that the teeth of primitive races are relatively immune to caries.

b. Relation of type and locality of stimulus to the neutralizing power of the secretion of saliva evoked by the stimulus

If the chronic disturbance of the neutralizing power be due to a chronic peripheral nervous affection or to a lesion or an infection remote from the salivary glands or teeth, such a disturbance would probably act through nervous reflexes. If the locality of the lesion is important, then by applying a definite stimulus to a circumscribed area in the oral mucosa such nervous irritation so produced might be expected to

TABLE 6

RACE	TEETH	DIET AND HABITS	AUTHORITY
Australian	"Toothache and indigestion common"	Large proteid diet. Flesh sometimes eaten raw. Only vegetable. "Nardu" grass, fern roots, yams	Eagle Bank and Crow, J. Mathieu. David Nutt, London
Australian	Teeth very tender	Chewing fiber from making of twine wears down teeth and makes them tender	The Australian Race, E. M. Curr. Tribunder & Co., London
Australian		Eat almost anything in the shape of animal food	Customs, Rites, etc., of the Aboriginal Tribe of the Gulf of Carpentaria, W. G. Stretton and E. C. Stirling. Trans: Roy. Soc. So. Australia, vol. 17, part 2, p. 240
Australian		Eat all the mammalian fauna	Report of the Horn Expedition, E. C. Stirling. Part IV, p. 51
Northern Australians		Natives eat anything that is edible	Native Tribes of the Northern Territory of Australia, Spencer. MacMillan & Co., 1914, New York
Brazilian	Unusually liable to decay	Milk, cheese, farina, sun dried meat. Food is bolted and all expectorate rather excessively	Highlands of Brazil, R. F. Burton. Tinsley Bros., London
Eesa or Bedoin branch of Somal	Teeth do not project, are poor and stained from surat tobacco	Mutton, milk, butter, rice, millet bread, dates	First Footsteps in East Africa, R. F. Burton. Dent & Co., London

Egyptians,* Ancient and Modern	Toothache common	Diet mainly vegetables, bread of millet or maize; milk, eggs, melons, beans, lupins. The cleaning of teeth a religious rite.	Account of Manners and Customs of Modern Egyptians, E. W. Lane. John Murray, London
Filipinos	Caries present in 20.90 per cent of cases examined	Diet includes great quantities of sugar	Ottofy Lancet, 176, 1909, 263
Friendly Isles	In general good teeth, but poorer than those of other nations	(See natives of Island of Otaheite)	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Gallois Tribe	(See Somal)	Gourds the only vegetable. Habitual drunkards. Habitually use the tooth stick	First Footsteps in East Africa, R. F. Burton. Dent, London
Igorots	Caries present in only 2.05 per cent of a large number of cases examined	Carnotes, a sweet potato, 30 per cent of diet. Rice, 30 per cent of diet. Other vegetables, maize, etc., 30 per cent of diet. Fish, meat and eggs, 10 per cent. Sugar is distasteful and is not used	Ottofy Lancet, 176, 1909. 253
Otaheite	White and regular	Mainly carbohydrate in character. Teeth cleansed six times a day	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Natives of Sarawak	"Toothache and decayed teeth are almost unknown"	Rice, yams, cucumbers, fowls, and fish	Natives of Sarawak and British North Borneo, H. L. Roth. Truslone & Hansone, London, p. 77.
Somal	Scattered teeth considered a sign of warm temperament	(See Eesa)	First Footsteps in E. Africa, R. F. Burton. Dent, London

* Note that Patrick quoted by Pickerell cites the Egyptians as one of the immune races, the ratio of caries in 3,306 teeth examined being only 3.418 per cent.

TABLE 6—Continued

RACE	TEETH	DIET AND HABITS	AUTHORITY
Somal	Teeth do not project, are poor and stained by tobacco	The teeth are cleaned by tooth stick. Flesh, holcus, few vegetables. Millet beer	First Footsteps in East Africa, R. F. Burton. Dent, London
Tasmanians	Among forty-two natives examined all had "very good teeth"	Refuse European food; eat rats, many vegetables of various kinds. Have enormous appetites. Meals are eaten with great greediness and great quantities are eaten at one time. Live chiefly on animal food	Aborigines of Tasmania, H. E. Roth. King & Son, Halifax, England, pp. 8 and 87
Tasmanians	Are reported by Cook, La Billardiere and Widowson as having good teeth		
Tasmanians	Teeth much worn; decay uncommon	Soundness of teeth due to chewing hard and tough material	Daily Life of the Tasmanians, Bonwick
Makololo	Beautiful teeth	General mixed diet, including:—porridge, milk, butter, corn, honey, beer, wild fruit, elephant meat	Narratives of an Expedition to the Zambesi, etc., David and Charles Livingstone. John Murray, London, 1865
Kebrabasa		Fowls, eggs, sugar cane, sweet potatoes, tomatoes, rice, maize; Kaffir corn	Ibid.
Manganja		Millet, maize, beans, nuts, yams, rice, pumpkins, cassava, sweet potatoes, etc.	Ibid.
Matumboka	"Teeth here are more solid and often wear down to the gums in old persons without decay. Cases of toothache not at all uncommon nevertheless"	Mixed diet	Ibid.

influence the neutralizing power. One of the easiest methods of stimulation is the use of an electric current which has been passed through an inductorium, and experiments along these lines were projected.

In this series of experiments it was desired to determine what comparative differences would result in titratable acidity and alkalinity by the use of the electric current at different parts of the oral mucosa. It has been demonstrated that the mechanical stimulus obtained by the chewing of paraffine excites a flow of saliva which is markedly different from that found normally in the mouth. With the employment of the electric current, obtained from an inductorium, a third sample was secured which differed in titration value from either the normal resting or paraffine saliva. The amount of current used and the locality at which it was applied did not appear to produce any marked deviation from the general result. Although the strength of current varied with different individuals, only that strength was used which at the end of two minutes produced a tingling sensation at the point of contact. Whenever this amount was appreciably increased it was found to be prejudicial to salivary activity, as an unnecessary nervous tension was thus produced.

The apparatus consisted of two Edison Lelande cells, type Z connected in series with an inductorium and a key. The electrodes consisted of two platinum points mounted on a vulcanite handle. The electrode was applied to the mucous membrane at some predetermined point and the saliva thus obtained titrated in the usual manner. The different localities at which the electrode was applied were, first, the opening of Stenson's duct opposite the upper second molar in the buccal mucosa; second, the openings of Wharton's ducts and the ducts of Bartholin on either side of the frenum linguae; third, on the dorsum of the tongue at the juncture of the posterior with the middle third near the apex of the V formed by the convergence of the two lines of the circumvallate papillae; fourth, at the gingivae. In applying the current at the bilateral structures one side was stimulated for two minutes and then the opposite side. No inflammation of the mucosa at the point of contact was developed at any time. The results of these experiments are reported in table 7. In the first column is noted the serial number of the patient, in each column "A" the alkalinity of 10 cc. of sample expressed in cubic centimeters of N/200 HCl.; in "B" the acidity of 10 cc. sample expressed in cubic centimeter of N/200 NaOH., and in "C" the total neutralizing power. The salivary factor appears whenever the paraffine saliva was taken; the distance of the

TABLE 7

"Electrical stimulus"

NUMBER	NORMAL RESTING SALIVA			PARAFFINE STIMULATED SALIVA			ELECTRICALLY STIMULATED SALIVA			FACTOR	COIL	DATE
	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.			
	A	B	C	A	B	C	A	B	C			
Stenson's duct												
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*			*cm.*	
E 1	14.05	5.25	19.30	25.50	2.35	27.85	4.80	16.50	21.30	69.30	27.50	December 28, 1915
E 2	15.30	5.80	21.10	34.40	3.25	37.65	18.20	5.50	23.70	56.50	25.50	January 5, 1916
E 2	4.10	15.30	19.40	24.50	3.40	27.90	9.45	11.00	20.45	69.50	27.00	December 28, 1915
E 4	6.60	13.75	20.35	24.15	4.75	28.90	7.55	17.75	25.30	70.40	30.00	January 7, 1916
E 5	17.70	24.75	42.45	32.75	3.35	36.10	17.80	8.50	26.30	117.60	27.00	January 10, 1916
E 6	19.50	6.50	26.00	48.30	−1.20	47.10	25.45	2.50	27.95	55.20	27.00	January 8, 1916
E 7	8.75	9.80	18.55	25.20	4.55	29.75	10.65	7.00	17.65	62.40	27.00	January 12, 1916
G 8	20.40	3.30	23.70	39.80	0.50	40.30	27.10	11.30	38.40	58.80	27.00	September 1, 1916
Wharton's and Bartholin's ducts												
E 1	9.00	9.50	18.50	17.75	5.50	23.25	10.75	5.30	16.05	79.60	30.00	January 5. 1916
E 2	16.95	1.30	18.25	34.70	−3.75	30.95	20.15	−1.50	18.65	58.90	32.00	January 12, 1916
E 4	17.80	4.60	22.40	19.40	8.25	27.65	15.90	7.10	23.00	81.00	29.50	January 8, 1916
F 1	16.25	6.95	23.20	29.50	2.70	32.20	19.05	6.25	25.30	72.09	30.00	January 15, 1916
F 2	21.10	3.40	24.50	34.20	1.90	36.10	19.30	3.10	22.40	67.90	29.75	January 16, 1916
F 3	20.10	3.10	23.20	24.15	2.75	26.90	15.00	2.30	17.30	86.20	29.50	January 17, 1916
F 3	16.10	6.25	22.35	22.00	1.85	23.85	14.40	2.50	16.90	93.70	29.50	January 18, 1916
F 5	17.70	4.50	22.20	37.40	2.50	39.90	14.25	3.95	18.20	55.70	29.00	January 18, 1916
G 7	22.85	2.50	25.35				19.90	2.70	22.60		29.50	February 22, 1916
G 8	15.15	6.50	21.65	44.85	1.50	46.35	27.55	11.00	38.55	46.71	27.80	July 22, 1916
G 8	26.05	7.75	34.80	44.55	3.10	47.65	26.40	11.80	38.20	74.70	26.00	August 16, 1916

Tongue

E 1	8.70	10.50	19.20	15.75	4.85	20.60	11.35	4.45	15.80	93.20	28.00	January 22, 1916
E 4	11.00	14.70	25.70				12.50	8.40	20.90		28.50	November 11, 1915

Gingivae

E 6	18.25	7.60	25.85				22.95	3.30	26.25		31.00	January 10, 1916
E 7	10.50	8.15	18.65				12.25	5.75	18.00		29.50	January 13, 1916
G 43	17.25	8.00	25.25	21.60	2.75	24.35	16.55	5.35	21.90	103.70	25.50	November 5, 1916
E 5	18.40	23.55	41.95				25.55	6.90	32.45		31.00	January 11, 1916

secondary coil from the primary shows the comparative strength of the current and is expressed in centimeters.

The data submitted demonstrate first, that the alkalinity of the electrically excited saliva is lower than that of the paraffine sample; second, that the acidity of the former is relatively higher than that of the latter; and third, that the neutralizing power of the saliva obtained by electric stimulus is lower in every instance than that collected by the paraffine method. This fact indicates that the use of the inductorium does not promote salivary activity to the same extent as the paraffine.

It was hoped that differential areas of irritation in the mouth could be demonstrated by means of this electrical stimulation for it was desired to determine whether one part of the oral mucosa is more sensitive to extraneous influences than another, so that a local irritation in one circumscribed locality would tend to produce a greater change in the salivary neutralizing power than in another locality. The experiments, however, so far reported, have failed to supply any conclusive evidence.

The repeated use of the inductorium on one individual produces a marked effect upon the relationship of the normal resting saliva to the activated paraffine saliva. This electric irritation alters the factor in a few days from one which is comparatively low to one with a much higher valuation. As examples of this condition may be cited subjects E1, E4 and G8. In the first instance the experiment was commenced on December 28, 1915. The salivary factor at this time was 69.3. Electrical stimulation was applied and after eight days a second test was made. The normal resting saliva and the paraffine saliva were first collected and the application of the current repeated. It was found that the factor rose steadily from 69.3 to 79.6 and finally to 93.2. Similarly with E4 the factor at the start was 69.5 but rose in eleven days to 81.0. For subject G8 the factor evaluated on July 23, 1916 was 46.7; on August 1 it had risen to 74.7. One month later it had returned to nearly the same ratio as at the start and duplicate samples on successive days gave a factor of 58.8. The results reported are based on duplicate analyses and on duplicate samples obtained on successive days.

The determination of the reaction of the taste impulses upon salivary secretion was attempted by comparing the results obtained with a normal resting sample with those secured by the use of different substances of marked taste. Howell (12) and other investigators describe the taste sense as being composed of four fundamental sensations, namely, bitter, sweet, acid and salty. Tastes other than these are combinations of any two or more of the primary sensations and produce, therefore, a

mixed stimulation of the sense organ. There was used for this experiment quinine on one day and sucrose on the second or third day following.

In securing the samples there was collected first a resting saliva and then the saliva secreted from the use of the taste stimulant. The results are reported in table 8. In the first column appears the subject number. Figures in columns A, B and C represent respectively the alkalinity, acidity and total neutralizing power of each sample. It may be stated in general that the action of sucrose, the sweet stimulant, tends to lower the total neutralizing power of the saliva. Quinine, the bitter stimulant, appears to produce the opposite condition yielding saliva resembling that secreted in response to the stimulus of chewing

TABLE 8

Comparison of effects of different types of stimulation upon salivary secretion

NUMBER	SALIVA NORMAL RESTING			SALIVA FROM QUININE STIMULUS			SALIVA FROM SUCROSE STIMULUS		
	A	B	C	A	B	C	A	B	C
F1	16.25	6.95	23.20	20.65	4.55	25.20	13.8^0	10.70	24.50
F2	19.55	4.20	23.75	33.95	2.00	35.95			
F2	25.50	5.00	30.50	25.80	3.75	29.55	17.15	8.00	19.15
F2	21.15	7.50	28.65				18.45	5.50	23.95
F3	20.70	3.70	24.40				13.25	8.00	21.25
F3	18.40	5.40	23.80				9.95	8.25	18.20
F4	19.25	4.75	24.00				15.00	6.20	21.20
F5	19.10	5.75	24.85				10.65	8.00	18.65
G7	22.85	2.50	25.35				12.60	3.60	16.20
F1	18.05	4.70	22.75	19.60	13.40	33.00	13.85	7.00	20.85

paraffine. The well known work of Miller (13) demonstrates that fermenting carbohydrates such as would be found on the teeth tend to promote caries. From the above experiments it would be logical to infer that saliva favors the condition as well, since the neutralizing power of the sucrose-stimulated saliva approaches that of the normal resting saliva, and a factor evaluated on this basis would be of a magnitude corresponding to that which under the paraffine stimulus would indicate the presence of caries.

SUMMARY

Dialysis of saliva shows that the total neutralizing power is chiefly due to inorganic constituents.

The use of the Van Slyke apparatus in the determination of amino-nitrogen in saliva is a new application of this method. The results so

obtained show that there is a definite correlation between the concentration of inorganic constituents, the amino-nitrogen content, and the neutralizing power of saliva; namely, that a high neutralizing power is associated with a correspondingly high percentage of inorganic constituents and with a low percentage of protein.

Data are presented which confirm Pickerill's observations concerning the effect of different constituents of the diet upon dental caries.

The use of the electric current as a salivary stimulant excites a secretion markedly low in alkalinity and correspondingly high in acidity when compared to the saliva resulting from the paraffine stimulus. The neutralizing power of saliva secreted in response to electrical stimuli is less than that secreted in response to the chewing of paraffine.

Differential areas of stimulation in the oral mucosa cannot be demonstrated.

The comparison of analyses of saliva obtained by a sweet stimulus (sucrose) with that obtained by a bitter stimulus (quinine) proves that the former yields a saliva low in neutralizing power and that the latter produces the opposite result.

In conclusion I wish to express my deep appreciation to Dr. T. Brailsford Robertson for his interest evinced throughout the work as well as for the valuable suggestions offered. My thanks are also due to those students and clinic patients who have provided the necessary material for the experimental purposes.

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THE EPINEPHRIC CONTENT OF THE BLOOD IN CONDITIONS OF LOW BLOOD PRESSURE AND SHOCK[1]

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Very few investigations have been made to determine the existence of a possible relationship between the activity of the adrenal bodies and shock although the reactions of the vascular system to injections of epinephrin and its condition during shock indicate that some such relationship may exist.

The results thus far reported apparently indicate that during shock the chromaffin material disappears from the adrenal bodies, and the conclusion has been drawn that low blood pressure and shock are accompanied by a lack of epinephrin in the blood. As logical as this conclusion appears to be, it does not seem to harmonize with a number of observed phenomena. Furthermore an examination of the published reports of these researches indicates a possible error in arriving at this conclusion.

Consequently the present investigation was undertaken with the view to determine by an examination of the amount of epinephrin in the blood of the adrenal vein, the activity of the adrenal bodies during the following conditions, productive of shock.

a. Handling of the intestine.

b. Low blood pressure from hemorrhage.

c. Occlusion of the inferior vena cava.

The only record of experimental evidence as to the quantity of epinephrin in the blood during conditions associated with shock is unaccompanied by details.

METHODS

All experiments were performed on dogs. Examination of the literature indicated that the use of the intestinal strip of the rabbit

[1] Preliminary report of this work was published in the Proceedings of the Society for Experimental Biology and Medicine, xiii, No. 5, 1916.

recommended by Hoskins (9) because of its specificity and degree of reaction, furnished the best means for the detection of variations in the amount of epinephrin in the blood.

It was necessary to provide an apparatus that permitted immersion of the intestinal strip in the medium to be tested at body temperature. Provision also had to be made for the rapid replacement of one medium with another and for passing through the fluid a regular supply of oxygen. Intestinal strips were obtained in the usual manner from a urethanized rabbit.

The abdominal wall was opened, and pieces of small intestine 3 cm. to 4 cm. in length, removed, if possible, from a region devoid of intestinal contents. Each piece (apparatus was made in duplicate), to be used immediately, was arranged in the glass tube used for holding the medium tested and attached by pin hooks to a thread connected with a recording needle. Other pieces to be used as a reserve were placed in a beaker of Ringer's solution through which was passed a continuous stream of oxygen and kept on a water bath at a temperature of 37°C. After considerable experimentation, it was found that the intestinal strip contracted most satisfactorily in a Ringer's solution containing NaCL 0.85 per cent, $CaCl_2$ (crystals dried) 0.026 per cent and KCl 0.03 per cent. The strips usually began at once to contract rhythmically at the rate of about 13 contractions per minute. Strips of intestine frequently continued to react for four or five hours with only slight diminution in amplitude and rate. During this time, however, the Ringer's solution was replaced from time to time and constantly a stream of oxygen bubbled through the liquid.

Intestinal strips from very young rabbits gave poor results and occasionally strips from adult rabbits contracted poorly.

The rhythmic contractions of the intestine were diminished in amplitude and the tone of the strip decreased by replacing the normal Ringer's solution with Ringer's holding in solution, small quantities of adrenalin (adrenalin put up by Parke, Davis and Company was used). A very definite reaction was produced in dilutions as great as 1–500,000,000. Dilution of 1–1,000,000 brought about complete inhibition of movement and great loss of tone. It was noticed that the strip after a time became less sensitive to the adrenalin so that a larger quantity was required to completely stop the contractions. At the same time the ability of the strip to detect minute quantities was lessened.

The rhythmic contractions of the strips were tested in rabbit's blood

and then in dog's blood. The strip immersed in blood contracted in the same manner as in Ringer's solution, except that usually the tone increased very markedly when the blood first replaced the Ringer's. Sometimes the first contractions in blood were very irregular especially in amplitude, but in a few minutes they became quite uniform. It was found that the most satisfactory results were obtained when the blood was diluted one-half with Ringer's solution. This dilution was used in all of the experiments.

The reaction of the intestinal strip to this diluted dog's blood containing small quantities of adrenalin was tested with results similar to those of Ringer's solution and adrenalin.

The general procedure in the experiments was as follows:

A physiological dose of morphine was injected into the dog before ether anesthetization. During the course of the experiment, no more ether was administered. As soon as the dog failed to display the ordinary reflexes, the right jugular vein was dissected free, a cannula inserted and from this about 50 cc. of blood drawn to be used as a control. All blood was defibrinated, as soon as drawn, and placed on ice. The left carotid artery was laid bare, and a cannula was inserted and connected by a rubber tube to a mercury manometer for recording blood pressure. The abdomen was then opened along the linea alba. By means of towels, wrung out in water at 37°C., the intestines were pushed to the left of the mid-line to expose the abdominal vena cava. The vena cava, right renal vein and inferior mesenteric vein were dissected free from surrounding tissue. The renal vein branches of the vena cava and a branch of the inferior mesenteric were ligatured, the latter as far distally as possible, usually an inch or an inch and a half from its entrance into the vena cava. A cannula was inserted into the inferior mesenteric proximal to the ligature, and pushed in until its end was well within the vena cava, and opposite to the opening of the adrenal vein. It was necessary to obtain blood, both before and during a condition of shock from the adrenal vein unmixed with vena cava blood, yet in the interval between the two drawings, the general circulation and the pouring of adrenal blood into the vena cava must not be interfered with. At first for the purpose of preventing the mixture of vena cava with adrenal blood during the period of drawing blood, loose ligatures were laid around the vena cava, proximal and distal to the entrance of the adrenal vein into the vena cava. At the time of drawing the blood the ligatures were firmly pulled to completely occlude the vena cava at these two points, making a closed chamber with the adrenal vein

as the only opening into it. It was found that the use of these ligatures was not satisfactory, as in the process of drawing the blood, it was difficult to make certain that the vena cava was completely occluded during the entire time; and the traction on the vena cava had a tendency to pull the adrenal vein from its normal position, thus interfering with the regular output of blood from it. After experimenting with various kinds of clamps, some with curved jaws protected by rubber and fitted with handles, were obtained, which gave very satisfactory results.

The first blood drawn, equal approximately to that enclosed in the section of vena cava, was rejected; the following blood was carefully measured as to rate of flow in order to eliminate the possibility that results obtained might be due to a greater concentration of epinephrin because of the less rapid flow of blood through the organ although its activity might not have been increased. The blood was defibrinated. After sufficient blood had been drawn, the clamps on the vena cava were released and a small amount of citrate put into the cannula to prevent coagulation. The cannula was then clamped off and the abdominal cavity closed as completely as possible, until after shock had been brought about by one of the methods employed.

At the time of drawing blood, during or after the development of shock, the same procedure was followed. At this time the blood pressure was usually low and trouble was frequently experienced, at first, with coagulation interfering with the flow of the blood, but later, with more care in cleansing the cannula, this was obviated.

After the termination of the experiment, a post mortem examination was made to be certain that no unligatured veins were connected with the portion of the vena cava between the clamps.

The following designations have been given to the various samples of blood:

Control blood. Blood taken from jugular vein before operation.

Blood No. 1. Blood taken from adrenal vein before shock has been induced.

Blood No. 2. Blood taken from adrenal vein after development of shock.

Blood Nos. 3, 4, 5. Blood taken at successive periods during and after development of shock.

The intestinal strip was found to contract regularly in jugular blood, this blood was replaced by blood No. 1 (blood taken from adrenal vein before shock). After a short time the strip was placed in blood taken during or after development of shock (blood Nos. 2, 3, 4 or 5).

As will be seen by examination of tracings, the results were checked by varying the order of replacements. To determine the quantitative relation of epinephrin in the samples tested, two methods were employed. In the one the tracings obtained were compared with those obtained by the addition of known quantities of adrenalin. In the second method the blood giving the reaction of epinephrin was diluted with control blood until the reaction of this blood was similar to that of blood No. 1.

Two classes of control experiments were conducted:

1. Experiments in which the manipulative processes and time elements were the same as in the regular experiments, but a condition of shock was not induced.

2. Experiments in which the adrenal bodies were ligatured in such a way that while the blood from the lumbar branch of the adrenal vein was permitted to enter the vena cava, no material could pass from the adrenal gland into the circulation.

The means of inducing low blood pressure and shock were as follows: (1) manipulation of the intestine, (2) hemorrhage, (3) occlusion of vena cava.

1. Manipulation of the intestine. After drawing blood from the adrenal vein, a considerable portion of the intestine was drawn out and handled until a condition of shock was brought about in the animal. This occurred in from one to two hours. The blood pressure at the end of the period of handling varied from 40 mm. to 60 mm. Hg.

2. Hemorrhage. Blood was allowed to flow slowly from either the vena cava, femoral or jugular vein. Usually from 50 cc. to 100 cc. of blood were taken at intervals of ten or fifteen minutes. After clamping off the cannula there was a tendency for an increase in blood pressure to occur. As long as this tendency manifested itself, blood at intervals was taken. After the blood pressure had remained stationary at 40 to 50 mm. Hg for from thirty to sixty minutes, blood No. 2 was drawn.

3. Occlusion of vena cava. Interrupted intratracheal insufflation was given.

The skin and tissue were cleaned away from one of the ribs. By means of a periosteal elevator, the rib was separated from its periosteum and a piece about 2 inches in length cut away with bone forceps. The periosteum and pleura were carefully cut through and by means of a curved aneurysm needle, a thread was passed around the thoracic inferior vena cava. The two ends were brought through the incision so that the vena cava might be occluded to any desired extent

by pulling upon the two ends of the thread. The incision was sewed up; the respiration machine was disconnected and the animal resumed its normal method of respiration.

As will be seen from descriptions of the experiments the pressure was reduced considerably and held at that point for some time. In many cases it was still further reduced, and the drawing of the blood did not occur until a tendency to fall became evident in the blood pressure.

DESCRIPTION OF EXPERIMENTS

Handling Experiments

Dog 2. Weight, 7 kgm.

Pressure before operation, 140 to 150 mm. Hg
11.00 a.m. Rate of blood flow from adrenalin vein, 6 cc. per thirty seconds

No. 1 blood

11.00 a.m. Pressure at beginning of handling, 120 mm. Hg
1.00 p.m. Pressure after two hours handling, 120 mm. Hg
1.15 p.m. Pressure after two hours fifteen minutes handling, 120 mm. Hg
1.15 p.m. Rate of blood flow after two hours, fifteen minutes handling, 6 cc. per thirty seconds

No. 2 blood

3.00 p.m. Pressure after four hours handling, 70 mm. Hg
Blood in cannula clotted. No blood could be drawn.

Note that in this experiment there was no fall of pressure after over two hours handling, although the dog exhibited the usual symptoms of shock, with exception of low blood pressure. The blood taken after handling of intestine for two hours and fifteen miuntes exhibited relatively large quantities of epinephrin. Even after dilution of thirty-two times with jugular blood No. 2 blood showed greater quantities of epinephrin than blood No. 1.

Dog 5. Weight, 11 kgm.

9.50 a.m. Blood pressure before operation, 120 to 130 mm. Hg
10.15 a.m. Rate of flow from adrenal vein, 5 cc. per thirty seconds or 1 cc. per sixty seconds.

No. 1

10.15 a.m. Blood pressure at beginning of handling, 110 to 120 mm. Hg
10.45 a.m. Blood pressure 110 to 120 mm. Hg
11.00 a.m. Blood pressure 120 to 130 mm. Hg
11.15 a.m. Blood pressure 120 to 130 mm. Hg
11.30 a.m. Blood pressure 120 to 130 mm. Hg
12.10 p.m. Blood pressure 80 to 90 mm. Hg
12.15 p.m. Blood pressure after handling of intestine two hours 60 to 70 mm. Hg

12.15 p.m. Rate of flow from adrenal vein, No. 2 blood, 4 cc. per sixty seconds or 1 cc. per fifteen seconds

After diluting blood No. 2 two and one-half times to compensate for the slower flow (on the supposition that the adrenals manufacture the same amount of epinephrin regardless of the amount of blood supplied to them), it was necessary to further dilute the blood two and one-half times with jugular blood before a tracing was given which resembled that given by blood No. 1.

Fig. 1. Dog 2. Handling intestine. Blood after shock (2) diluted thirty-two times with jugular blood still showing presence of epinephrin.

Dog 6. Weight, 4.5 kgm.

9.50 a.m. Blood pressure before operation, 110 to 120 mm. Hg

10.15 a.m. Rate of blood flow from adrenal vein, No. 1 blood, 4.5 cc. per sixty seconds

10.20 a.m. Blood pressure at beginning of handling, 85 to 95 mm. Hg

10.55 a.m. Blood pressure, 65 to 75 mm. Hg

11.05 a.m. Blood pressure, 50 to 60 mm. Hg
11.20 a.m. Blood pressure, 30 to 40 mm. Hg
11.20 a.m. Rate of flow from adrenal vein, 2 cc. per sixty seconds. No. 2 blood.

After dilution of No. 2 blood with jugular blood to compensate for difference of flow, the tracing made by No. 2 blood showed approximately the same difference from the tracing made by No. 1 blood as the difference between the tracings of normal jugular blood and jugular blood to which had been added adrenalin sufficient to make a content of 1–10,000,000.

HEMORRHAGE EXPERIMENTS

Dog 8. Weight, 8 kgm.

9.50 a.m. Blood pressure before operation, 110 to 120 mm. Hg
9.50 a.m. Rate of flow of blood from adrenals 3.5 cc. per sixty seconds
10.15 a.m. Blood drawn, pressure fell to 40 mm. Hg
10.40 a.m. Blood pressure rose to 100 mm. Hg
10.52 a.m. / 10.57 a.m. } Blood drawn, pressure fell to 40 mm. Hg
11.10 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.12 a.m. / 11.18 a.m. } Blood drawn, pressure fell to 60 mm. Hg
11.24 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.25 a.m. / 11.27 a.m. } Blood drawn, pressure fell to 40 mm. Hg
11.35 a.m. Blood pressure rose to 60 to 70 mm. Hg
12.20 p.m. Blood pressure began to fall 40 mm. Hg
2 cc. Blood drawn from adrenal vein, pressure fell to 20 to 30 mm. Hg
50 cc. Normal salt injected into vena cava blood pressure rose to 40 mm. Hg
2 cc. Blood drawn from adrenal vein. Rate of flow from adrenal vein 2 cc. per sixty seconds

After dilution with jugular blood to compensate for difference in rate of flow No. 2 blood showed indications of containing considerably more epinephrin than No. 1, but the difference in this experiment was not so marked as in some other experiments. This difference amounted to an addition of sufficient epinephrin to make a dilution of 1–50,000,000.

Dog 15. Weight, 11 kgm.

Blood pressure before operation, 120 to 130 mm. Hg
11.00 a.m. Blood from adrenal vein, rate of flow, No. 1, 9 cc. per thirty seconds
11.00 a.m. Blood pressure, 110 to 120 mm. Hg
11.15 a.m. Blood drawn, blood pressure fell to 80 to 90 mm. Hg
11.25 a.m. Blood pressure rose to 100 to 110 mm. Hg
11.30 a.m. Blood drawn, blood pressure fell to 50 to 60 mm. Hg
11.35 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.36 a.m. Blood drawn, blood pressure fell to 50 to 60 mm. Hg

11.50 a.m. Blood pressure rose to 60 to 70 mm. Hg
11.52 a.m. Blood drawn, pressure fell to 35 to 45 mm. Hg
12.10 p.m. Blood from adrenal vein, rate of flow No. 2, 3 cc. per thirty seconds
12.10 p.m. Blood pressure 20 cc. normal NaCl injected, 20 to 30 mm. Hg
12.30 p.m. Blood pressure fell to 15 to 20 mm. Hg
Blood from adrenal vein, rate of flow No. 3, 2 cc. per thirty seconds
Pressure fell while drawing blood.
12.40 p.m. Pressure 0. Animal died.

Examinations of tracings show that even after dilution seventeen times with jugular blood, blood No. 2 contained more epinephrin than blood No. 1.

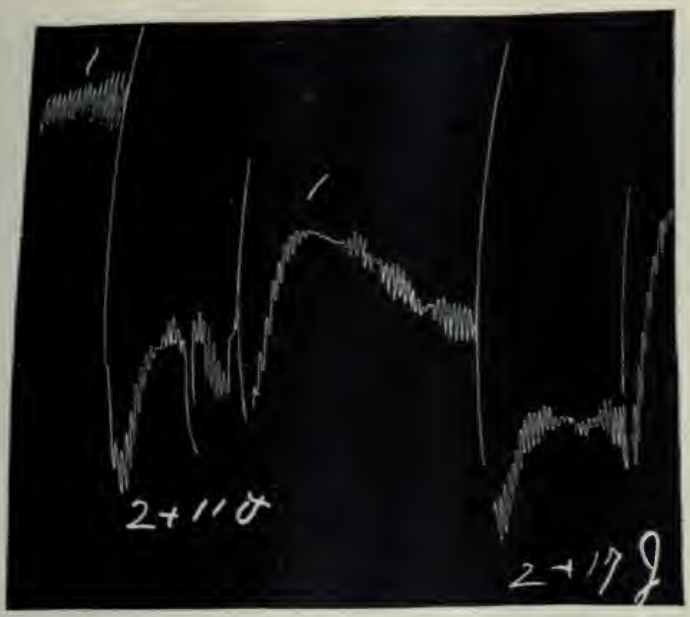

Fig. 2. Dog 15. Bleeding.

Dog 16. Weight, 17 kgm.

10.00 a.m. Blood pressure before operation, 135 to 140 mm. Hg
10.30 a.m. Rate of flow from adrenal vein, No. 1, 8 cc. per thirty seconds
10.30 a.m. Blood pressure, 110 to 120 mm. Hg
10.30 a.m. } Blood drawn, (40 cc.), pressure remained at 110 to 120 mm. Hg
10.35 a.m. }
10.40 a.m. Blood drawn, (50 cc.), pressure remained at 110 to 120 mm. Hg
10.45 a.m. Blood drawn, (50 cc.), pressure remained at 110 to 120 mm. Hg
10.50 a.m. Blood drawn, (70 cc.), pressure remained at 110 to 120 mm. Hg
11.00 a.m. } Blood drawn, (100 cc.), pressure 100 mm. Hg
11.05 a.m. }

11.10 a.m. Blood drawn, (50 cc.), pressure 100 mm. Hg
11.25 a.m. Blood drawn, (50 cc.), pressure rose to 115 mm. Hg
11.35 a.m. Blood drawn, (100 cc.), pressure 110 mm. Hg
11.40 a.m. Blood drawn, (70 cc.), pressure fell to 95 mm. Hg
12.00 m. Blood drawn, (100 cc.), pressure fell to 60 mm. Hg
12.05 p.m. Blood drawn, (50 cc.), pressure fell to 50 mm. Hg
12.25 p.m. Blood drawn, rose to 60 mm. Hg
12.35 p.m. Blood drawn, fell to 50 mm. Hg
12.40 p.m. Blood drawn, fell to 30 mm. Hg
1.00 p.m. Rate of flow of blood from adrenal vein, No. 2, 4 cc. per thirty seconds
Pressure fell rapidly, animal died.

It will be noticed that pressure failed to fall for a long period, and that when it began to fall it did so very rapidly.

From examination of tracings, it was seen that both No. 1 and No. 2 blood contained large quantities of epinephrin as compared with jugular blood, but no striking difference in amount in blood No. 1 and blood No. 2. Blood No. 2 contained additional epinephrin equal to an amount sufficient to make a dilution of 1–25,000,000.

VENA CAVA OCCLUSION EXPERIMENTS

Dog 12. Weight, 17 kgm.

Blood pressure before operation, 120 to 125 mm. Hg
11.40 a.m. Rate of flow of blood from adrenal vein, 12 cc. per thirty seconds

No. 1 blood

11.40 a.m. Blood pressure, 120 mm. Hg
11.45 a.m. Vena cava occluded, blood pressure, 60 mm. Hg

Kept at this pressure until 1.15

1.15 p.m. pressure began to fall rapidly falling to almost 0 mm. Hg

Vena cava was clamped above and below adrenal vein and blood from enclosed section removed with pipette. This blood was evidently blood returning from posterior part of body, by way of vena cava, inferior mesenteric and adrenal veins, practically general venous blood. It will be seen by referring to tracings that this blood contains a very much greater amount of epinephrin, equal to an amount sufficient to make a dilution of 1–10,000,000. If no increased output of epinephrin occurred, then this No. 2 blood should show less epinephrin than No. 1 blood, because of the great admixture of general venous blood.

Dog 14. Weight, 31 kgm.

Blood pressure before operation, 140 mm. Hg
Rate of flow of blood from adrenal vein, No. 1, 10 cc. per thirty seconds
12.00 m. Blood pressure, 120 mm. Hg
12.10 p.m. Blood pressure reduced by occlusion, 50 mm. Hg
12.55 p.m. Blood from adrenal vein, rate of flow No. 2, 7 cc. per thirty seconds

Blood pressure kept at 50 mm. until 1.30 p.m.

1.30 p.m. Blood from adrenal vein, rate of flow No. 3, 7 cc. per thirty seconds, 50 mm. Hg

2.05 p.m. Blood from adrenal vein, rate of flow No. 4, 7 cc. per thirty seconds

2.18 p.m. Blood pressure lowered to 35 mm. Hg

Blood pressure kept between 30 and 35 mm. for twenty minutes

2.38 p.m. Blood pressure showed indication of falling.

2.40 p.m. Blood from adrenal vein, rate of flow No. 5, 5 cc. per thirty seconds

2.50 p.m. After rapid fall of pressure, animal died.

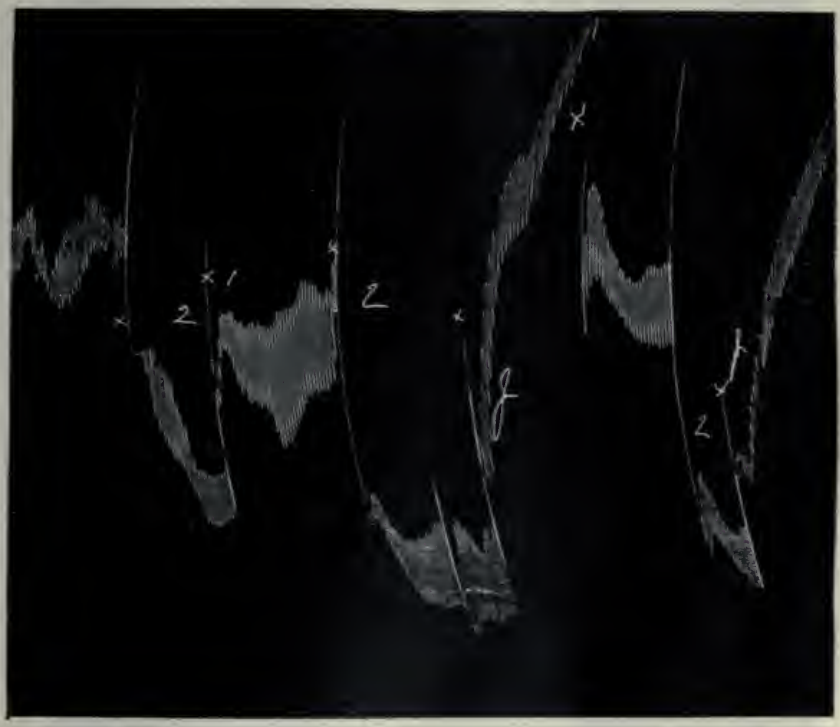

Fig. 3. Dog 12. Occlusion of vena cava

Examination of tracings show somewhat increasing amounts of epinephrin in the blood with the continuance of the low blood pressure of 50 mm. Hg. The greatest differenc is evident in blood which had been kept at a pressure of 30 to 35 mm. Hg, after the one hundred and five minutes at pressure of 50 mm. Hg. Evidently a very greatly increased amount of epinephrin has been added to blood during the period of this very low pressure.

Fig. 4. Dog 14. Occlusion of vena cava

Dog 17. Weight, 17.5 kgm.

Blood pressure before operation, 140 mm. Hg
11.00 a.m. Blood from adrenal, rate of flow No. 1, 8 cc. per thirty seconds
11.05 a.m. Blood pressure, 130 mm. Hg
Blood pressure held at 60 mm. until 12.05.
12.05 p.m. Blood from adrenal vein, rate of flow No. 2, 9 cc. per thirty seconds
12.10 p.m. Blood pressure reduced to 40 mm. Hg
Blood pressure held at 40 mm. Hg until 12.55.
12.55 p.m. Blood from adrenal vein, rate of flow No. 3, 10 cc. per thirty seconds
1.00 p.m. Blood pressure reduced to 30 mm. Hg
Blood pressure held at 30 mm. Hg until 1.10 p.m.
1.10 p.m. Blood from adrenal vein, rate of flow No. 4, 8 cc. per thirty seconds
1.15 p.m. Blood pressure held at 30 mm. until 1.25.
1.25 p.m. Blood pressure began to fall, pressure failed to react on release of ligature around vena cava, although up to this time the pressure rose somewhat on release of tension of ligature.
1.26 p.m. A few cubic centimeters of blood were obtained from adrenal vein. No. 1.

Fig. 4a. Dog 14. Occlusion of vena cava

Examination of tracings shows that blood No. 2 and blood No. 3 contain no greater amount of epinephrin than blood No. 1. Blood No. 2 was drawn after pressure had been kept at 40 mm. Hg for one hour; blood No. 2 was drawn after pressure had been kept down to 30 mm. Hg, for an additional period of fifty minutes. Blood No. 4 and especially blood No. 5 show the presence of larger additional amounts of epinephrin. Blood No. 4 was taken after pressure had been kept to 30 mm. for an additional fifteen minutes and at a time when the power of reaction of the blood vessel had disappeared. Evidently an increased amount of epinephrin was poured into the blood as the period of low pressure became prolonged and at a time when the blood pressure mechanism was becoming ineffective.

CONTROL EXPERIMENTS

Dog 7. Weight, 15 kgm.

9.40 a.m. Pressure before operation, 120 to 130 mm. Hg
10.20 a.m. Blood from adrenal vein, rate 25 cc. per thirty seconds of flow No. 1
10.20 a.m. Blood pressure, 100 mm. Hg
11.20 a.m. Blood pressure reduced by bleeding to 40 to 50 mm. Hg
11.20 a.m. Blood from adrenal vein, rate of flow No. 2, 12 1.2 cc. per second
Blood No. 2 showed no greater amount of epinephrin than blood No. 1.

This experiment shows several things; first, that the manipulation, incident to the operation, and remaining under ether, for the length of time of an experiment, does not cause an increased amount of epinephrin in the blood; second, that the low pressure from bleeding must be maintained for a considerable time before an increased amount of epinephrin in the blood can be observed.

Dog 18. Weight, 1.5 kgm.

Blood pressure before operation, 120 mm. Hg

Abdominal cavity was opened and adrenals were tied off so that blood flow was interfered with as little as possible.

This was accomplished for the left suprarenal but ligature of right apparently interfered somewhat with blood flow. The manipulation caused a fall of blood pressure to 80 mm. Hg.

Blood was taken from vena cava through inferior mesenteric vein, blood No. 1.

By occlusion of vena cava pressure was reduced to 50 mm. Hg and kept at that pressure for one hour and thirty minutes. The blood pressure was then reduced to 30 mm. and kept at that point for thirty minutes, when the blood pressure began to fall. Blood was then drawn as before, from the vena cava, through the inferior mesenteric vein.

Examination of tracings shows that blood No. 2 had no greater tendency to cause relaxation of strip of rabbit intestine than blood No. 1. On the contrary just the reverse was true as might be expected if the change in the intestinal strip was due to the presence of epinephrin in the blood. The tracings indicate that as soon as blood No. 1 was replaced by blood No. 2, the tone of the strip increased.

This experiment indicates that no other substance than epinephrin poured into the blood, e.g., excretion of pituitary body, produces the characteristic reaction of the intestinal strip.

Dog 19. Weight, 10 kgm.

Blood pressure before operation, 120 mm. Hg
Adrenal glands ligatured in the same manner as in the preceding experiment
During the operative processes, blood pressure fell to 90 mm. Hg
12.00 m. Blood drawn from vena cava through inferior mesenteric vein
Pressure reduced to 50 mm. Hg. By tension on thoracic inferior vena cava and kept at that point until 1 p.m.

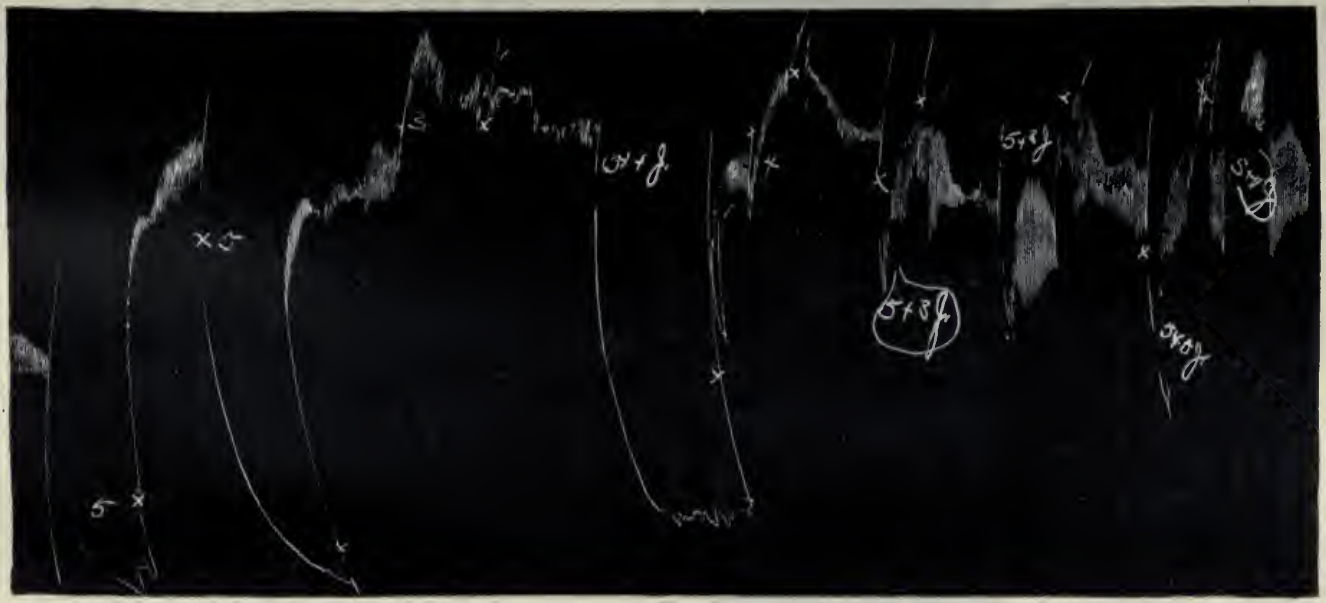

Fig. 5. Dog 17. Occlusion of vena cava. Pressure reduced to 30 mm. No. 3: Pressure reduced to 40 mm. after one hour five minutes reaction to 80 mm. No. 4: Pressure reduced to 30 mm. after one hour fifty-five minutes reaction to 80 mm. No. 5: Pressure reduced to 30 mm. after two hours twenty minutes; no reaction.

1.00 p.m. Blood pressure reduced to 40 mm. Hg
1.15 p.m. Blood pressure reduced to 30 mm. Hg
1.30 p.m. Blood drawn from vena cava through inferior mesenteric vein, No. 2
1.30 p.m. Blood pressure reduced to 20 mm. Hg
1.40 p.m. Blood pressure falling, blood drawn from vena cava through inferior mesenteric, No. 3

Examination of tracings shows that neither No. 2 blood nor No. 3 blood had any effect in causing a loss of tone or relaxation of the intestinal strip of rabbit.

It is evident therefore, from this experiment and the previous one that the relaxation of the intestinal strip in a condition of prolonged low blood pressure condition is due to epinephrin from the adrenal glands.

Fig. 6. Dog 18. Low blood pressure by occlusion of vena cava, suprarenals ligatured.

SUMMARY OF RESULTS OF EXPERIMENTS

In all three types of experiments, the epinephric content of the adrenal blood was increased provided that the pressure was sufficiently low and the condition of low pressure maintained for a sufficient length of time. Since the blood was diluted with control blood to compensate for the difference in the rate of flow through the adrenal organ, an increased activity of these organs was indicated.

It is a question whether this dilution of the blood to compensate for the difference in rate of flow through the adrenal bodies is legitimate. The dilution is made on the supposition that possibly the absolute output of the glands is not affected by the blood supply; that when the rate of flow of blood through the gland is one-half as great, this blood will contain twice as much epinephrin per cubic centimeter as blood flowing through the gland at the normal rate. As such a difference is not probable, the blood taken after shock in these experiments has evidently been over diluted and the results are more positive than they appear to be.

It is of interest to note in this respect that in the experiments with animals 2 (fig. 1) and 7, the rate of flow of the blood taken after shock was identical with that of the blood taken before shock. In both these cases, however, a relatively greatly increased amount of epinephrin was found in the blood drawn from the adrenal vein, after shock had developed.

In one case it was necessary to dilute the experimental adrenal blood with thirty-two times (fig. 1) its volume of jugular blood and in another thirty-one times (fig. 4) before a tracing could be obtained similar to that of adrenal blood drawn before low blood pressure was induced. In other cases the reaction was similar to the reaction given by control blood to which had been added adrenalin sufficient to make a 1–10,000,000 dilution, animal 12, which, in comparison with the normal amount of epinephrin in the blood, is a much more concentrated solution.

While the accurate determination of the actual amount of epinephrin in the blood may be difficult, yet there has never been any doubt as to the general relative amounts in samples of blood taken during one experiment.

In experiments in which the degree of low blood pressure could be most accurately controlled samples taken at intervals showed that the marked increase of epinephric content of the blood occurred only after a considerable duration of a condition of low blood pressure, varying from one to two hours (figs. 4 and 5). In these experiments, the later samples indicated an increasing amount of epinephrin.

None of the experiments showed any indication of a lessened amount of epinephrin in the blood, even after the most prolonged period of low blood pressure and although the animal might be at the point of death.

The handling experiments showed that in cases of shock accompanied by high blood pressure, the blood contains relatively a large amount of epinephrin. In some instances negative results were obtained unless the pressure was permitted to fall below 50 mm. to 60 mm. Hg. These negative experiments served as controls, indicating that the anesthetization and general operative procedure did not bring about the results obtained. To be certain that the results were not due to the presence in the blood of the secretion of some other organ, for example the pituitary body, the adrenal bodies were eliminated by ligatures. The results in these cases, however vigorous the experiment, were negative (fig. 6).

Table of experiments

NUMBER OF DOG	MEANS OF INDUCING SHOCK	BLOOD PRESSURE BEFORE SHOCK	TIME BETWEEN TAKING BLOODS NUMBER 1 AND NUMBER 2	PERIOD OF VARIOUS STAGES OF BLOOD PRESSURE	RESULTS	
					By dilution	Increase of epinephrin by comparison with known quantities of adrenalin
		mm. Hg				
2	Handling of intestine	120	2 hr. 15 min.	120 mm. Hg–2 hr. 15 min.	32×+	
5	Handling of intestine	115	2 hr.	115 mm. Hg–45 min. 125 mm. Hg–30 min. 85 mm. Hg–40 min. 65 mm. Hg– 5 min.	2½×	
6	Handling of intestine	90	1 hr.	90 mm. Hg–35 min. 70 mm. Hg–10 min. 55 mm. Hg–15 min. 35 mm. Hg– 1 min.		Additional 1–10,000,000
8	Hemorrhage	115	2 hr. 30 min.	115 mm. Hg–65 mm. Hg–1 hr. 45 min. 65 mm. Hg–45 min. Pressure fell rapidly to 40 mm. Hg.		Additional 1–50,000,000
9	Hemorrhage	100	1 hr. 50 min.	100 mm. Hg–30 mm. Hg–1 hr. 30 mm. Hg–50 min.		Additional 1–50,000,000
15	Hemorrhage	125	1 hr. 10 min.	125 mm. Hg–65 mm. Hg–50 min. 65 mm. Hg–20 min.	17×+	

16	Hemorrhage	115	2 hr. 30 min.	115–110 mm. Hg–1 hr. 10 min. 95 mm. Hg–20 min. 60 mm. Hg–40 min. 30 mm. Hg–20 min.		Additional 1–25,000,000
12	Occlusion of vena cava	120	1 hr. 35 min.	60 mm. Hg–1 hr. 30 min. Pressure then fell rapidly		Additional 1–10,000,000
14	Occlusion of vena cava	120	55 min.	50 mm. Hg–45 min. Blood Number 2	10×	
				50 mm. Hg–80 min. Blood Number 3	15×	
				50 mm. Hg–115 min. Blood Number 4	20×	
				50 mm. Hg–115 min. 30–35 mm. Hg–20 min. Blood Number 5	31×+	
17	Occlusion of vena cava	130	60 min.	60 mm. Hg–60 min. Blood Number 2	1X	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. Blood Number 3	1×	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. 30 mm. Hg–15 min. Blood Number 4	2×	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. 30 mm. Hg–30 min. Pressure began to fall rapidly Blood Number 5	11×+	

Check experiments

NUMBER OF DOG	MEANS OF INDUCING SHOCK	BLOOD PRESSURE BEFORE SHOCK	TIME BETWEEN TAKING BLOODS NUMBER 1 AND NUMBER 2	PERIOD OF VARIOUS STAGES OF BLOOD PRESSURE	RESULTS	
					By dilution	Increase of epinephrin by comparison with known quantities of adrenalin
		mm. Hg				
7	Hemorrhage	100	60 min.	100–50 mm. Hg–60 min.	1×	
18	Occlusion of vena cava	120	90 min.	50 mm. Hg–90 min. 30 mm. Hg–30 min.	1×–	Adrenals ligatured
19	Occlusion of vena cava	120	90 min.	50 mm. Hg–60 min. 40 mm. Hg–15 min. 30 mm. Hg–15 min. Blood Number 1	1×	Adrenals ligatured
				50 mm. Hg–60 min. 40 mm. Hg–15 min. 30 mm. Hg–15 min. 20 mm. Hg–15 min. Blood Number 3	1×	Adrenals ligatured

× = the amount of epinephrin in Blood Number 1.

DISCUSSION OF RESULTS

The evidence which has been accumulating within the past few years relative to the activity of the adrenal glands during shock would appear to indicate that these organs existed in a state of fatigue or at least that the output of epinephrin was not increased during or following the appearance of this syndrome. Thus Crile (13) reported in 1913 that there was no augmentation of output of epinephrin into the blood resulting from trauma under anesthesia. Also Bainbridge and Parkinson (5) and Corbett (15) conclude from a histological study of the adrenal glands that shock is the result of adrenal deficiency. These investigators based this assumption upon the decreased amount of chromaffin substance found in the glands at the time of examination.

Such findings are obviously in complete accord with that explanation of shock which holds that the condition is due to a splanchnic dilation of the blood vessels in the absence of epinephrin with the consequent fall in blood pressure.

The results of the experiments described in the present paper apparently are not in accord with those of the investigation just mentioned, nor are they in keeping with that conception of the cause of shock.

However, recently several investigators have reported work from which the conclusion might be drawn that a constriction of the arteries may occur in shock. Thus Muns (17) showed that during the development of shock, a peripheral constriction of the arteries of the limb occurred and if this was sufficient to offset the dilation in the splanchnic area, the pressure did not fall. Malcolm gives clinical evidence along similar lines.

It would appear that the apparent lack of harmony in the results arises from a failure to appreciate the actual conditions under which secretion occurs. Histological evidence does not necessarily give us an adequate picture of the functional activity of the cell. The former represents the condition of the physiological state at a single cross section of time; the latter must be represented over a considerable duration of time. Secretion is a double process. First, the material must be produced in the cell, and second, it must be eliminated into the blood or lumen of a duct. Normally these two processes occur simultaneously, but in conditions of disturbed cellular equilibrium, the one may overbalance the other; in which case the amount of substance in the cell at any one time holds no necessary relation to the

amount eliminated over a certain period of time. Thus the findings of Corbett (15), that the cells appear chromaffin poor, might very well have been interpreted as the result of more easy permeability of the cell for the hormone and while more than the normal amount was being produced, the material passed into the blood as soon as formation occurred. A similar explanation holds for the appearance at times of symptoms of hyposecretion with the glands containing an excess of the hormone. In this case the permeability for the substance was decreased, and the cell came into equilibrium with an excessive storage of the hormone, but with no elimination or perhaps a decreased one.

According to this point of view, the findings in this paper fit in with the histological picture. Increased production of epinephrin exists with a decreased amount present at any instant of time. It is difficult to bring Crile's (13) results into harmony with this conception. This investigator gives no details of his experiment, and it is well possible that the period of traumatization was not sufficiently prolonged in order to bring about the excessive discharge of epinephrin. In the present work there has never been a failure to find increased values of the hormone in the blood, if the handling of the intestines was prolonged for a sufficient period.

In 1905 Elliott (4) suggested that chromaffin tissue has the function of compensating for injury of sympathetic fibres and that after sympathetic impulses fail, their place is taken by the epinephric stimulation of the nerve endings.

The researches of Cannon and de la Paz (8) and Cannon and Hoskins (9) indicate that the adrenal secretion is largely a reserve for times of special stress. Hoskins and McClure (11) showed that there seems to be provided an adaptive distribution of the blood favorable to extreme muscular effort and that this is in some way related to epinephric action. Our results accord with this point of view. In shock the adrenals function as a line of secondary defense against a falling blood pressure. The presence of epinephrin in increasing amounts as shock progresses points to an attempt on the part of the circulatory system to redistribute the blood and bring about a peripheral constriction of the arteries wherever possible. In this way the normal pressure may be retained. If this self preservative mechanism is sufficient to offset the unknown factors which are instrumental in causing the fall in pressure, shock does not result fatally.

GENERAL CONCLUSIONS

1. That increased quantities of epinephrin are thrown into the blood during conditions of low blood pressure and shock.

2. That this increased amount of epinephrin found in the blood is accompanied by a hyper-activity of the adrenal gland and is not simply the result of the release of epinephric material stored in the gland.

3. That the epinephric content of the blood increases only after a somewhat prolonged continuation of the conditions leading to shock.

4. That the quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock.

5. That this increased output of epinephrin into the blood may be a last effort on the part of the organism to resist the forces that are tending toward a fatal degree of low blood pressure.

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BILE PIGMENT METABOLISM

VII. Bile Pigment Output Influenced by Hemoglobin Injections, Anemia and Blood Regeneration

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This paper shows the results obtained by the intravenous injection of hemoglobin in bile fistula dogs with and without anemia. Impressions gathered from various authoritative writings and published investigations led us to expect a pretty uniform reaction following an injection of hemoglobin in these bile fistula dogs under uniform conditions. We can not report any such uniformity of reaction and the tables given below require very careful study and conservative analysis. There are various stimulating possibilities which suggest themselves, but they must not be taken too seriously until the experimental data are conclusive. On the other hand, we must review some of the positive statements of other workers and suggest that a more conservative attitude be adopted toward the whole chapter of pigment metabolism.

The relation of hemoglobin injection to its elimination by the kidney has been studied by many investigators, recently by Pearce, Austin and Eisenbrey (1). They have studied the differences in hemoglobin elimination by the kidney when icterus was present or after removal of the spleen as compared with normal controls. There is a lowering of this threshhold of kidney elimination for hemoglobin when hemolytic or obstructive jaundice is present. Quite recently Sellards and Minot (2) have reported confirmatory work with human cases. They show that hemoglobin injection will cause hemoglobinuria in cases showing evidence of increased blood destruction, much more frequently than in normal controls. They state that this tolerance to hemoglobin bears no relation to the red cell count but to the amount of blood destruction in the body. The tolerance to hemoglobin

is low when blood destruction is high. We intend to suggest other factors which may be of importance in a consideration of this problem.

In these experiments with occasional exceptions we have used a unit amount of hemoglobin (obtained from 5 cc. of washed packed red cells) given into the jugular vein. This amount of hemoglobin as a rule will cause no hemoglobinuria and no clinical symptoms.

We must refer again to the long accepted theory that the pigment radicle of hemoglobin is quantitatively changed to bile pigment. This theory has been so long accepted that its very life tenure gives it a certain amount of respectability and perhaps more authority than it deserves. We would urge those who may be particularly interested to scrutinize the experimental evidence upon which this important claim is based. The best work is that of Stadelmann and his pupils (3). Their experiments were relatively few but carefully performed and accurately controlled. They used large amounts of hemoglobin, and followed the rise in bile pigment secretion over a long period. These workers do not claim that there is conclusive evidence for a quantitative elimination of the pigment radicle as bile pigment in bile fistula dogs.

No such conservatism marks the statements of Brugsch and Yoshimoto (4) and Brugsch and Kawashima (5) who claim that there is a quantitative elimination of bile pigments for a given amount of hematin injected. Further, that knowing the amount of bile pigment (or its end products) one can easily estimate the rapidity of hemoglobin disintegration! Careful scrutiny of their papers shows few experiments, fewer experimental data and no control of many important factors such as general condition, diet, icterus and hemoglobin determinations.

We insist that it should remain an open question whether the pigment radicle of hemoglobin is quantitatively eliminated in the bile as bile pigment. We believe there is more evidence against than for this view, but do not believe the question is settled.

Still more firmly do we insist that any estimation of blood destruction or of the life cycle of the red cell based upon the analysis of bile pigment or urobilin is absolutely faulty (Eppinger and Charnas (6), Wilbur and Addis (7) and others). We have shown that diet can increase bile pigment (8) and liver atrophy (Eck fistula) (9) or bile salts or bile fat (10) can decrease bile pigment secretion with no evidence of any blood changes. Now we suggest that the pigment portion of the destroyed hemoglobin under certain conditions may not be quantitatively eliminated as bile pigment. What information of

value concerning hemoglobin metabolism can we derive from an analysis of stercobilin alone? Diet may influence pigment elimination one way, liver function influence it another way and hemoglobin injection may cause a prompt rise in bile pigment output or a negative reaction. Can we state what has been happening in the liver from analysis of intestinal or bile pigments? Can we tell the life history of a cirrhotic liver from any analysis of the end product found at autopsy? To answer either of these questions is equally simple and requires the vision of a prophet.

When it was found that an Eck fistula liver put out much less bile pigment than a normal liver (9), we suspected that the lowered liver function of the Eck fistula was responsible. It was suspected that the liver might be concerned in *pigment construction*, that the liver might build bile pigment or even blood pigment. What substances can be built into various pigments? It is clear that blood feeding does not influence this bile pigment secretion (11). But might not pigment building be influenced in other ways? Can the liver use hemoglobin to form other pigments than bile pigments? If an anemia is produced does the liver take a part in the formation of this new blood pigment which goes on so rapidly? Given an anemia, what will the liver do with hemoglobin injected intravenously? There must be pre-pigment substances or parent pigments. Can such substances be held in reserve and used at times to form hemoglobin or other body pigments or thrown into the bile as bile pigments? Does the body build up this parent pigment substance only from "building stones" of the food or can it use pigment over and over again?

These and many other questions come up in any consideration of this complex problem of pigment metabolism. We have too long viewed this question of pigment metabolism with complacency, as perhaps one field in which our information was satisfactory. We urge that this whole question be approached with an open mind: that the simple little story of pigment katabolism from hemoglobin to urobilin be studied with care because this story may require revision.

EXPERIMENTAL OBSERVATIONS

These dogs with bile fistulae are comparable to those used in other experiments previously described. They had been under observation with a fixed routine for months and were in excellent condition. The methods used in operations, collections and analyses have been care-

fully described in the first paper of this series (12). There are no deviations from these published methods unless otherwise noted.

The preparation and injection of the hemoglobin solution was in all experiments done in a uniform manner. Blood freshly drawn from a dog was defibrinated or collected in oxalate, the red corpuscles washed repeatedly and packed firmly by the last washing in the centrifuge. Of these red cells 5 cc. were pipetted out, laked with distilled water and made up to 0.9 per cent solution with sodium chloride. This solution was given through a needle in the jugular vein. In no instance did this amount of hemoglobin given intravenously in bile fistula dogs cause any untoward results.

This amount of red corpuscles (5 cc.) should give approximately 60 mgm. of bile pigment, provided the pigment radicle of the hemoglobin is split off and excreted quantitatively as bile pigment.

Table 41 (dog 15–22) gives the results of several injections of hemoglobin into a simple bile fistula dog. This dog had been under observation for over a year in perfect condition under the usual uniform routine. If all the other experiments fell in with this one, we could make some generalizations which would indicate that the pigment metabolism is more simple than is probably the case.

The mean daily pigment secretion in this dog is pretty uniform, and we note the increase above this mean which follows the unit injection of 5 cc. laked red corpuscles. The urine contains a trace of bile pigment which increases after each injection of hemoglobin. The increase in bile pigment following the hemoglobin injection rises from 15 mgm. after the first to 38 mgm. following the fourth injection.

If hemoglobin injections showed this uniform graded increase from the first to the last injections or to a maximum output, we might assume a possible storing of pigment material in the body. When such storage capacity was exhausted, we might assume that the excess would be eliminated in the bile. This storage of pigment in one form or another is a possibility to be kept in mind but later experiments show the striking variations in the elimination of bile pigment after a unit injection of hemoglobin.

Tables 42 and 43 (also refer to table 74 in the following paper) are quite alike in their irregular reaction following a unit injection of hemoglobin. These dogs were in good condition and under the usual routine care. It is to be noted that dog 16–60 (table 43) has a subnormal mean bile pigment output. There are several possibilities to account for these low periods which will be discussed later. It will appear

TABLE 41

Normal bile fistula. Hemoglobin injection

Dog 15–22

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs*	
June 28	34	28	26		88	4.6	7.2	5.8		17.6		trace	33.5	
June 29	24*	30	15	14	59	5.4*	20.0	12.3	12.8	45.1	15.1	trace	33.8	
June 30	13	21	25		59	9.1	13.1	11.3		33.5		trace	33.8	
July 1					44					29.2		trace	33.8	Stools contain stercobilin
July 3	15	24	22		61	10.0	12.4	8.9		31.3		trace	33.5	Hemoglobin 120 per cent
July 5	19	15	19		53	12.0	9.1	8.5		29.6		trace	33.0	Hemoglobin 118 per cent
July 6	14*	22	14	24	60	6.7*	11.2	23.8	17.2	52.2	22.2	+	33.5	
July 7	32	24	17		73	8.0	8.1	6.5		22.6		trace	33.8	
July 8					22					25.6		trace	33.5	
July 10	9	20	16		45	10.8	18.0	9.7		38.5		trace	33.3	
July 11	17*	15	15	21	51	4.6*	17.0	23.6	21.8	62.4	32.4	+	33.5	
July 12	9	12	17		38	10.8	14.6	15.2		40.6		+	33.8	Red blood cells, 6,368,000. Hb. 117 per cent
July 13	16*	20	25	24	69	7.6*	22.0	19.0	27.0	68.0	38.0	+	33.8	
July 14	6	13	25		44	8.0	14.6	13.3		35.9		trace	33.8	
July 15					22					30.8		trace	33.8	
July 17					46					24.8		trace	33.8	

* 5 cc. laked red blood cells given intravenously at the end of the second hour. Mean daily output in table = 30 mgm. per six hours.

that the reaction of this dog toward hemoglobin injections is much like the other animals. Variations all the way from 10 mgm. to 39 mgm. above the mean secretion of bile pigments are noted in these experiments. Moreover, too, injections only forty-eight hours apart may show striking differences, sometimes the first injection being fol-

TABLE 42

Control bile fistula. Hemoglobin injection

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
April 24	15	17	19		51	13.6	13.6	15.3		42.5		trace	36.3	Hemoglobin 106 per cent
April 25	22*	20	17	20	57	14.0*	18.8	19.2	19.8	57.8	18.3	trace	36.5	
April 26	18	18	19		55	13.8	11.3	15.9		41.0		trace	36.8	
April 27	17†	15	18	22	55	11.4*	18.2	18.8	18.4	55.4	15.9	+	37.0	
April 28	20	19	20		59	16.2	14.9	16.4		47.5		+	37.5	
April 29					48					29.8		trace	37.3	
May 1	14	14	16		44	11.6	15.2	18.8		45.6		trace	36.3	Hemoglobin 101 per cent
May 2	25†	24	18	17	59	16.4*	30.4	26.0	16.8	73.2	33.7	+	37.0	
May 3	21	22	15		58	11.5	11.9	10.4		33.8		trace	37.3	
May 4	16†	16	21	23	60	10.0*	22.2	21.8	18.9	62.9	23.4	+	37.0	
May 5	16	19	20		55	11.1	12.0	11.6		34.7		trace	37.3	
May 6					64					40.1		trace	37.0	
May 8	17	15	18		50	11.4	13.2	16.2		40.8		trace	37.0	Hemoglobin 116 per cent
May 9	20†	17	15	14	46	13.1*	20.6	22.4	15.8	58.8	19.3	+	37.0	
May 10	17	16	17		50	11.8	12.5	12.2		36.5		trace	36.5	
May 11	23†	21	24	24	69	11.9*	21.8	29.2	16.8	67.8	28.3	++	37.5	
May 12	24	19	18		61	15.2	12.8	16.2		44.2		+	37.5	
May 13					50					38.0		+	37.0	

* 4 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 39.5 mgm. per six hours.

Average of twelve control days before hemoglobin injection experiments was 35.9 mgm.

lowed by a greater increase in bile pigments than the second and vice versa. It is to be recalled that an injection of 5 cc. laked red corpuscles should yield 60 mgm. of bile pigment, if there is a quantitative elimination of the pigment radicle.

We do not wish to go into various possibilities at this time. We wish to emphasize this striking irregularity of bile pigment increase

TABLE 43

Control bile fistula. Hemoglobin injection

Dog 16-60

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS.	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								Mixed diet
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			
1916													*lbs.*	
May 1	17	21	20		58	3.4	5.2	6.4		15.0		trace	37.5	Hemoglobin 94 per cent
May 2	21*	22	23	22	67	4.7*	11.4	18.9	15.8	46.1	23.3	++	38.0	
May 3	26	24	19		69	6.4	8.1	6.8		21.3		trace	38.5	
May 4	11*	20	14	26	60	6.7*	8.6	8.8	15.8	33.2	10.4	++	38.3	
May 5	25	24	23		72	7.3	4.9	6.2		18.4		+	38.5	
May 6					56					16.4		trace	38.0	
May 8	18	20	14		52	8.4	7.6	5.3		21.3		trace	37.5	Hemoglobin 93 per cent
May 9	26*	23	23	21	67	9.4*	17.0	20.1	19.2	56.3	33.5	++	37.5	
May 10	24	31	31		86	14.0	15.4	16.0		45.4		+	38.0	
May 11	25*	34	26	29	89	9.6*	20.6	23.6	18.2	62.4	39.6	+	38.5	
May 12	22	21	21		64	9.9	8.4	3.8		22.1		trace	37.5	

5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 22.8 mgm. per six hours.
Average of twenty control days before this experiment 22.4 mgm.

following a unit injection of hemoglobin in bile fistula dogs in apparent health. It is *possible* that the pigment radicle of hemoglobin is quantitatively eliminated as bile pigment when injected intravenously. If, however, the reaction following hemoglobin injection is as simple as usually assumed (merely a splitting off of the pigment radicle and elimination as bile pigment) why is not the reaction in these animals more uniform? In some experiments the crest of the wave of secretions seems to come in the third and fourth hours after injection with a return to normal during the total period of observation. Again there is much delay.

The reaction following bile or bile salt feeding is uniform under carefully controlled conditions (10). Why is not this reaction following hemoglobin injections more uniform? We believe that this reaction

is not simple and probably the whole body pigment metabolism is concerned. Some experiments given below strengthen this belief.

We realize that our six hour experimental period may be criticised, but there are more reasons for than against it. Such experiments must be repeated very many times because of individual variations in bile pigment secretion and obvious experimental variations. Twelve

TABLE 44

Bile fistula. Bleeding. Hemoglobin injection

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	16	17	17		50	11.1	13.9	15.2		40.2		trace	36.0	Hemoglobin 113 per cent. Bled 122 cc.
May 16	17	23	18		58	7.6	10.4	6.8		24.8		0	35.8	R. B. C. 6,444,000. Hemoglobin 114 per cent
May 17	16*	17	20	15	52	8.2*	13.7	19.8	17.0	50.5	14.7	+	35.8	
May 18	19	17	15		51	12.0	13.0	14.2		39.2		+	36.0	Stools contain no stercobilin
May 19	9*	25	20	15	60	5.8*	11.3	18.6	17.0	46.9	11.1	+	35.5	
May 20					44					39.2		+	35.0	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 35.8 mgm. per six hours.

Average of twelve control days before hemoglobin injection experiments is 35.9 mgm.

or twenty-four hour collections are extremely trying for the dog and if repeated at short intervals will change the dog from a normal to an abnormal condition. As stated in our first paper, we are convinced that these short period collections give a more nearly normal picture than do the long or continuous observations where the dog can not even approximate a normal condition.

Tables 44 and 45 continue the observations on dogs 16–138 and 16–60 (tables 42 and 43). These tables show the effect of a slight bleeding before the injection of hemoglobin. The results of the hemoglobin injection are striking—there is only a small output of bile pigment above the mean in one dog and no bile pigment increase in the other dog. Here is a very suggestive observation which may have something

TABLE 45

Bile fistula. Bleeding. Hemoglobin injection

Dog 16–60

DATE	BILE											URINE TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	22	22	21		65	8.9	9.9	8.9		27.7		trace	36.8	Hemoglobin 97 per cent. Bled 130 cc.
May 16	28	19	20		67	6.3	6.0	5.0		17.3		trace	37.3	R. B. C. 5,728,000. Hemoglobin 95 per cent
May 17	20*	15	13	12	40	3.8*	4.1	2.3	2.4	8.8	0	trace	37.8	
May 18	37	17	13		67	8.9	2.3	2.3		13.5		trace	37.5	Hemoglobin 96 per cent
May 19	27*	16	22	15	53	8.5*	6.4	5.4	3.4	15.2	0	trace	37.0	
May 20					60					19.6		trace	36.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 19.5 per six hours.
Average of twenty control days before this experiment is 22.4 mgm.

of importance back of it. May we assume that the greater part of this injected hemoglobin pigment radicle may have been deviated in the body to supply some pigment demand which was precipitated by this removal of blood? Before we answer *yes* to this question, as we might wish to do, let us consult the following tables 46 and 47 where the anemia is marked but the pigment retention not definite nor uniform—in other words, the anemic dogs react like the normal controls.

Tables 46 and 47 continue the history of these two simple bile fistula dogs. Anemia of a definite grade is produced by aspiration of blood from the jugular vein in sufficient amounts to cause a drop in the dogs' hemoglobin to 50 per cent or less. This acute demand for hemoglobin pigment to make into new red cells might react on the general pigment metabolism (compare tables 44 and 45) as has been suspected. There is no direct evidence in these tables 46 and 47. We note the same wide variation in bile pigment excretion following a unit injection of hemoglobin. In fact, the variations are even more marked than in the normal periods of these same dogs (tables 42 and 43).

The escape of bile pigments in the urine is more noticeable.

The mean daily bile pigment output during this anemia period (dog 16–138, table 46) is subnormal, 25.6 mgm. as compared to the normal 35.9 mgm. per six hours. This is much more evident during the period of regeneration following the acute anemia (table 48). Dog 16–60 has a subnormal output to begin with, and does not show this drop in bile pigment secretion (table 47).

Tables 48 and 49 show one important fact. During the period of acute regeneration following anemia produced by bleeding bile fistula dogs, there is a very low mean bile pigment output. Dog 16–138 has a normal mean daily output of 35.9 mgm. bile pigment per six hours (table 42). During the acute anemia period this mean output of bile pigment falls to 25.6 mgm. per six hours (table 46); but during the regeneration period following the anemia this mean bile pigment output per six hours falls to 11.2 mgm. or even to 7.2 mgm.—a drop to one-third normal or even less. Dog 16–60 shows a less striking reaction but one which is quite similar. It is to be recalled that this dog's initial mean bile pigment output per six hours was subnormal (table 43).

Dogs during the periods of *blood regeneration* following hemorrhage will put out less bile pigments than normal. There are several possible explanations and probably several factors concerned. The circulating red cells are somewhat below normal, and the number destroyed presumably smaller than normal, consequently the amount of pigment furnished in this manner is subnormal. It is more than possible that there is a conservation of pigment substances under these conditions of blood regeneration, but this term pigment conservation must be limited to the body pigments at present, however much the temptation to extend it to pigments introduced from without (e.g., hemoglobin). We have no evidence here that any more injected hemoglobin may

TABLE 46

Bile fistula. Anemia. Hemoglobin injection

Dog 16–138

DATE	BILE										URINE TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
May 22	31	25	30		86	12.5	11.9	16.4		40.8		+	34.5	Hemoglobin 115 per cent. Bled 122 cc.
May 23	17	15	20		52	9.6	11.8	14.9		36.3		trace	34.3	Hemoglobin 102 per cent. Bled 282 cc.
May 24	18	15	17		50	9.8	11.2	11.8		32.8		trace	34.5	Hemoglobin 94 per cent. Bled 200 cc.
May 25	16*	22	20	21	63	7.9*	19.6	24.2	18.6	62.4	36.8	++	34.3	Hemoglobin 66 per cent
May 26	20*	26	21	18	65	9.1*	23.6	19.8	15.4	58.8	33.2	++	36.0	
May 27	29	20	18		67	7.9	5.4	6.0		19.3		trace	36.3	
May 29	26	16	22		64	8.2	8.6	9.9		26.7		+	34.8	Hemoglobin 88 per cent. Bled 350 cc.
May 30	16	15	12		43	7.2	8.1	9.4		24.7		trace	34.8	Hemoglobin 63 per cent. Bled 200 cc.
May 31	19*	18	22	22	62	5.6*	14.5	33.6	15.8	63.9	38.3	++	34.5	Hemoglobin 52 per cent
June 1	20*	25	25	23	73	7.2*	13.5	15.8	14.0	43.3	17.7	++	34.8	
June 2	16	16	23		55	7.9	9.0	9.9		26.8		trace	34.3	
June 3					60					18.8		trace	34.5	Hemoglobin 71 per cent. Bled 350 cc.
June 5	18	18	19		55	8.9	9.4	8.5		26.8		trace	33.5	Hemoglobin 60 per cent. Bled 350 cc.
June 6	14*	19	18	14	51	4.7*	15.3	17.8	14.6	47.7	22.1	+	33.5	Hemoglobin 38 per cent
June 7	24	21	21		66	7.6	4.7	7.6		19.9		trace	34.3	
June 8	25*	25	24	32	81	5.1*	10.8	14.0	12.2	37.0	11.4	+	34.5	Hemoglobin 51 per cent
June 9	22	22	23		67	4.9	4.4	5.2		14.5		+	34.5	
June 10					52					20.0		+	34.0	Stools contain no stercobilin

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 25.6 mgm. per six hours.

Average for twelve control days with no anemia is 35.9 mgm.

TABLE 47

Bile fistula. Anemia. Hemoglobin injection

Dog 16–60

DATE	BILE										URINE TOTAL BILE PIGMENT, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
May 22	31	25	30		86	12.5	11.9	16.4		40.8		trace	36.3	Hemoglobin 94 per cent. Bled 130 cc.
May 23	23	18	24		65	9.9	9.4	11.9		31.2		trace	36.3	Hemoglobin 85 per cent. Bled 262 cc.
May 24	17	17	34		68	6.8	6.8	11.5		25.1		trace	35.8	Hemoglobin 70 per cent. Bled 100 cc.
May 25	16*	13	18	20	51	6.4*	7.3	13.8	17.3	38.4	17.3	trace	35.5	Hemoglobin 63 per cent
May 26	26*	28	34	27	89	8.8*	15.8	26.4	20.1	62.3	41.2	trace	36.3	
May 27	28	19	20		67	7.6	6.0	8.1		21.7		trace	37.8	
May 29	18	19	19		56	8.1	5.6	8.1		21.8		trace	35.8	Hemoglobin 78 per cent. Bled 325 cc.
May 30	24	12	19		55	5.9	4.4	6.4		16.7		trace	35.8	Hemoglobin 59 per cent. Bled 160 cc.
May 31	21*	23	24	21	68	5.2*	8.2	11.0	5.2	23.4	2.3	trace	35.3	Hemoglobin 51 per cent
June 1	22*	30	40	22	92	6.4*	8.2	20.8	8.9	37.9	16.8	+	36.0	
June 2	24	20	32		76	5.4	3.2	4.4		13.0		trace	35.3	Stools contain no stercobilin
June 3					50					17.0		trace	35.5	Hemoglobin 51 per cent
June 5	30	19	20		69	6.8	4.8	5.0		16.6		trace	34.5	
June 6	15*	20	28	27	75	6.0*	16.2	34.0	20.6	70.8	49.7	++	35.0	Hemoglobin 54 per cent
June 7	29	24	26		79	6.5	5.9	5.8		18.2		trace	35.3	Hemoglobin 62 per cent
June 8	30*	31	32	34	97	5.2*	14.0	22.3	16.0	52.3	31.2	++	36.3	
June 9	27	32	25		84	2.4	5.1	3.3		10.8		trace	36.0	

* 5 cc. laked red blood cells introduced into the jugular at the end of the second hour.

Mean daily output in table = 21.1 mgm. per six hours.

Average of twenty control days before anemia is 22.4 mgm

TABLE 48

Simple bile fistula. Anemia after-period. Hemoglobin injections

Dog 16–138

DATE	BILE: Amount in cubic centimeters 1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Bile pigments in milligrams 1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean	URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS: Mixed diet
1916													*lbs.*	
June 26	29	27	27		83	2.0	2.4	1.8		6.2		0	33.5	Hemoglobin 83 per cent
June 27	20*	23	26	25	74	2.2*	9.9	10.5	10.8	31.2	24.0	trace	33.8	
June 28	24	25	23		72	3.2	2.8	2.6		8.6		trace	34.3	Feces contain no stercobilin
June 29	22*	23	25	22	70	3.0*	5.7	9.0	9.4	24.1	16.9	trace	34.1	
June 30	16	25	25		66	1.4	3.3	2.3		7.0		trace	35.0	

* 5 cc. laked red blood cells given intravenously at beginning of third hour.
Mean daily output in table = 7.2 mgm. per six hours.
Average for twenty control days during this anemia after-period, 11.2 mgm.

TABLE 49

Simple bile fistula. Anemia after-period. Hemoglobin injections

Dog 16–60

DATE	BILE: Amount in cubic centimeters 1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Bile pigments in milligrams 1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean	URINE TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS: Mixed diet
1916													*lbs.*	
June 26	37	24	32		93	3.3	1.7	2.8		7.8		0	34.0	Hemoglobin 76 per cent
June 27	19*	13	30	23	66	5.2*	3.8	10.0	10.4	34.2	24.3	0	34.0	
June 28	39	20	22		81	4.1	2.2	2.5		8.8		0	34.5	Feces contain no stercobilin
June 29	30*	21	28	30	79	5.2*	2.9	3.2	11.4	17.5	7.6	0	34.3	
June 30	39	31	33		103	5.9	3.5	3.7		13.1		0	35.0	

* 5 cc. laked red blood cells given intravenously at the end of the second hour.
Mean daily output in table = 9.9 mgm. per six hours.
Average of twenty control days in this anemia after-period is 13.0 mgm.

be retained in the body during active blood regeneration than during normal periods or intervals of acute anemia. It is probable that the body can not use hemoglobin as such when injected intravenously, but must break it down into its constituent parts before actual pigment construction can take place. How much of this indirect conservation of body pigment does actually take place we cannot say at present. There is at least a possibility that some of the hemoglobin

TABLE 50

Simple bile fistula. Hemoglobin injections

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
July 5	19	22	24		65	3.4	3.0	2.7		9.1		0	33.5	
July 6	16*	20	16	22	58	4.0*	5.4	6.1	7.9	19.4	4.4	0	34.0	
July 7	19	17	20		56	6.8*	7.7	8.1		22.6		0	34.0	
July 10	32	24	26		82	2.8	2.7	3.5		9.0		trace	33.8	Hemoglobin 91 per cent. R. B. C. 5,856,000
July 11	23*	15	18	20	53	3.6*	2.6	3.6	8.6	14.8	0	++	34.0	
July 12	23	22	22		67	9.4	5.4	5.4		20.2		trace	34.3	Stools contain no stercobilin
July 13	15*	15	31	19	65	2.0*	4.4	11.8	9.9	26.1	11.1	+++	34.5	
July 14	18	22	24		64	4.0	5.4	4.9		14.3		+	34.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table —15.0 mgm. per six hours.

Average for twenty control days during this anemia after-period is 11.2 mgm.

pigment radicle which does not appear in the bile in some of these injection experiments may be taken up by the body cells, digested and used for other purposes in the body. There is no evidence that the body cells acquire greater efficiency in this respect after repeated hemoglobin injections.

Tables 50 and 51 continue the observations and experiments upon these same two simple bile fistula dogs. Regeneration of red cells

following the anemia is still in progress and the general picture is very like that noted in tables 48 and 49. The very low bile pigment output of dog 16–138 after the usual hemoglobin injections is of interest. The urine contains more bile pigment than usual, but at the most only a very few milligrams. The level of blood hemoglobin has returned close to normal, but the mean bile pigment elimination is still much below normal—50 per cent of normal in one dog (16–138).

TABLE 51

Simple bile fistula. Hemoglobin injections

Dog 16–60

DATE	BILE										URINE TOTAL BILE PIGMENTS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
July 5	23	19	26		68	7.7	3.4	2.4		13.5		0	34.0	Hemoglobin 94 per cent
July 6	22*	26	23	23	72	6.4*	12.4	18.8	9.9	41.1	27.0	+	34.5	
July 7	24	19	21		64	6.7	4.3	4.7		15.7		+	34.3	
July 8					50					17.0		trace	34.0	
July 10	19	23	26		68	6.0	3.1	4.0		13.1		trace	33.3	Hemoglobin 102 per cent. R. B. C. 5,940,000
July 11	27*	26	23	25	74	4.2*	13.4	16.1	10.8	40.3	26.2	+	34.0	
July 12	25	12	32		69	6.1	3.5	7.2		16.8		+	34.3	Stools contain no stercobilin
July 13	19*	25	20	32	77	5.2*	6.7	9.9	10.8	27.4	13.3	+	33.8	
July 14	28	25	27		80	3.2	3.3	2.4		8.9		trace	33.8	

* 5 cc. laked red blood cells given intravenously at the end of the second hour.
Mean daily output in table = 14.1 mgm. per six hours.
Average of twenty control days in this anemia after-period is 13.0.

CLINICAL AND AUTOPSY SUMMARY

Simple bile fistula

Dog 16–60. Adult, fat, brindle bull dog, male, 44 pounds.

December 21, 1915. Ether anesthesia, bile fistula operation, general condition good during post-operative period.

May 1, 1916. Dog in good condition, 37.5 pounds. See table 43 for details—experimental period.

July 14, 1916. Dog in good condition—end of this experiment, 34 pounds.

July 21. General condition good, vigorous appetite, normal activity, 33 pounds. Hemoglobin 104 per cent.

Ether anesthesia and killed by bleeding.

Autopsy at once. Thorax negative. Spleen is normal size, the malphigian corpuscles are perhaps a bit enlarged. Liver shows a definite increase in brown pigment. Bile passages are clear and pale. Common duct is completely obstructed and no bile can enter duodenum. Its lower end is dilated to about 1 cm. in diameter. Stomach and intestines show a normal mucosa. The muscle coat of the small intestine is pigmented a maple sugar color which is characteristic of these dogs. Some of the retroperitoneal lymph glands are large and contain calcified foci. The other organs present nothing abnormal.

Microscopical sections are negative except from the liver, which shows a slight but definite increase in portal stroma which contains many mononuclear cells. There is a slight increase in pigment in the liver cells. There is no proliferation of the bile ducts.

Simple bile fistula

Dog 16–138. Adult bull dog, male, 38.8 pounds.

March 25, 1916. Ether anesthesia; bile fistula operation; general condition is excellent during post-operative period.

April 24, 1916. Dog in good condition, 36.3 pounds. See table 42 for details—experimental period.

July 14, 1916. Dog in good condition, 34.5 pounds.

End of experiments cited above.

August. Dog remains in good condition.

September 6. Dog in coma, bloody urine; blood shows very high urea and non-protein nitrogen.

Ether anesthesia and killed.

Autopsy at once. Thorax negative. Spleen is small and fibrous. Liver shows pale ducts and complete exclusion of bile from the duodenum. Stomach and intestines show nothing of interest. A urethral stone is found just below the prostate. Bladder is large and its wall is thick. There is a definite hemorrhagic cystitis with early ulceration. Both ureters and kidney pelves are dilated. The kidneys show multiple miliary abscesses in the cortex.

Microscopical sections add no information of importance to this experiment.

SUMMARY

It has been generally assumed from the publications of other workers that hemoglobin injection will be followed by a uniform elimination of a corresponding excess of bile pigment—in other words, hemoglobin injection is followed by its quantitative elimination as bile pigment.

Our experiments show that the reaction of a bile fistula dog under uniform conditions following a unit injection of hemoglobin intraven-

ously is certainly *not uniform.* That the elimination of the pigment radicle of the hemoglobin *may be quantitative* we can not deny, but surely all our experiments speak against any such probability.

Some hemoglobin injections may be followed by little or no increase in bile pigment secretion. It is possible that this pigment is taken up by the body cells and used for some purpose, perhaps to build other body pigments.

During periods of acute anemia from bleeding, the bile fistula dogs may show a fall in bile pigment output and at times a lessened elimination of bile pigment following a unit injection of hemoglobin. The latter is quite inconstant, and yet may indicate some conservation of hemoglobin either directly or indirectly. Compare the influence of splenectomy and the associated *increase* in bile pigments in the next paper.

Bile pigment elimination may be greatly depressed during the period of acute regeneration following an anemia, yet the response to hemoglobin injections may be even more irregualr and inconstant than under normal conditions. It is highly probable that we have a certain pigment conservation under these conditions, but the mechanism of this conservation may not be simple and direct but possibly very complex. The whole question of pigment construction is concerned in this problem and much more experimental work is required.

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BILE PIGMENT METABOLISM

VIII. Bile Pigment Output Influenced by Hemoglobin Injection; Splenectomy and Anemia

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This paper gives the results of experiments upon bile fistula dogs with an added splenectomy. Unit amounts of hemoglobin are injected intravenously during a control period, during an anemic period and during the period of blood regeneration. These experiments supplement those of the preceding paper as well as giving additional controls. The two bile fistula dogs are followed through a normal period, a period of bleeding and anemia, followed by an interval of acute blood regeneration. Here some peculiar differences due to the splenectomy are noted and will be discussed later.

A few points bearing on splenectomy and pigment production may be appropriately taken up in this place. It is known that splenectomy in dogs may often be followed by a moderate anemia (Musser (1), Pearce, Austin and Musser (2), Musser and Krumbhaar (3)), but it is not decided whether this anemia is due to blood destruction or to less active blood formation. It has been claimed by Martinotti and Barbàci (4) that after splenectomy the output of bile pigments drops to one-half normal. Enough experiments are reported below and in a preceding paper (5) to disprove this claim. We feel safe in asserting that under nòrmal conditions a bile fistula dog will put out the same average amount of bile pigments whether with or without a spleen. It must be kept in mind that bile fistulae with splenectomy *plus* anemia from bleeding will often show remarkable periods of abnormally high pigment output alternating at times with periods of low bile pigment secretion.

This is a very interesting chapter in our splenectomy studies which calls for much more work. It is to be noted that the bile fistula dogs

with splenectomy may remain in a normal condition indefinitely and put out the usual amount of bile pigments per six hours. After a moderate anemia has been produced we may see a striking reaction—not a drop in mean bile pigment output as noted in controls with spleen intact but a period of great increase in pigment output. There is jaundice and heavy pigmentation of the urine. The blood cells may decrease still further and we may assume that all this increase in bile pigment secretion is due to red cell destruction. There may be another possibility or several more in fact.

We have stated that a bile fistula dog with splenectomy plus anemia may show periods of abnormally high bile pigment secretion during the long interval of blood regeneration which is greatly prolonged under such conditions. May we say that pigment production is depressed below normal? On the contrary we see an abnormal amount eliminated in the bile and urine (icterus). Further, it is to be noted that the color index of the blood during such periods is about unity as compared with simple bile fistula dogs with anemia when the color index is below one, as is usually observed in a secondary anemia. This may mean that pigment is present in excess, and the corpuscles are saturated with hemoglobin. It is not inconceivable that the frame work of the red cell may be formed through some mechanism and as it approaches maturity may be loaded with hemoglobin which it may select from the materials brought to it by the blood. Under some circumstances the formation of hemoglobin may be in excess of the stroma and the color index may approach unity. These experiments do not show many figures giving the blood color index, and we prefer to leave this point for future consideration.

How may we explain the periods of icterus which occur during the regeneration period following anemia in a bile fistula-splenectomy dog? We could show chart after chart in which periods of icterus appear suddenly with no obvious cause and as suddenly vanish with a long slow rise in the hemoglobin curve toward normal. There may be a sharp drop in the number of red corpuscles indicating disintegration of red cells to be in part responsible for the icterus. Yet the spleen is absent and cannot be blamed for this "hemolytic icterus." May we assume that the corpuscles manufactured during such periods are in some way faulty? We may believe that the stroma is less abundantly produced than the hemoglobin; perhaps the stroma is actually defective.

The hemoglobin injections give the same remarkable bile pigment fluctuations in relation to the mean output. It will be recalled that 5

cc. of laked red cells should be equivalent to 60 mgm. of bile pigment, if there is a quantitative change of the hemoglobin pigment radicle to bile pigment. Do we find any constant output of bile pigments above the mean following a unit injection of hemoglobin? On the contrary we find all variations from 4 mgm. to 42 mgm. increase above the mean daily bile pigment output—the same inexplicable variations

TABLE 61

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16–10

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
June 26	24	29	25		78	12.9	12.4	11.3		36.6		trace	29.0	Hemoglobin 106 per cent
June 27	27*	20	17	23	60	14.0*	14.9	8.8	26.8	50.5	17.5	+	29.0	
June 28	23	22	25		70	11.4	8.9	6.7		27.0		trace	29.1	Stools contain no stercobilin
June 29	18*	27	21	20	68	8.1*	17.0	17.1	16.1	50.2	17.2	+	29.0	
June 30	26	24	19		69	11.0	11.0	9.9		31.9		+	29.5	
July 1					46					31.8		trace	29.3	
July 5	25	21	19		65	12.9	9.4	8.6		30.9		trace	29.0	
July 6	21*	26	15	19	60	11.9*	15.4	19.6	18.8	53.8	20.8	+	29.5	
July 7	24	22	20		66	11.9	14.0	14.0		39.9		+	29.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 33 mgm. per six hours.
Average of twenty control days 34.4 mgm. per six hours.
Bile fistula operation and splenectomy done September 30, 1915.

in pigment output under as near uniform conditions as can be attained. The reaction following hemoglobin injection is the same in a simple bile fistula as with a combined bile and Eck fistula or a combination of bile fistula and splenectomy. The dogs used in these experiments had been under observation for months in apparent perfect health and under the usual routine described previously (6). The methods used have

been described in detail in former papers. These experiments are to be compared with those outlined in the preceding paper. These splenectomy animals were kept under the same routine as the bile fistula

TABLE 62

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16–27

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
April 24	11*	20	26		57	8.8	7.6	8.8		25.2		trace	25.8	Hemoglobin 103 per cent
April 25	18*	14	17	21	52	5.2*	6.9	12.7	9.4	29.0	4.3	trace	26.5	
April 26	15	25	25		65	6.8	7.9	7.3		22.0		trace	26.3	
April 27	26†	27	22	18	67	5.8†	12.8	14.0	5.6	32.4	7.7	trace	26.8	
April 28	23	18	25		66	7.9	8.9	10.2		27.0		0	26.5	
April 29					48					22.0		trace	26.0	
May 1	27	17	16		60	9.7	8.4	6.8		24.9		trace	25.5	Hemoglobin 87 per cent
May 2	14†	22	24	12	58	5.6†	20.8	18.9	8.2	47.9	23.2	++	26.5	
May 3	13	14	19		46	5.8	5.6	7.3		18.7		trace	26.8	
May 4	15†	20	17	23	60	11.5†	14.9	13.3	17.0	45.2	20.5	+++	26.8	
May 5	14	22	12		48	7.2	12.8	7.3		27.3		++	26.8	
May 6					38					23.8		+	26.3	
May 8	16	29	16		61	6.4	8.6	5.4		20.4		trace	26.3	Hemoglobin 86 per cent
May 9	12†	20	15	21	56	5.9†	17.0	7.4	4.9	29.3	4.6	++	26.0	
May 10	18	21	19		58	14.5	12.3	4.3		31.0		+	26.3	
May 11	19†	20	29	23	72	6.0†	7.2	19.4	16.4	43.0	18.3	++	26.3	
May 12	12	29	30		71	8.9	11.0	6.8		26.7		+	25.8	Stools contain no stercobilin
May 13					54					28.3		trace	26.0	

* 4 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood ceils given intravenously at end of second hour.

Mean daily output in table = 24.7 mgm. per six hours.

Average of twenty control days is 23.6 mgm. per six hours.

Bile fistula operation and splenectomy done December 8, 1915.

dogs without splenectomy, and the anemia was produced in the same way at the same time as can be seen by comparison of the various tables.

The three preceding tables (61, 62 and 63) show three bile fistula dogs with splenectomy, with normal blood hemoglobin and normal output

TABLE 63

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16–41

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Tota 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
April 24	28	19	24		71	10.0	7.7	11.5		29.2		trace	36.0	Hemoglobin 111 per cent
April 25	19*	16	34	22	72	11.0*	12.9	26.0	13.4	52.3	27.3	+	36.3	
April 26	12	17	16		45	3.8	4.6	5.0		13.4		trace	36.8	
April 27	17*	23	40	32	95	3.1*	8.9	18.8	12.2	39.9	14.9	+	37.3	
April 28	36	40	33		109	9.6	11.6	11.2		32.4		trace	36.8	
May 1	18	16	19		53	14.4	12.0	10.4		36.8		trace	35.5	Hemoglobin 92 per cent
May 2	24*	22	25	32	79	9.8*	15.2	23.6	23.8	62.6	17.3	+	36.5	
May 3	36	29	20		85	22.8	18.2	16.1		57.1		trace	37.5	
May 4	32*	34	29	31	94	13.7*	26.6	30.4	30.8	87.8	42.5	+	37.5	Stools contain no stercobilin
May 5	23	19	29		71	12.4	12.8	17.0		42.2		+	37.0	
May 8	19	24	21		64	11.5	14.0	9.4		34.9		trace	35.8	Hemoglobin 103 per cent
May 9	21*	23	27	30	80	9.4*	18.8	18.9	8.8	46.5	11.7	+	35.5	
May 10	27	32	34		93	9.1	11.6	10.8		31.5		+	36.3	
May 11	35*	18	23	25	66	11.8*	9.4	18.8	19.0	47.2	12.4	+	36.5	
May 12	24	22	31		77	13.5	11.9	12.5		37.9		+	35.8	
May 13					46					35.2		trace	35.3	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 35 mgm. per six hours.

Average of twenty control days is 32.2 mgm. per six hours.

Bile fistula operation and splenectomy done December 2, 1915.

of bile pigments for the unit period of six hours. These experiments are to be compared with those in the preceding paper (tables 41, 42 and 43) in which splenectomy had not been performed. It will be noted that the total output of whole bile as well as of bile pigments is identical in the two groups of dogs. Splenectomy under these conditions does not influence the output of bile pigments.

TABLE 64

Bile fistula. Splenectomy. Bleeding. Hemoglobin injection

Dog 16-27

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	13	22	17		52	6.2	9.9	8.8		24.9		trace	25.5	Hemoglobin 90 per cent. Bled 85 cc.
May 16	17	17	24		58	5.7	6.5	9.8		22.0		trace	25.8	R.B.C. 4,880,000. Hemoglobin 83 per cent
May 17	12*	16	23	17	56	5.8*	9.0	12.4	8.8	30.2	6.2		26.0	
May 18	18	20	18		56	3.2	4.6	5.2		13.0		trace	26.0	Stools contain no stercobilin
May 19	15*	17	18	17	52	5.7	11.4*	16.2	17.6	45.2	21.2	++	25.8	
May 20					54					36.4		+	25.8	

* 5 cc. laked red blood cells injected into jugular at end of the second hour.
Mean daily output in table = 24.0 mgm. per six hours.
Average for twenty control days is 23.6 mgm.

Hemoglobin injections (5 cc. of laked red cells) are followed by the same fluctuations in bile pigment output whether a dog retains his spleen or has been deprived of its services. The same dog under identical conditions may respond to the hemoglobin injection with a rise of 11 mgm. bile pigment above the mean, again with a rise of 42 mgm.

Tables 64 and 65 show a continuation of the experiments with dogs 16–27 and 16–41 (tables 62 and 63). Each dog is bled a small amount from the jugular and later tested again with hemoglobin injections. These experiments are to be compared with the control non-splenec-

tomized dogs (tables 44 and 45). There are no particular differences noted in these tables (64 and 65) as a result of this initial bleeding. The dogs react to the hemoglobin injections as they did before the bleeding. Note particularly the figures in table 65 where it is seen that the first injection of 5 cc. hemoglobin caused a rise above the mean of only 3.4 mgm. bile pigment but two days later the same injection caused an increase of 38.4 mgm. bile pigment.

TABLE 65

Bile fistula. Splenectomy. Bleeding. Hemoglobin injection

Dog 16–41

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	14	16	24		54	9.8	8.2	15.8		33.8		trace	34.8	Hemoglobin 106 per cent. Bled 120 cc.
May 16	19	25	26		70	6.4	8.1	11.0		25.5		trace	34.5	R.B.C. 5,680,000. Hemoglobin 103 per cent
May 17	33*	30	41	28	99	6.7*	8.2	16.2	9.4	33.8	3.4	trace	34.8	
May 18	32	32	43		107	10.1	10.3	12.8		33.2		trace	35.0	Hemoglobin 100 per cent
May 19	14*	25	38	31	94	11.3*	22.6	26.6	19.6	68.8	38.4	trace	34.8	Stools contain no stercobilin
May 20					64					29.4		trace	34.8	

* 5 cc. laked red blood cells introduced into jugular vein at end of second hour.
Mean daily output in table = 30.4 mgm. per six hours.
Average of twenty control days is 32.3 mgm. per six hours.

The two preceding tables, 66 and 67, show some very unusual figures as compared with the control tables in the same dogs before the anemia, and compared also with the simple bile fistula dogs in the preceding communication. Table 66 shows a great rise in bile pigment output to double normal with persistent and increasing icterus. The hemoglobin falls after the bleeding has been discontinued, and remains low for weeks with alternating periods of icterus and high bile pig-

TABLE 66

Bile fistula, Splenectomy, anemia. Hemoglobin injection

Dog 16–27

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 22	15	22	20		57	8.1	15.2	10.3		33.6		trace	26.0	Hb. 90 per cent. Bled 85 cc.
May 23	7	16	15		38	8.4	21.4	14.8		44.6		+	25.8	Hb. 81 per cent. Bled 215 cc.
May 24	19	17	17		53	12.0	12.7	14.5		39.2		+	25.8	Hemoglobin 59 per cent
May 25	17*	12	13	25	50	19.2*	15.2	17.2	37.4	69.8	16.7	++	26.0	Hemoglobin 58 per cent
May 26	17*	19	20	16	55	20.6*	27.4	32.4	28.8	88.6	35.5	++	26.8	Moderate icterus
May 27	22	17	17		56	21.8	18.4	20.6		60.8		++	27.3	Hemoglobin 36 per cent
May 29	17	14	19		50	16.8	16.7	19.8		53.3		++	26.0	Hemoglobin 28 per cent
May 30	15	17	11		43	14.4	19.2	14.4		48.0		+++	26.0	Definite icterus
May 31	11*	24	20	21	65	10.0*	29.2	30.6	23.8	83.6	30.5	+++	25.5	Hemoglobin 27 per cent
June 1	20*	21	19	17	57	11.0*	17.6	23.0	21.2	61.8	8.7	+++	25.3	Distinct icterus
June 2	23	15	15		53	20.4	17.0	17.1		54.5		++	24.8	
June 3					50					54.0		++	25.0	Hemoglobin 28 per cent
June 5	11	24	17		52	10.0	24.8	16.8		51.6		++	24.0	Hemoglobin 33 per cent
June 6	17*	19	21	17	57	15.2*	30.6	34.2	26.6	91.4	38.3	++++	24.3	Urine contains no urobilin
June 7	13	14	16		43	18.8	20.8	21.4		61.0		+++	24.5	
June 8	14*	16	16	17	49	16.4*	25.0	26.0	24.4	75.4	22.3	+++	24.3	
June 9	20	19	18		57	24.2	23.0	21.0		68.2		++++	24.5	R.B.C. 1,760,000. Hb. 31 per cent
June 10					24					68.8		++++	24.5	Marked icterus

* 5 cc. laked red blood cells injected into the external jugular at end of second hour.
Mean daily output in table = 53.1 mgm. per six hours.
Average of twenty control days before anemia 23.6 mgm.

TABLE 67

Bile fistula. Splenectomy. Anemia. Hemoglobin injection

Dog 16–41

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
May 22	31	20	32		83	13.2	11.0	14.7		38.9		trace	34.3	Hemoglobin 101 per cent. Bled 120 cc.
May 23	20	16	15		51	14.4	10.0	6.8		31.2		trace	34.5	Hemoglobin 94 per cent. Bled 280 cc.
May 24	32	27	25		84	7.2	6.0	6.7		19.9		trace	35.0	Hemoglobin 71 per cent. Bled 100 cc.
May 25	18*	16	22	23	61	7.2*	10.7	19.6	10.4	40.7	11.0	trace	34.8	Hemoglobin 60 per cent
May 26	27*	28	31	27	86	7.9*	17.0	28.0	17.6	62.6	32.9	trace	35.3	
May 27	35	24	20		79	8.6	7.6	6.4		22.6		trace	35.3	Stools contain no stercobilin
May 29	25	13	15		53	15.2	10.5	16.2		41.9		trace	34.8	Hemoglobin 72 per cent. Bled 300 cc.
May 30	12	13	12		37	6.2	7.3	8.2		21.7		trace	34.5	Hemoglobin 60 per cent. Bled 160 cc.
May 31	27*	27	34	30	91	6.7*	8.5	21.4	12.8	42.7	13.0	+	34.3	Hemoglobin 48 per cent
June 1	49*	35	45	32	112	12.2*	11.0	22.6	17.2	50.8	21.1	++	35.3	
June 2	32	29	31		92	8.7	7.2	6.3		22.2		+	34.5	No icterus
June 3					88					33.6		+	34.8	Hemoglobin 57 per cent. Bled 250 cc.
June 5	23	22	22		67	10.9	9.4	10.9		31.2		+	33.5	Hemoglobin 46 per cent
June 6	14*	19	29	23	71	9.8*	22.0	37.6	22.8	82.4	52.7	+++	34.0	Hemoglobin 47 per cent. Urine contains no urobilin
June 7	22	22	21		65	12.8	14.0	14.4		41.2		++	34.5	R.B.C. 3,200,000. Hemoglobin 61 per cent
June 8	31*	30	27	28	85	14.0*	21.0	24.2	21.4	66.6	36.9	++	35.5	
June 9	35	31	29		95	12.5	9.1	7.2		28.8		+	35.8	Stools contain no stercobilin
June 10					78					24.2		trace	34.8	No icterus

* 5 cc. laked red blood cells introduced into the external jugular at the end of the second hour.

Mean daily output in table = 29.7 mgm. per six hours.

Average of twenty control days before anemia 32.2 mgm.

ment excretion followed by periods of blood regeneration and low bile pigment output.

The other dog (table 67) shows a less striking picture during this period, but in later periods showed even more striking icterus and high pigment output which interrupted the regeneration of red cells toward

TABLE 68

Bile fistula. Splenectomy. Anemia. Hemoglobin injection

Dog 16–27

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
June 28	17	22	22		61	5.0	6.9	4.9		16.8		+	24.0	Red blood cells 3,542,000. Hemoglobin 61 per cent
June 29	10*	16	32	26	74	3.6*	5.4	15.8	10.5	31.7	25.0	+	24.5	Stools contain no stercobilin
June 30	19	27	23		69	4.8	4.2	2.6		11.6		trace	24.8	
July 1					62					7.0		trace	25.3	
July 3	15	28	22		65	4.4	5.6	4.0		14.0		trace	24.8	Hemoglobin 73 per cent
July 5	11	19	17		47	1.7	3.0	1.9		6.6		trace	24.5	
July 6	11*	16	19	20	55	2.0*	2.9	5.6	3.2	11.7	1.0	trace	24.8	
July 7	23	21	23		67	2.6	1.9	3.1		7.6		0	25.0	
July 8					46					12.4		0	25.3	
July 10	15	22	20		57	4.1	4.0	2.8		10.9		0	24.8	R.B.C. 3,880,000 Hemoglobin 78 per cent
July 11	11*	17	18	17	52	3.5*	8.0	25.0	11.4	44.4	33.7	++	24.5	
July 12	12	30	22		64	3.7	3.4	2.5		9.6		trace	24.8	Stools contain no stercobilin
July 13	15*	23	20	19	62	1.7*	6.2	11.6	10.8	28.6	17.9	+++	24.5	
July 14	12	25	24		61	3.8	4.5	2.7		11.0		++	24.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 10.7 mgm. per six hours.

Average of twenty control days = 23.6 mgm. per six hours.

a normal count. This dog (16–41) required more prolonged bleeding to cause an anemia, but after some delay reacted very much like the first animal.

The reaction to uniform injections of hemoglobin (5 cc.) is truly remarkable, and ranges from 11 to 53 mgm. above the average mean

TABLE 69

Bile fistula. Splenectomy. Anemia

Dog 16–41

DATE	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	Total 6 hrs.			Mixed diet
1916										*lbs.*	
June 19	35	24	22	81	31.4	25.8	28.0	85.2	++	34.0	Hemoglobin 63 per cent. No icterus
June 20	29	25	26	80	35.2	39.4	40.2	114.8	++	33.8	
June 21	20	32	34	86	27.2	28.0	29.0	84.2	++	34.0	Urine contains no urobilin
June 22	27	25	30	82	30.6	27.0	32.4	90.0	++	34.5	
June 23	24	30	31	85	25.8	50.6	56.7	133.1	++	34.3	Feces contain stercobilin
June 24				82				125.6	++	34.0	
June 26	37	33	32	102	39.6	37.0	43.2	119.8	++	33.5	Hemoglobin 67 per cent
June 27	28	30	35	93	37.6	34.0	34.6	106.2	++	33.0	
June 28	30	33	32	95	25.1	28.6	23.8	77.5	++	33.5	Hemoglobin 69 per cent. R.B.C. 3,440,000
June 29	29	28	32	89	21.0	31.8	33.2	86.0	++	33.3	
June 30	26	28	28	82	37.6	40.2	35.2	113.0	++	33.5	
July 1				72				124.0	++	34.0	No icterus
July 3	36	33	31	100	40.8	46.0	50.0	136.8	+++	34.3	Hemoglobin 69 per cent
July 5	28	27	29	84	56.4	54.6	52.8	163.8	+++	33.8	
July 6	24	36	22	82	38.7	51.2	81.2	171.1	+++	33.8	Hemoglobin 42 per cent. R.B.C. 1,920,000
July 7	37	27	29	93	61.8	72.8	77.6	212.2	+++	34.0	
July 8				78				198.4	+++	33.8	Moderate icterus
July 10	24	25	22	71	64.8	78.8	69.2	212.8	+++	33.5	Hemoglobin 43 per cent. R.B.C. 1,720,000
July 11	25	23	24	72	58.4	77.4	78.8	214.6	+++	33.0	Urine contains no urobilin
July 12	22	32	28	82	59.6	86.4	75.6	221.6	+++	33.0	Distinct icterus
July 13	29	32	26	87	68.7	75.9	61.5	206.1	+++	33.5	
July 14	34	27	25	86	78.3	73.2	70.5	222.0	+++	33.5	Stools contain stercobilin
Average				85				146.3		33.7	

level. There is not even any parallelism with the amount of icterus, although the high figures in table 67 are noted in conjunction with icterus.

These two tables, 68 and 69, continue the observations upon same two dogs. The younger dog (16–27) has recovered from the spontaneous icterus (table 68), and shows a low bile pigment output, a rising hemoglobin curve and the same wide fluctuations in bile pigment excretion following a unit injection of hemoglobin. This same dog at later periods again developed spontaneous icterus with high bile pigment excretion and a drop in hemoglobin.

Table 69 (dog 16–41) shows a truly remarkable picture. Bile pigment is constantly present in considerable amounts in the urine and definite icteroid coloration of the skin and mucous membranes develops. The output of bile is normal, but the bile pigments are enormously increased—even more than *six times normal.* Note at this time the low hemoglobin curve and the color index which remains so constantly close to unity. It seems that this dog was putting out about the maximum amount of bile pigment, and perhaps he was manufacturing the maximum amount of pigment substance, which may account for the high color index of the blood.

CLINICAL AND AUTOPSY ABSTRACT

Bile fistula and splenectomy

Dog 16–10. Yellow, mongrel, female, 34.5 pounds.

September 30, 1915. Ether anesthesia; bile fistula and splenectomy. Condition remained unchanged after operation. Weight varied between 29 and 31 pounds.

June, 26, 1916. See Table 61 for details.

July 15, 1916. Dog in good condition except for mange. Ether anesthesia and killed.

Autopsy at once. Thorax negative. Liver is of normal size. There is an increase in pigment in the parenchyma. Bile passages are pale and clear. The duct is completely obliterated just above the duodenum. Mesenteric and portal lymph glands are slightly enlarged and a bit pigmented a yellowish color.

Bone marrow shows slight hyperplasia.

Kidney shows a little pigmentation of the cortex as seen in human cases of pernicious anemia.

Intestine shows a normal mucosa and considerable pigmentation of muscle coats which are of a maple sugar color.

Other organs are negative.

Microscopical sections. Liver shows definite increase in the portal stroma which in places contains nests of mononuclears. The bile ducts are normal. The liver cells close to the hepatic veins contain bile pigment in small and large colloid like grains and lumps.

Bile fistula and splenectomy

Dog 16–27. Young mongrel terrier, female, 23 pounds.

December 8, 1915. Ether anesthesia. Bile fistula operation and splenectomy. Condition excellent after operation. Weight varied between 23 and 26.5 pounds.

April 24, 1916. See table 62 for details.

July 14, 1916. End of experimental period; 24.8 pounds; condition constantly good until September, when there was some bleeding from the bile fistula.

September 30, 1916. Ether anesthesia and killed.

Autopsy at once. Thorax negative. Liver is deep greenish and much pigmented as may be seen with long standing obstruction; portal tissue not conspicuous, except in region of fistula, which is thickened and warty looking. Common duct is cut across and isolated from the duodenum. Intestines and pancreas show a definite pigmentation as described above. Lymph glands about pancreas are slightly enlarged and pigmented. Other organs are negative.

Microscopical sections. Liver shows a considerable increase in bile pigment deposited in the liver cells close to the hepatic veins. The portal stroma is very slightly increased and contains a few mononuclear cells. Bone marrow of femur shows a distinct hyperplasia which is explained by the period of bleeding during the two weeks preceding death. About one-half of the marrow is made up of fat cells. The other organs show nothing of importance.

Bile fistula and splenectomy

Dog. 16–41. Large normal bull dog, male, 38.5 pounds.

December 2, 1915. Ether anesthesia, bile fistula and splenectomy. Postoperative condition excellent.

April 24, 1916. See table 63 for details; 36 pounds.

July 14, 1916. End of this experiment, 33.5 pounds.

March 7, 1917. Dog in fair condition, 30 pounds.

Dog has shown repeated periods of spontaneous icterus and blood regeneration as tabulated above.

DISCUSSION

At present we can not offer a satisfactory explanation for these periods of icterus and high bile pigment elimination which are observed in bile fistula dogs with splenectomy. It is not certain how much of the bile pigment in dog 16–41 has been built up into hemoglobin and then broken down into bile pigment, but we can say that if this did happen, then the construction of red corpuscles must have been going on at a terrific pace. This dog over a period of two weeks put out on an average of 200 mgm. of bile pigment per six hours, over six times normal. We may safely calculate on a minimum output of 600 mgm. per twenty-four hours, which represents 50 cc. of red corpuscles. This dog weighed 33 pounds, and we may estimate his blood volume as 1200 cc. and dur-

ing normal periods about 600 cc. of red cells. His count at this time is two-fifths normal, and we must assume that he possessed about 240 cc. red cells. To account for this amount of bile pigment as coming only from the red cells, we must assume the destruction of his total red cells every four or five days. This is scarcely conceivable when we consider the slow regeneration of red cells which occurs after simple anemia in control dogs. This calculation does not take into consideration the pigment escaping into the urine.

Moreover, we wish to point again to the color index of unity which indicates probably a saturation of the corpuscles with hemoglobin. This fits in perfectly with the tentative suggestion that such animals are manufacturing pigment substance at a maximum capacity. Some of the pigment substance is formed into hemoglobin until the red cells can hold no more and some of the pigment substance escapes in the urine. Much of it escapes in the bile. We do not believe for an instant that all this pigment substance has been built up into hemoglobin and then degraded to other pigment substances. We do not believe the body is capable of doing this to such an extent as is shown in the last table, 69. There is evidently some extremely powerful stimulus present under these conditions of associated anemia, splenectomy and bile fistula which drives the body to maximum speed in its manufacture of pigments for the blood, bile and other tissues. We believe the liver is the mainspring in this mechanism. We hope to report in the near future on some of the interesting points which are suggested by these experiments.

SUMMARY

Splenectomy added to a simple bile fistula modifies in no way the secretion of bile pigments in the bile. A large number of experiments control this statement.

Hemoglobin injected intravenously gives no *constant* increase in the output of bile pigments in the bile. Splenectomy does not modify this reaction. Variations from 4 to 42 mgm. are noted following a unit injection.

Dogs with bile fistulae, anemia and an added splenectomy may show some remarkable deviations from the control experiments.

Experimental anemia in these bile fistula splenectomy dogs may give a very remarkable reaction—periods of spontaneous icterus, blood destruction and high pigment output may alternate with periods of regeneration and low bile pigment output without any demonstrable

cause. An interaction of the liver and spleen in the *construction* as well as in the *destruction* of the hemoglobin and red cell stroma may be indicated by these experiments.

Regeneration of red cells with consequent recovery from the experimental anemia is very greatly prolonged and may occupy months in the splenectomy experiments as compared with weeks in the simple bile fistula experiments without splenectomy.

The color index may remain uniformly high during this long period of blood regeneration in the splenectomy experiments. The output of bile pigments may average considerably above normal, and we may suspect an overproduction of blood and bile pigments with perhaps a deficiency of red corpuscle stroma.

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BILE PIGMENT METABOLISM

IX. Bile Pigment Output Influenced by Hemoglobin Injection in the Combined Eck-Bile Fistula Dog

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The combination of a bile fistula with an Eck fistula has been studied in an earlier communication (1). The Eck fistula shunts the portal blood directly into the vena cava, and limits the blood supply of the liver to the arterial blood coming through the hepatic artery. The Eck fistula liver is smaller than normal, shows central fatty degeneration and usually a subnormal liver function. The Eck fistula liver secretes less bile pigment than the normal liver—often 50 per cent of normal or less. There is strong evidence that bile pigment formation is dependent in part upon the functional activity of the liver and not solely upon the disintegration of red cells.

With these points in mind, we must consider carefully the tabulated experiments given below. Is there any evidence that the Eck fistula dogs have difficulty in supplying the usual amount of blood and body pigment as one might expect from the low bile pigment output? The Eck fistula dog shows periods of anemia which we suspect may be referable to an inadequate supply of pre-pigment material (manufactured in the liver?). This point can not be settled without much further work which we hope to supply in the near future. We have observed that removal of 150± cc. of blood from an Eck fistula dog may depress markedly the blood hemoglobin level while the same procedure in a control dog may influence very slightly the blood hemoglobin level. This applies to observations made twenty-four hours after bleeding. This observation may be explained in part by the experiments of Lamson (2) who demonstrated the storage of red cells held in reserve in the liver. This reserve, which is present in the normal liver, may not be found in the Eck fistula liver.

Combined Eck and bile fistula dogs have less tendency to develop icterus with bile pigments in the urine than do the simple bile fistula dogs. We have no evidence that the Eck fistula liver can not excrete the pigment radicle of hemoglobin as promptly as the normal liver. If the hemoglobin were not removed with some promptness from the blood stream, we might expect more formation of bile pigments in the tissue outside of the liver and more bile pigment in the urine. This is contrary to fact, as there is much less tendency to icterus and pigmentation of the urine in the Eck fistula dog than in the control.

The injection of hemoglobin intravenously in Eck fistula dogs gives much the same reaction as in simple bile fistula dogs. The unit injection of 5 cc. of laked red cells should give an increase of 60 mgm. of bile pigment above the mean, provided the pigment radicle of hemoglobin is quantitatively eliminated in the form of bile pigment. Compare the control table 74 with tables 71, 72 and 73 of the Eck fistula. It is seen that the variations in bile pigment output after hemoglobin injection are perhaps more pronounced in the Eck fistula than in the control. If anything, the Eck fistula dog can take care of more hemoglobin than the normal dog with less bile pigment showing in the urine and less bile pigment increase from the fistula. But one cannot be sure of this point when the same dog will put out an excess of 25 mgm. of bile pigment above the mean following a unit injection of hemoglobin and a few days later put out zero milligrams of bile pigment above the mean following the same unit injection of hemoglobin (tables 72 and 73).

EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments were also used in experiments previously reported (1). Reference should be made to this paper for more complete details of control periods. The operative procedures have been reviewed. The general methods and experimental procedures have been described in detail in the first paper of this series (3).

The clinical history of each of these three dogs has been detailed in a recent publication (1), and need not be again outlined. The dogs were all in a uniformly good condition as regards weight, activity and diet. The tables give all experimental data.

The three preceding tables show several points of interest (tables 71, 72 and 73). The same dog with combined Eck and bile fistula shows a slow gain in hemoglobin from 38 per cent to 98 per cent during a period of three and one-half months. During the anemic period

(table 71) we see a marked reaction to a unit injection of hemoglobin. One injection of 5 cc. laked red blood cells given intravenously causes a rise in bile pigment secretion of 24 mgm. The curve of secretion is very sharp, and the apex falls usually during the second two-hour period after hemoglobin injection, the final period often showing a return to normal. The urine after such hemoglobin injections at the most shows a trace of bile pigment. The Eck fistula animals on the

TABLE 71

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE											WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean	URINE TOTAL BILE PIGMENTS, SIX HOURS	Bread, bone and milk diet
1916													*lbs.*	
March 27	15	12	18		45	2.3	4.4	4.0		10.7		trace	20.3	Hemoglobin 45 per cent
March 28	21*	14	13	9	36	3.3*	10.4	13.4	10.2	34.0	24.3	trace	20.5	
March 29	21	18	15		54	3.3	2.4	3.0		8.7		trace	20.0	
April 3	25	21	20		66	3.9	2.9	2.2		9.0		trace	20.8	Hemoglobin 38 per cent
April 4	14†	18	16	6	40	2.2†	2.4	3.2	2.0	7.6	0	trace	21.3	
April 6	22	22	18		62	4.9	4.4	4.0		13.3		trace	20.3	
April 7	25	16	18		59	2.3	3.1	1.9		7.3		0	20.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood cells given intramuscularly at end of second hour.

Mean daily output in table, 9.8 mgm. per six hours.

Average output for 30 control days is 11.8 per six hours.

Eck fistula operation September 13, 1915. Bile fistula operation February 17, 1916. Excellent condition throughout observations.

whole seem to store away the injected pigment more effectually than the simple bile fistulas. This statement is made with reservation in the face of wide fluctuations following hemoglobin injections in simple bile fistula dogs and bile fistula-Eck fistula dogs.

The after anemia period (table 73) shows even less reaction to hemoglobin injections. Two injections are followed by no bile pigment increase and one injection causes a rise of 19 mgm. in bile pigment

secretion. We get no evidence in any of these experiments that the Eck fistula liver can not eliminate the pigment radicle as promptly as the normal liver following hemoblogin injections. If anything, the Eck fistula liver seems to react more promptly than the normal liver in pouring out any excess of bile pigments which may be formed as a result of hemoglobin injections.

TABLE 72

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Bread, bone and milk diet
1916													*lbs.*	
June 5	26	18	12		56	2.4	2.0	0.8		5.2		0	21.0	Hemoglobin 74 per cent
June 6	19*	17	17	16	50	2.1*	6.1	16.8	12.0	34.9	27.0	trace	20.9	
June 7	20	11	8		39	8.6	4.4	1.6		14.6		trace	21.0	
June 8	16†	13	7	4	24	4.0†	7.0	10.2	2.1	19.3	11.4	trace	21.5	Hemoglobin 78 per cent. R. B. C. 5,352,000
June 9	15	11	18		44	1.7	0.7	2.0		4.4		0	21.8	
June 10					48					5.4		0	21.3	
June 12	15	15	12		42	2.6	3.0	1.1		6.7		0	20.8	Hemoglobin 72 per cent
June 13	16†	14	14	11	39	4.0†	7.9	8.2	4.4	20.5	12.6	trace	20.5	
June 14	13	6	5		24	8.6	2.0	0.6		11.2		0	20.3	
June 15	9†	12	16	12	40	6.8†	9.8	16.4	7.0	33.2	25.3	trace	20.3	
June 16	15	7	5		27	5.0	1.9	1.0		7.9		trace	20.5	

* 7.5 cc. laked red blood cells given intravenously at end of second hour.
† 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table — 7.9 mgm. per six hours.
Average output for thirty control days is 11.8 mgm.

Another fact deserves a word. It is to be noted in tables 72 and 73 that the color index of this dog is constantly low. Other periods show a very striking deviation from normal, and there is some evidence that the red cells contain a subnormal amount of hemoglobin. A similar condition may be found in simple bile fistulae with anemia, but it is

not as marked nor does it persist with a high red count as we have observed in the combined Eck-bile fistula. We may suspect that this lack of hemoglobin in the red cells may be due to inefficient pigment production in this dog. This point calls for much more work, and we believe more study should be directed toward pigment *construction*

TABLE 73

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Bread, bone and milk diet
1916												*lbs.*	
June 19	17	15	22		54	4.2	3.4	7.4		15.0		0	20.0	Hemoglobin 78 per cent
June 20	12*	12	11	8	31	5.4*	7.9	14.4	9.2	31.5	19.2	trace	20.5	
June 21	7	7	6		20	12.8	8.4	4.5		25.7		trace	19.9	
June 26	17	12	13		42	2.3	1.9	0.9		5.1		0	20.5	Hemoglobin 80 per cent
June 27	10*	11	9	8	28	3.6*	5.0	1.6	1.2	7.8	0	0	20.3	
June 28	13	9	8		30	2.9	1.2	0.8		4.9		0	20.5	Feces contain stercobilin
July 3	16	13	9		38	3.6	2.6	1.2		7.4		0	20.5	
July 5	20	15	14		49	2.8	1.7	1.3		5.8		0	20.3	Hemoglobin 98 per cent. R. B. C. 6,332,000
July 6	14*	11	9	10	30	4.1*	5.7	5.4	4.9	16.0	3.7	0	20.5	
July 7	9	7	11		27	7.3	6.6	2.7		16.6		0	20.3	
July 8					28					18.2		0	20.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table — 12.3 mgm. per six hours.
Average output for thirty control days of this period, 11.8 mgm.

rather than pigment *destruction*. We hope to submit experiments which may give information concerning pigment construction in the body.

Dog 16–146 (table 74) acts as control to the three preceding Eck fistula tables. This control dog is of about the same weight, activity

and temperament as the Eck fistula dog (16–15). Both the control dog and the second Eck fistula (table 75) show much variation in bile pigment secretion following the unit injection of hemoglobin.

TABLE 74

Simple bile fistula control. Hemoglobin injection

Dog 16–146

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
June 5	11	16	18		45	6.4	7.6	6.8		20.8		0	17.5	Hemoglobin 84 per cent
June 6	16*	14	21	18	53	5.4*	17.0	21.8	14.5	53.3	33.5	+++	17.5	Urine contains no urobilin
June 7	14	17	17		48	3.8	6.1	6.1		16.0		trace	17.8	
June 8	14*	16	19	13	48	5.4*	13.2	17.1	7.3	37.6	17.8	++	18.3	
June 9	17	16	14		47	6.8	7.2	5.4		19.4		trace	18.3	
June 10					36					14.8		0	18.2	
June 12	16	17	16		49	8.6	7.6	7.6		23.8		0	17.8	Hemoglobin 90 per cent
June 13	9*	13	19	15	47	5.1*	13.4	17.8	10.4	41.6	21.8	++	18.0	
June 14	11	14	14		39	7.6	6.9	6.9		21.4		trace	18.3	
June 15	16*	19	17	17	53	8.6*	15.6	16.0	14.4	46.0	26.2	++	18.5	
June 16	12	12	11		35	8.2	9.2	7.0		24.4		trace	18.3	Stools contain no stercobilin
June 17					42					18.8		trace	18.0	
June 19	20	17	15		52	6.8	6.5	7.2		20.5		trace	18.0	Hemoglobin 89 per cent
June 20	13*	16	14	15	45	3.8*	9.7	12.8	12.0	34.5	14.7	trace	17.8	
June 21	14	12	16		42	6.4	6.4	5.4		18.2		trace	17.5	

* 5 cc. laked red blood cells given intravenously at the end of second hour.
Mean daily output in table = 19.8 mgm. per six hours.
Average of twenty control days is 18.1 mgm.

Table 75 (dog 16–139) shows an unusually high mean bile pigment output, in fact, practically normal for a control dog of equivalent weight. It can be seen in an earlier communication (1), table 36, that this same dog in the previous month put out 14.2 mgm. per six

hours. After the hemoglobin injections were begun, there is a constant high level of bile pigment elimination. We do not believe that one depends upon the other, but must admit that there may be some possible connection. Secondly, it is to be noted that this dog devel-

TABLE 75

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–139

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Bread, bone and milk diet
1916												*lbs.*	
May 31	8	12	11		31	5.8	6.4	5.0		17.2		trace	29.0	Hemoglobin 86 per cent
June 1	9*	9	11	11	31	4.9*	3.6	11.4	10.0	25.0	0.6	trace	29.0	
June 2	10	12	14		36	9.2	8.2	5.4		22.8		trace	29.3	
June 5	13	13	11		37	10.5	9.4	5.9		25.8		trace	28.8	Hemoglobin 88 per cent
June 6	7*	10	10	10	30	6.6*	10.4	16.2	15.0	41.6	17.2	++	29.3	Urine contains no urobilin
June 7	6	9	12		27	7.2	8.0	6.7		21.9		+	29.3	
June 8	14*	12	11	9	32	11.2*	9.7	12.2	11.6	33.5	9.1	++	29.5	Hemoglobin 82 per cent. R. B. C. 5,416,000
June 9	7	12	13		32	9.2	11.9	8.8		29.9		trace	29.5	
June 10					30					25.8		trace	29.8	
June 19	14	13	12		39	6.4	6.2	6.2		18.8		trace	29.0	Hemoglobin 91 per cent
June 20	17*	17	14	15	46	6.8*	13.0	17.0	16.2	46.2	21.8	++	29.0	
June 21	13	12	14		39	12.8	10.8	9.4		33.0		++	28.8	
June 22	7*	11	11	12	34	16.0*	20.0	15.0	21.2	56.2	31.8	++	29.0	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 24.4 mgm. per six hours.
Mean daily output in month previous, 14.2 mgm. per six hours.

oped frank clinical distemper within two weeks after this experiment ended, and it is possible that the disease was latent in this animal during this period of experimentation. Distemper is an infection in dogs which can cause a variety of unusual complications.

SUMMARY

We have submitted evidence that the Eck fistula liver secretes less bile pigment than the control liver. It is possible that bile pigment formation is dependent in part upon liver function rather than upon the disintegration of red cells.

It is probable that the Eck fistula liver can eliminate hemoglobin from the blood stream as promptly as the control liver.

The Eck-bile fistula dog has less tendency to icterus and staining of the body tissues with bile pigment. Even with a high red cell count the color index will often be low in the Eck fistula. This is evidence to show that the Eck fistula dog has a subnormal pigment building capacity.

The Eck-bile fistula dog shows the same great fluctuation in bile pigment excretion following a unit injection of hemoglobin. It is possible that these dogs can store more pigment substance than the control dogs after unit injections of hemoglobin.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

II. Volume Changes and Venous Discharge in the Spleen

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In the first paper of this series (1) attention was called to the desirability of further investigation of the effects of adrenin on the blood-flow in various organs with adequate attention to dosage and duration of administration of the drug.

Apparently in previous studies along this line the spleen has received relatively little attention. Oliver and Schaefer (2) were the first to investigate the effects of suprarenal extract in this organ. In the several cases studied the reaction to the extract was an "enormous" contraction. In none was any dilatation observed except for a short time preceding the reaction proper. This was regarded as probably a passive effect. Bardier and Frenkel (3) recorded the results of a study on a single animal, which was, apparently, under the influence of curare. Their extract was made by macerating desiccated or fresh gland for twenty-four hours at 37°C. After three injections they noticed: (a) Dilatation for three minutes followed by constriction while systemic blood pressure rose from 110 to 220 mm.; (b) Contraction followed by dilatation while the systemic pressure varied between 100 and 120 mm.; (c) Dilatation followed by contraction while the arterial pressure varied from 80 to 180 mm. Judging from the initial arterial pressures in each case the animal was relapsing into shock. The vasomotor reactions in the first and third cases indicate that the dosage transcended physiologic limits. What part was played in the reactions in the spleen by the curare and by protein decomposition products in the extracts was not determined. Falta and Priestley (4) observed that the spleens of animals exposed several hours after subcutaneous injections of large doses of adrenin appeared anemic. Vincent (5) without giving any details states that adrenin administered intravenously causes a

contraction of the spleen. All the foregoing observations indicate that the outstanding effect of adrenin in the spleen is a marked contraction but both primary and secondary dilatations have been recorded. We have extended the investigations as herein reported.

Technique. In all cases dogs have served as experimental animals. In most instances ether or morphin-ether anesthesia was employed but a few were decerebrated. The experimental results in both cases were similar. In some instances the vagi were cut but this procedure made no apparent difference in the outcome. The adrenin (Parke, Davis "Adrenalin") was introduced into a femoral vein, sometimes instantaneously and sometimes slowly. Various dosages were used depending upon whether pressor or depressor effects were desired. Simultaneous records were made of changes of splenic volume or venous outflow or both and of changes of femoral arterial pressure.

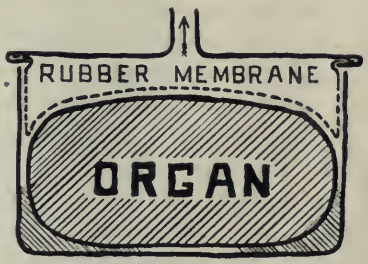

Fig. 1. Diagram showing construction of plate plethysmograph.

Various types of plethysmographs were tried, including a Roy's oncometer. The most satisfactory type was one improvised in the laboratory; it is somewhat like that of Edmunds. The general plan of the apparatus is shown in figure 1. A box was selected of suitable size to contain the organ under investigation. For this purpose a celluloid soap box proved satisfactory. Such boxes are light, inexpensive and easily procurable at any shop where toilet accessories are sold. The volume changes were transmitted by means of a plate plethysmograph which served as a lid for the box. It was made as follows: A sheet of copper, gauge 24, was cut the same shape as the top of the box and about 2 cm. greater in diameter. A sheet of rubber tissue was then cut in turn 2 cm. greater in diameter than the copper plate. A hole was drilled through the middle of the plate to communicate with a brass tube 3 mm. in diameter soldered to it to form the outlet of the plethysmograph. The edges of the plate were then bent up around the four sides to make a shallow box about 0.5 cm. deep. The margin was incised at intervals to permit this bending. Melted collophonium wax or marine glue was introduced as a narrow zone around the outer edge of the box. This served to affix the rubber membrane which was next introduced. The excess circumference of the membrane was taken up in a series of pleats

which were held in place by bending inward and down the sides of the box. A marginal zone of glue or collophonium wax applied just before the copper was bent down served to make the joint hermetically tight. In case this result was not secured at the first attempt a bit more of the cement was introduced to stop the leak or else the rim at that point was simply held for a moment in hot water to melt the cement already applied and allow it to flow into the crevice.

In using this type of plethysmograph the organ is isolated and placed on a wet cotton pad in the box. A slit is cut in the side of the box to accommodate the blood vessels of the organ. The plate plethysmograph is then applied as a lid to the top of the box and secured in place by two rubber bands. It is then attached by rubber tubing to a recorder. A T-tube interpolated in the connecting tube permitted the inflation of the plethysmograph with air under a slight pressure sufficient to cause the membrane to adapt itself to the inclosed organ but not great enough to interfere in any way with the circulation.

In a few instances the Cushny cardiograph was found to give satisfactory results in recording changes in the dimensions of the organ but its use involved a greater degree of exposure of the viscera than did the plethysmograph.

Venous outflow was recorded by drops from an oiled cannula tied in a relatively small vessel. In some cases the spleen was divided and volume change and outflow determined simultaneously.

The float recorder previously described (1) was employed in all cases.

Results. In determining the effects of adrenin in the spleen 17 dogs were used. In the plethysmograph studies 65 injection and 18 infusion experiments were made while the venous outflow series included 34 injection and 20 infusion experiments.

In nearly all cases the effects on organ volume were similar to those described by Oliver and Schaefer for pressor injections, namely, a brief, supposedly passive dilatation followed by marked contraction. The same results were also obtained with depressor injections and infusions as well as with pressor infusions. Figure 2 which was obtained with a slightly depressor infusion will suffice to illustrate all these cases. It was found to be possible to hold a spleen in a state of uniform contraction by adrenin infusion for ten minutes. Longer periods were not tried. The effect in the spleen lasted from a half to five minutes after blood pressure had returned to normal. In no case with either large or small dosage was a secondary dilatation observed during an infusion period.

Occasionally, however, as shown in figure 3, a dilatation occurred after the administration of the adrenin was discontinued. This effect was not passive since it was noted when the arterial pressure was either normal or depressed. With no dosage was a pure splenic dilatation observed.

The threshold for the reaction in the spleen was highly variable but generally it was lower than that for changes of arterial pressure. In the most sensitive preparation investigated splenic contraction first appeared with an injection of 0.5 cc. of a 1: 2,000,000 solution. This means of course that the spleen is one of the most sensitive organs in the body and reacts before a sufficiently widespread effect occurs to influence the general blood pressure.

Fig. 2. Spleen contraction under the influence of adrenin, depressor infusion. Dose 2.8 cc. 1: 200,000 in 65 seconds. Dog weight 15 kilos. Time, five seconds.

In several instances spleens that were previously quiescent began to undergo rhythmic changes in volume after the injection of adrenin. In such cases a new injection would check the rhythmic contractions but they would begin again as the effect of adrenin wore off.

The effect of adrenin on venous outflow was just what would be expected from a consideration of the volume changes. A typical graph is reproduced as figure 4. During the preliminary dilatation period the flow was augmented. The augmentation persisted during the first part of the contraction period as the blood already in the organ was being expelled. Then during the remainder of the contraction period the flow was depressed, reaching the normal rate at about the same time splenic volume was restored to normal. The depressed outflow during the latter part of the period was obviously due partially to retention of the blood in the expanding organ.

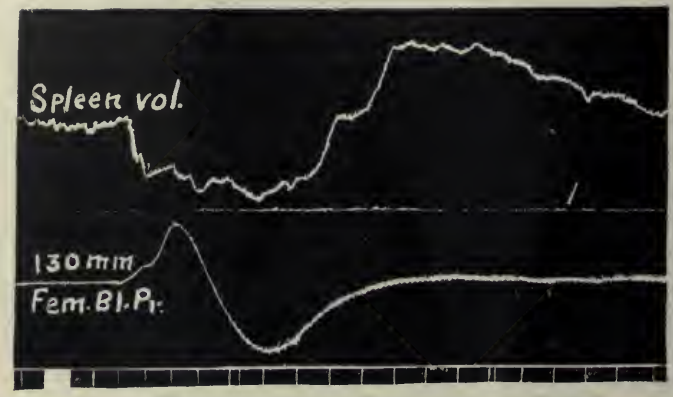

Fig. 3. Shows secondary dilatation of the spleen after injection of adrenin, 1 cc. 1: 50,000. Time, five seconds.

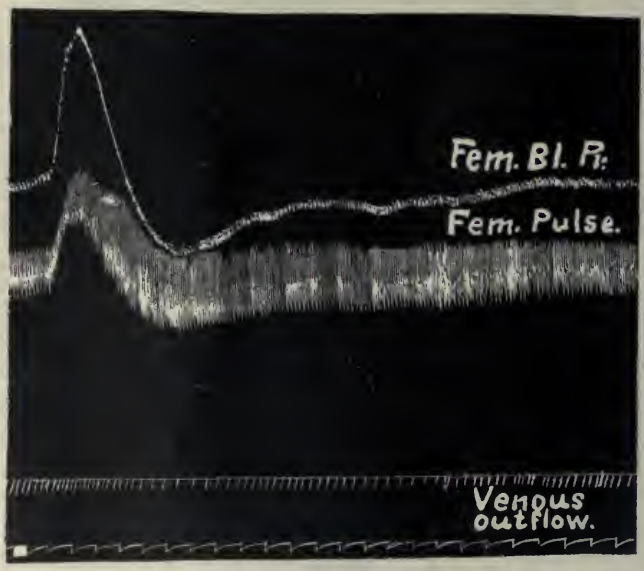

Fig. 4. Shows effect of injection of adrenin, 1: 100,000, on outflow from splenic vein.

SUMMARY

Adrenin in all effective dosages whether injected instantaneously or infused causes in the spleen a brief dilatation followed by a contraction.

Occasionally the contraction is followed by a secondary dilatation after the administration of the adrenin is discontinued.

Aside from the brief preliminary effect in no case was a pure (primary) dilatation observed.

The threshold for splenic contraction is lower than for changes in arterial pressure.

Occasionally a quiescent spleen is stimulated by adrenin to rhythmic contractions.

Adrenin causes a brief increase, then a decrease in the outflow from the splenic veins.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

III. Volume Changes and Venous Discharge in the Kidney

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The effects of adrenin on vascular conditions in the kidney, as in several other organs, were first studied by Oliver and Schaefer (1). Without going into details as to any possible differential effects of large and small doses, of the effects of long continued administration of the extracts or of after effects, they reported that suprarenal extracts cause a marked diminution in kidney volume. Largely on the basis of this fact and the observation that the spleen is similarly affected the generalization was offered that such extracts cause a marked vasoconstriction throughout the splanchnic area.

Four years later, in 1899, Bardier and Frenkel (2) investigated the relation of suprarenal extracts to diuresis but included also in their study the vasomotor effects. Their experiments were made on anesthetized dogs, apparently under the influence of curare. Their extracts were made either from desiccated glands or from fresh glands macerated for twenty-four hours at body temperature. Judging from the effects on arterial pressure relatively large doses were employed. The extracts were administered intravenously. These authors described as typical effects a contraction of the kidneys and a depression of urine flow followed by a dilatation of the organ and a diuresis. In certain exceptional cases, however, the injections were followed at once by dilatation and polyuria. Whether the use of curare or the presence of protein decomposition products in their extracts played any part in the results was not determined.

In the same year Gottlieb (3) included the kidneys in a series of experiments on the effects of adrenal extracts on the heart and blood vessels. He worked on isolated kidneys of hogs and dogs. It was noted that when such extracts were added to the perfusate that was being

passed through the organs a marked decrease in outflow resulted. This finding was corroborated by Gioffredi (4). Similar experiments were made by Sollmann in 1905 (5). But in addition to the venous outflow the rate of urine discharge was also studied by this investigator. When adrenin was added to the perfusate to make a dilution of 1:50,000 both the venous and urine flow were markedly decreased and the kidney volume as determined by an oncometer was also lessened. The dogs from which the kidneys were obtained had received large doses of morphine. It may be noted that Sollmann used solutions from twenty to one hundred times as concentrated as the adrenin solution in the adrenal veins as determined under ordinary experimental conditions.

In three perfusion experiments Pari (6) found that solutions of 1:20,000 to 1:100,000 caused renal vasoconstriction, but in one case a solution of 1:500,000 caused dilatation for a few minutes followed by constriction.

Jonescu (7) recorded the effects of intravenous injections of adrenin on the blood pressure and kidney volume of rabbits. Doses which caused a moderate rise of blood pressure caused a slight dilatation of the kidneys which was followed by a marked contraction that persisted for some time after the arterial pressure had returned to normal. When smaller doses were used the kidneys showed contraction while the blood pressure remained practically unchanged. From this observation a theory was deduced that the kidney vessels have a special affinity for adrenin. It would be possible, therefore, for the adrenals by a slight continuous overactivity to set up a nephritis without any significant vascular hypertension. The theory is obviously untenable on the grounds cited. The work of Hartman (8) and that reported in the first of this series (9) accounts for the effects observed without invoking any special "affinity" of the renal vessels for adrenin. While the kidneys were contracting blood-vessels in other parts of the body were expanding, hence the systemic pressure was little affected.

In a short paper published in 1911 Froehlich (10) reported that both *l*- and *d*-suprarenin as well as "adrenalin" cause a protracted contraction of the kidneys. A more extensive investigation along the same line was reported by Ogawa (11) a year later. He used both *l*- and *d*-adrenin and synthesized *l*- and *d*-suprarenin. Instead of the oncometer method, however, he utilized perfusions to determine the effect of the drugs on the kidney vessels. Rabbit kidneys in the most sensitive preparations reacted slightly to a dilution of *l*-adrenin of 1:20,000,000 showing a diminished outflow. In case of a solution of 1:1,000,000

the primary effect was a sharp decrease in the rate of outflow followed by a rate above normal when the adrenin was discontinued. The augmented outflow was noted also as a secondary effect if the adrenin perfusion was continued for a relatively long period. These secondary dilatations appeared only if relatively strong solutions were used—that is, dilutions of from 1: 1,000,000 to 1: 5,000,000. In two instances primary dilatation was noted with solutions of 1: 40,000,000 and 1: 50,000,000, respectively. The same results were obtained with *d*- as with *l*-adrenin except that stronger solutions had to be used. The synthetic product also gave qualitatively similar effects. In cats and dogs the same results were obtained but the threshold was higher.

In all the foregoing reports renal vasoconstriction following the administration of adrenin is a prominent feature. In most cases, however, the doses used were probably greater than the quantity that can be discharged from the suprarenal glands in a corresponding length of time.

The evidence, so far as it goes, indicates that urine secretion follows *pari passu* the vasomotor effects produced in the kidneys by adrenin. This renders important a definite determination of the question whether the vasodilatation reported by Bardier and Frenkel and by Ogawa is a significant feature of the response to adrenin injections. If it is characteristic then adrenin diuresis such as has been described by Kleiner and Meltzer (12) may well be due, partially, at least, to local vasomotor effects in the kidneys. The suprarenal glands might then justly be brought into question as involved in "spontaneous" diuresis. The fact that vasodilatation was observed only as a secondary effect with larger doses when the adrenin would be largely destroyed or the mechanism fatigued, or as a primary effect only when very small concentrations were employed points toward this as a physiologic mechanism, since it is probable that it is only with very high dilutions that the body normally has to deal. This might be correlated with the fact observed by Kleiner and Meltzer that in order to produce diuresis adrenin must be administered as to be slowly absorbed whereas in cases in which it reaches the kidneys promptly it acts as a renal depressant (Cow, 13). In our experiments, the report of which follows, we have accordingly been especially interested in any possible dilator effects that might appear.

Technique. In general we have followed the same methods recorded in the two preceding papers of the series. The plate plethysmograph and float recorder therein described were utilized. Etherized dogs have been used exclusively in the work on the kidneys. In most

cases only plethysmograph studies were made but in a few instances venous outflow was also recorded.

Results. In this series 16 dogs were used. In the investigation of volume changes 151 injection and 5 infusion experiments were made. In determining the effects on venous outflow 14 injections and 3 infusions were given.

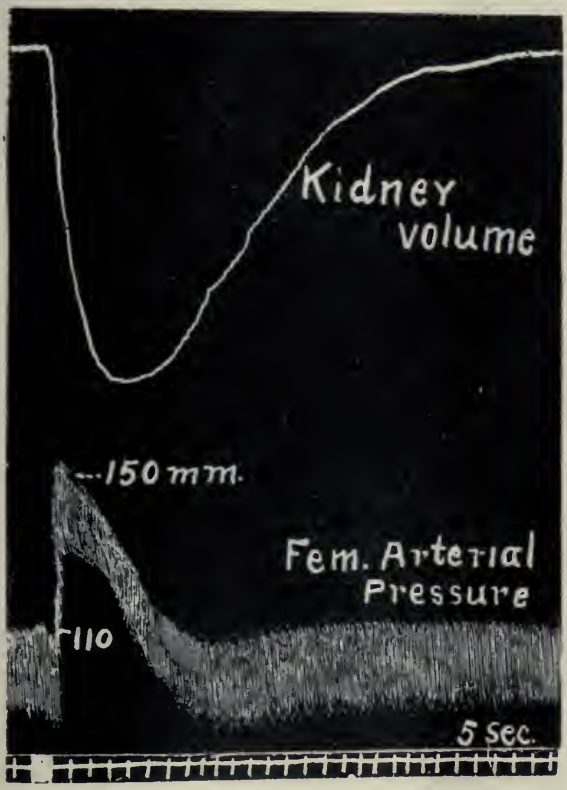

Fig. 1. Kidney contracting under influence of adrenin, 4 cc. 1: 100,000. Blood pressure from femoral artery. Time, five seconds. Dog, weight 18 kilos.

The results in the kidney were in general much the same as those in the spleen. The outstanding feature of the reaction was a sharp contraction such as that shown in figure 1. The graph reproduced is unusual, however, in one respect: neither the arterial pressure nor the organ volume show the preliminary augmentation that generally appears after adrenin injections. This augmentation which is short

lasting and inconsequential in degree is regarded as purely a passive effect due to the sudden stimulation of the heart before the outlying tissues are affected. It will be noted that the volume change outlasts for some time that of the arterial pressure. This lag is a characteristic part of the reaction picture. In various cases it was found to persist from a half to two minutes. It was usually longer towards the end of a series of experiments.

In one case only was a different type of reaction observed. This is illustrated in figure 2. After the characteristic passive preliminary dilatation as the arterial pressure rose the organ contracted. Then as arterial pressure began to fall the kidney dilated only to return to its

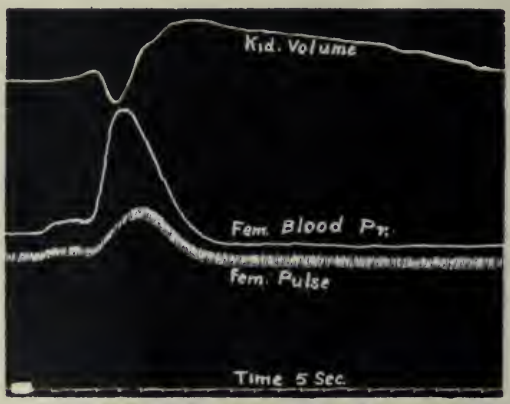

Fig. 2. Kidney expanding under influence of small dose of adrenin, 0.5 cc. 1: 200,000. Pulse and pressure from femoral artery. Time, five seconds. Dog, weight 8 kilos.

initial volume a minute after the normal blood pressure was restored. This result was observed when such doses as 0.5 cc. of 1: 100,000 were administered. When this dose was doubled the ordinary contraction of the kidney appeared and outlasted the change of blood pressure.

In no case did a pure dilatation occur such as was reported by Ogawa in two of his rabbit experiments when very small doses were used. Neither in any of our experiments was a secondary dilatation observed either during infusions or after injections or infusions such as was regarded by Bardier and Frenkel as a characteristic feature of the reaction. It should be noted, however, that only five infusion experiments were made. The results shown in figure 2 suggest that in a larger series

animals might occasionally be found which would show a dilatation of the kidneys during infusions with adrenin in high dilution. In view of the fact that infusions gave qualitatively the same results as injection in all cases observed, this possibility was not extensively investigated.

It was found that the kidneys could be held for at least ten minutes in a uniform state of contraction. Longer periods were not tried. The threshold for changes in kidney volume and for changes of blood pressure were about the same. The reactions in the kidney were qualitatively similar irrespective of whether pressor or depressor dosages were employed.

Our observations consistently support the reports of previous investigators that adrenin decreases the venous outflow from the kidney.

Our experiments as a whole do not support the theory that adrenin diuresis is due to a dilator effect of small quantities of adrenin in the kidney. On the other hand they do not definitely exclude the possibility that such may be the case in a normal unanesthetized animal. The situation as regards adrenin diuresis may well be not unlike that as regards pituitrin diuresis. From the fact that pituitrin in anesthetized animals often gives a short lasting polyuria a theory has been deduced and widely held that this substance is a diuretic agent in the normal organism, whereas, as a matter of fact it is an efficient anti-diuretic (14). In view of the evidence (a) that adrenin in doses which cause renoconstriction depresses urine formation; (b) that adrenin administered subcutaneously, and consequently, absorbed slowly, causes polyuria; and (c) that in anesthetized animals renodilatation has occasionally been reported as a result of administering adrenin in high dilutions or as a secondary reaction with larger quantities, the theory is not improbable that in normal animals adrenin in relatively small quantities causes a dilatation in the kidneys. Possibly the matter could be definitely determined by attaching metal guide strips to the poles of a kidney and then after recovery from the operation had occurred studying with a fluoroscope the renal volume reactions to adrenin when no anesthetic was used.

Marshall and Davis (15) have reported that ablation of the suprarenal glands results in depression of the functions of the kidneys even while systemic blood pressure remains normal. If, as is generally assumed, the adrenals keep the blood supplied with minute quantities of adrenin, the depression noted might in harmony with the above mentioned theory be ascribed to a deficiency of circulating adrenin, leaving the renal constrictor factors in the ascendancy. The supposition is,

however, intrinsically improbable. That renal functioning should be dependent upon minute quantities of such a dilator substance, that a hormone reaction should have been evolved to function in this purely negative way to correct a gratuitous overactivity of some other factor would seem to be a useless complication.

SUMMARY AND CONCLUSIONS

Adrenin in both depressor and pressor doses ordinarily causes contraction of the kidneys of dogs and a corresponding decrease in the venous outflow.

Instantaneous injections and slower infusions of adrenin give qualitatively similar results.

One animal gave renodilatation with smaller and renoconstrictions with larger doses.

The threshold for renal changes and blood pressure changes is about the same.

The observations as a whole do not support but also do not definitely disprove the theory that in normal animals adrenin diuresis is due to renal dilatation.

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FURTHER OBSERVATIONS ON THE DIFFERENTIAL ACTION OF ADRENALIN

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In the course of a series of experiments performed by one of us and reported in this Journal (1), it was found that the fall in general blood pressure, which is caused by the intravenous injection of small doses of adrenalin, is not brought about by dilatation in the vessels of all parts of the body alike. In an animal in which the arteries to the abdominal organs have been clamped, injections of a standard small dose of adrenalin caused much the same fall as had previously occurred in the intact circulation. On the other hand, when the arteries to the limbs were occluded, those to the splanchnic area being intact, the reaction to the standard dose was changed from a pure fall to a rise of blood pressure, as registered from the carotid artery. In that research the only distinction drawn was the broad one as between the reactions of the "peripheral" circulation on the one hand, which included the vessels of bone, muscle, and skin, and that of the "splanchnic" circulation on the other, which, as well as comprising the vessels of the abdominal and thoracic viscera, necessarily included those of the muscles of the thorax and back. The present research was undertaken with a view to following out the subject of the differential action of adrenalin somewhat more in detail, and in the hope of arriving at some conclusion as to the mechanism involved in the vascular adjustment caused by it.

Oncometric experiments were carried out on intestine, spleen, and kidney. While our research was in progress the appearance of the paper by Hoskins, Gunning and Berry (2) made further investigation of the reactions in skin and muscle unnecessary.

In every case simultaneous records were taken of the reactions of at least two organs in response to adrenalin, since we considered it of importance to determine whether the same range of dose which caused constriction in any one abdominal organ also caused constriction in all the others, and whether the amount giving rise to dilatation in one was

necessarily the same as that which caused another to dilate. Since the reaction of any organ to a given dose may vary not only among different individuals, but also in the same individual during the period of an experiment, it is necessary to record the changes occurring in the same animal, at the same time.

The animals used were dogs and cats. In two of the experiments on the latter we injected urethane subcutaneously; in all the others the anesthetic was ether. Blood pressure was registered from the right carotid artery. Injections of adrenalin were made with a graduated syringe, the needle of which was thrust through the wall of a piece of rubber tubing fitted to a cannula, which was inserted low in the left jugular vein. The adrenalin solution used was that manufactured by Parke, Davis and Company, 1:1,000, diluted with distilled water to the required strength immediately before use. In each experiment the first injections were made of a solution 1:100,000; when large doses were required, as was often the case in working with dogs, in preference to injecting large quantities of distilled water into the animal's circulation, we substituted for the more dilute solution one of a strength of 1:10,000. The duration of each injection was signaled on the record below the time marker. No special precautions were taken as to absolute uniformity in the rate of injection, but it was kept fairly constant, and was in all cases slow, as shown by the records.

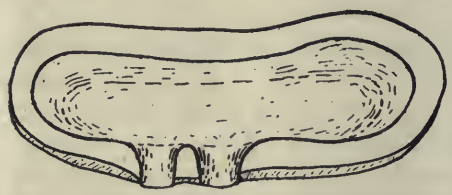

Fig. 1. Gutta percha oncometer for spleen

In some experiments we left the vagi intact; in the majority they were cut. We were unable to observe, however, any specific effect of these on the reaction of any organ to adrenalin except the familiar one of cardiac inhibition caused by large doses, with the consequent great rise in blood pressure.

The oncometers which we used for kidney and for intestine were gutta percha ones of the ordinary type, fitted with glass lids. The early experiments on the spleen were done with the same oncometers; later we had a series of special ones made. These were modelled after the shape of the spleen (see fig. 1) and were provided with two lips for stalks, separated by about 0.6 cm. in the smaller and 1.5 cm. in the larger.

As recorders in the first few experiments we used Marey's drums;

later we substituted for these bellows recorders, which have the advantage of recording volume changes without introducing alterations of pressure within the system itself. In several experiments the recorders were calibrated by injections of known volumes of air.

The pressure inside the system differed little from atmospheric; in practice we raised the pressure until the bellows were about half filled, and were thus adjusted to give maximum variations in either direction.

INTESTINE

A loop of the small intestine, about one-third of its total length, was selected, generally that immediately above the caecum, since the blood vessels there are long and form a convenient stalk. Two pairs of double ligatures were tied about its lower end, about 2 inches apart, the blood vessels supplying the piece between the ligatures tied off, and the piece of intestine removed. A similar operation was performed at the upper end of the required length, and the loop was ready to be placed in the oncometer without the necessity of further dissection, other than simply slitting the mesentery for an inch or two on either side of the stalk. Before putting the loop into the oncometer we washed out its contents with warm saline. This prevents the slow formation of gas which otherwise takes place within the lumen, and which interferes with the records of volume changes due to the circulation alone. The whole operation from the time the abdomen was opened until the intestine was put into the oncometer was not longer than fifteen or twenty minutes. During this time the intestinal loop and the other abdominal contents were kept covered with warm saline pads.

Fig. 2. Constriction of the intestine following injection of a small dose of adrenalin (0.4 cc., 1 : 100,000). Dog 14 (Reduced $\frac{1}{3}$)

In the great majority of cases, the effect of doses of adrenalin, both large and small, was to cause constriction of the intestine. In all of

these experiments small doses caused only constriction (figs. 2 and 6); as the quantity of adrenalin was increased, however, a prolonged and marked dilatation supervened on the preliminary constriction (see figs. 3 and 7). There were two exceptions, in which the least effective dose caused dilatation. These occurred during the early experiments, before we had fully realized the importance of completely removing gas from the lumen, and we consider it probable that this increase in volume of the loop was caused by the relaxation of the muscles of its walls, under the influence of the adrenalin.

The threshold for the constrictor effect was shown, in the six experiments in which it was determined, to vary within fairly wide limits, from 0.014 to 0.07 cc. adrenalin 1: 100,000 per kilogram body weight, that is, it was reached by doses such as also caused a slight fall in blood pressure. The general resemblance of these two curves, indeed, make it at first glance appear possible that the one effect may be dependent on the other, and that the constriction in the intestine may be nothing more than a decrease of blood supply to its vessels, caused by the lowering of the general blood pressure. Latent periods, duration, and degree of decrease of intestinal volume, also bear some relationship to the same changes in the general blood pressure. Closer inspection of the tables (1 and 2), however, shows clearly that this is not the case. Though the diminution in intestinal volume generally occurs several seconds after the beginning of the fall of blood pressure, this is not always the case; for instance in experiments 13, 14, and 20, the records show the intestinal decrease to precede that of the blood pressure by several seconds, and our notes, made during the course of the experiments, corroborate this as actually occurring, and not being due to a possible faulty alignment. The time of the least intestinal volume does not correspond to that of the lowest blood pressure, nor is the duration of the constriction the same as that of blood pressure fall. (See experiments 7 and 13, where it is greater, and experiments 3, 18 and 20, where it is materially less.) Above all, the constriction does not take place only when the dose is such as to cause a fall of blood pressure; constriction of the intestine occurs time and time again when the blood pressure is above and not below its normal level (see figs. 3 and 7).

The dose of adrenalin necessary to cause a dilatation of the intestine to follow on this constriction is as variable as is the threshold dose for the constriction itself. It varies from 0.04 to 0.31 cc. of a solution 1: 100,000 per kilogram in dogs, and in cats it is about 0.4 cc. The latent period of the dilatation is longer than that of the constriction,

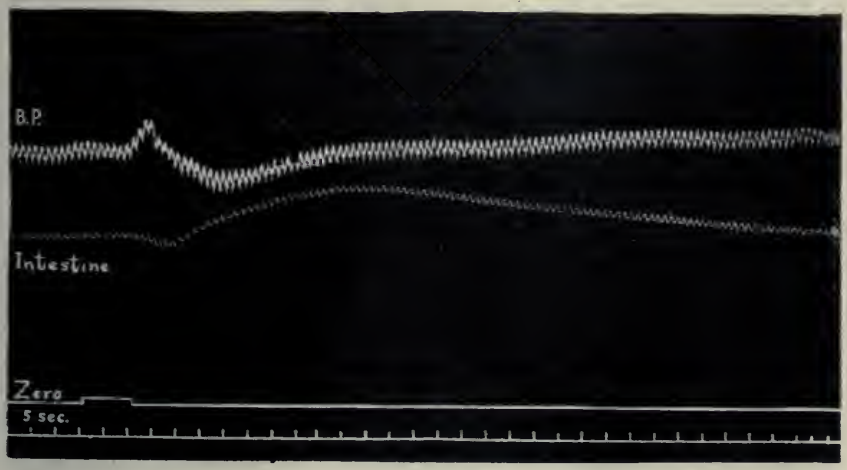

Fig. 3. Preliminary constriction followed by prolonged dilatation of the intestine, caused in the same animal as that of figure 2, by a larger dose (0.2 cc., 1 : 10,000) (Reduced ⅓)

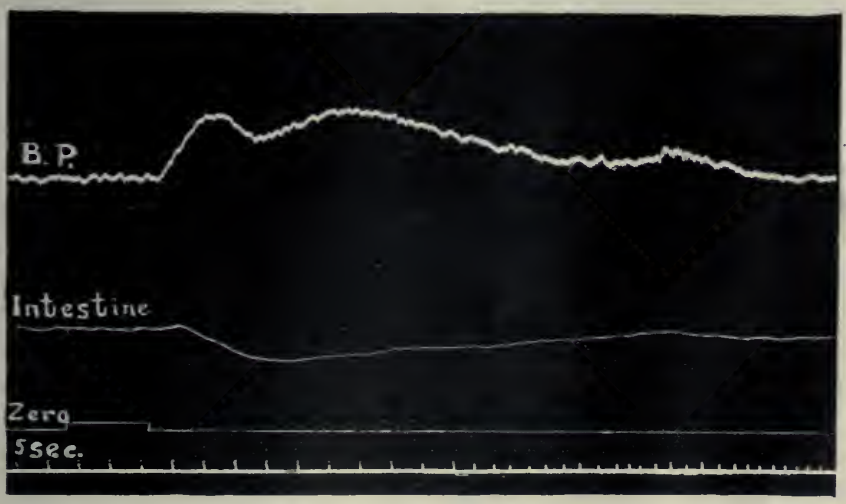

Fig. 4. Reaction of same loop of intestine as that of figures 2 and 3, to a dose of adrenalin of much the same magnitude (0.3 cc., 1 : 10,000) as that of figure 3, after the coeliac and superior mesenteric ganglia had been removed. (Reduced ⅓)

and the effect is as if the one were superimposed upon the other. As the doses are increased from the threshold dose on, the resulting constriction becomes more and more marked and its duration longer. Once the dose is great enough, however, to cause dilatation, this cuts short the first effect, with the result that the latter is reduced by from one-fourth to two-thirds of its former length. The volume change brought about by dilatation is much larger than that caused by constriction. For instance, in a dog of 25 kilograms the constriction reduced the volume of the system of the intestinal oncometer by 1 cc., while the dilatation increased it by more than 5 cc.

The observations on the resemblance of the curve of constriction to that of the general blood pressure may be applied in much the same way to this case also, for in increasing the doses of adrenalin sufficiently to cause a dilator effect in the intestine, in a few cases we crossed the threshold for pressor effect on the blood pressure. The same arguments, however, which prevented our accepting the explanation of a passive effect in constriction, are also valid in this case. The latent periods of intestinal effect are longer than those of blood pressure fall, the time of maximum dilatation never coincides with that of maximum rise of pressure, and its duration is far greater, in many instances two to three times as long (experiments 21, 22, 23, table 2). As before, too, the occurrence of the intestinal effect does not depend on the nature of the blood pressure change; we have several records which, like experiments 3 and 7, show a marked increase in intestinal volume during a fall in general blood pressure.

As a possible explanation for the occurrence of increase in the intestinal volume, after injections of doses of adrenalin above a certain size, it might be suggested that such doses are just sufficient to affect the intrinsic nervous system of the intestine and to bring about relaxation of its walls, thus permitting expansion of the blood vessels within them. To investigate this, we inserted a rubber balloon into a part of the intestine immediately adjacent to that which furnished the loop in the oncometer, injected a small quantity of air, and connected it to a small bellows recorder, which made a tracing below the oncometer record. By this it was found that injections so small as to cause only a slight fall in blood pressure were sufficient to bring about a relaxation of the intestinal wall, and that as the dose was increased no well-marked difference could be observed in the reaction of the intestinal wall to that dose which first gave dilatation in the oncometer, nor to any of the succeeding ones.

In an attempt to decide upon the origin of this dilator effect of adrenalin we severed connection between the loop of intestine involved and the central nervous system, by dissecting and removing the two coeliac ganglia and the superior mesenteric ganglion, or by cutting the splanchnic nerves. In all experiments, five in all, after this operation was performed, the dilatation by adrenalin was entirely done away with, and doses which previously had caused a preliminary constriction followed by a dilatation now gave nothing but a simple constriction of the loop (see fig. 4).

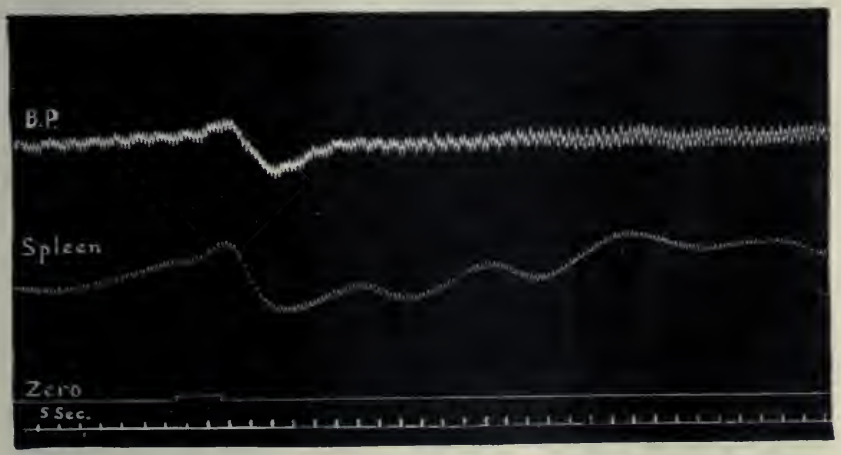

Fig. 5. Constriction in spleen, followed by a series of waves, after injection of a small dose of adrenalin (0.1 cc., 1 : 10,000). Dog 19 (Reduced ½)

THE SPLEEN

In the dissection of the spleen the gastrosplenic ligament with its numerous fat vessels was ligatured off, bit by bit, and cleared away from the neighborhood of the splenic blood vessels. In the early experiments those of the latter which supply the upper half of the spleen were also tied off. After two or three of these dissections, however, we were so dissatisfied with the appearance of the spleen under these conditions that we adopted a splenic oncometer with two lips. This enabled us to leave all vessels supplying the splenic substance intact, except sometimes that to the extreme tip of the upper end, which bound the organ too closely to allow of its being put into the oncometer. With the exception of this small piece, the spleen remained in

excellent condition, even during the course of an experiment lasting over several hours. During dissection we protected it with saline pads, and we warmed the oncometer to receive it.

Of the dogs, ten in all, which we investigated, seven showed only constriction in the spleen in response to the whole range of doses of adre-

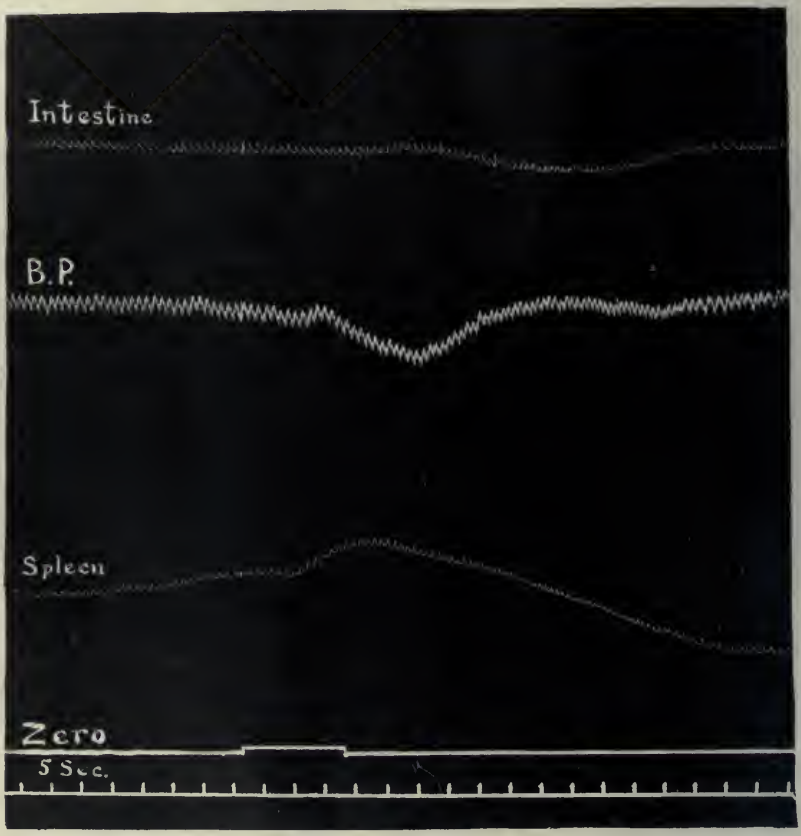

Fig. 6. Dilatation in spleen, and constriction in intestine, after small dose of adrenalin (0.3 cc., 1 : 10,000). Dog 21 (Reduced ⅓)

nalin employed. This constriction was more marked and more prolonged as the doses were increased in magnitude (see figs. 5 and 7). The three others each gave dilatation at some dose of adrenalin. Two of the three showed as a first effect dilatation with small doses; or, to speak more accurately, small doses of adrenalin set up in these

spleens (which had previously been relatively inactive) a series of waves, of which the first was in the direction of dilatation (see fig. 6). In both these organs the effect of increasing the dose was to increase the constriction in the waves at the expense of the dilatation, until large doses caused only decrease in volume. In the third of these spleens doses of adrenalin also set up series of waves, but its reaction differed

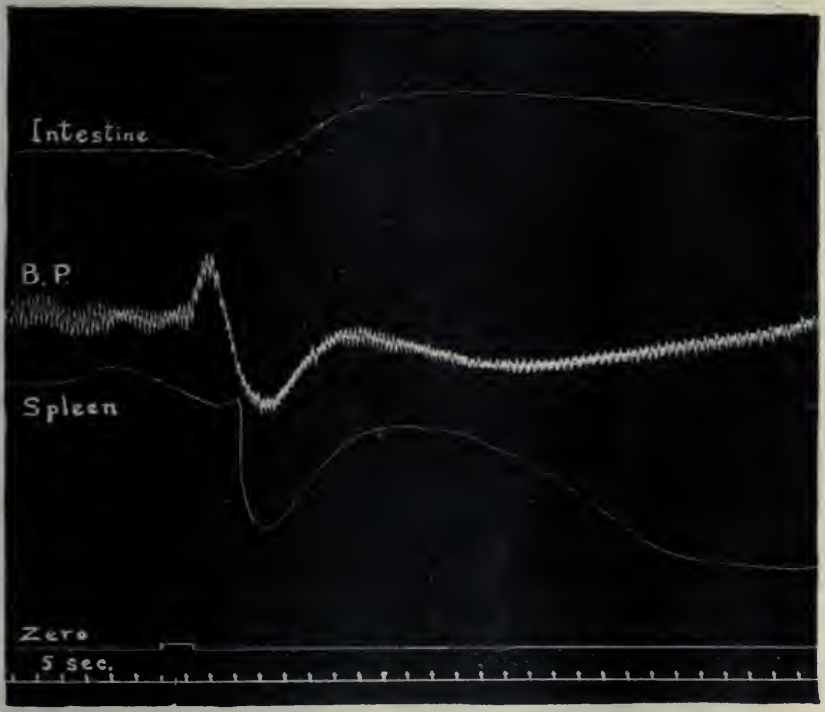

Fig. 7. Effect on same animal as that of figure 6, of a larger dose (1 cc., 1 : 10,000); slight dilatation followed by marked constriction in spleen, preliminary constriction and marked dilatation in intestine (Reduced $\frac{1}{2}$)

from that of the other two in that, on administration of relatively large doses (0.2 cc., 1 : 100,000 per kilogram) the constriction was followed by dilatation.

KIDNEY

The upper part of the ureter and the kidney vessels of one side throughout their entire length were dissected out to form a stalk. The mesentery was removed as gently as possible from the surface of the

kidney. A few of the larger veins running from it into the capsule were ligatured. During the dissection the kidney was protected as completely as possible with warm pads.

Four experiments in all were performed, two on cats and two on dogs. In every case injections of adrenalin caused constriction in the kidney (see figs. 8 and 9); with small doses of low concentration this

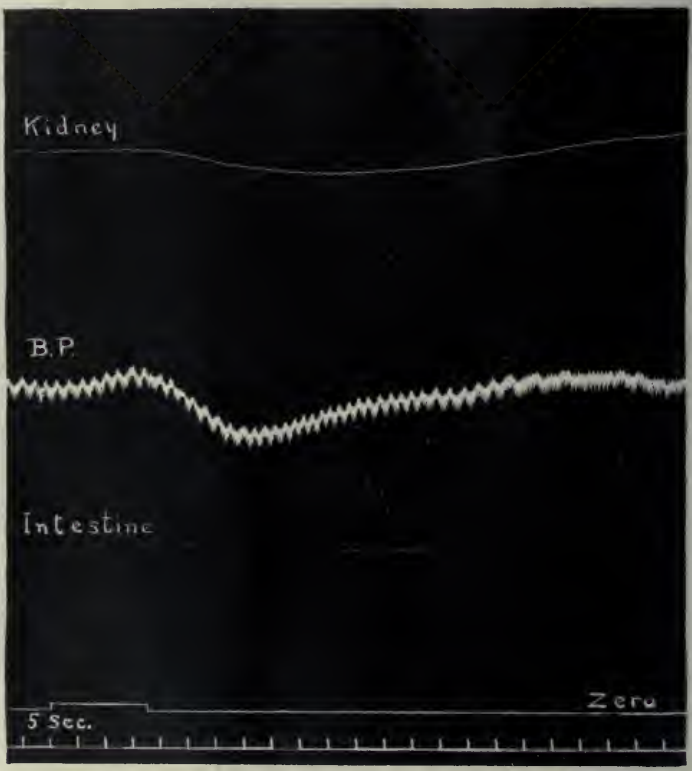

Fig. 8. Constriction of kidney after a dose of adrenalin 0.2 cc., 1 : 100,000 (Reduced $\frac{1}{2}$)

was the only effect (see fig. 8); in two cases the preliminary constriction caused by large doses (e.g., 0.32 cc. 1: 100,000 per kilogram), such as occasioned a preliminary rise followed by a fall of blood pressure, was followed by a dilatation of the organ. The curve of this dilatation was similar in form to that familiar to us in the reaction of the intestine to large doses of adrenalin. It showed a rise of

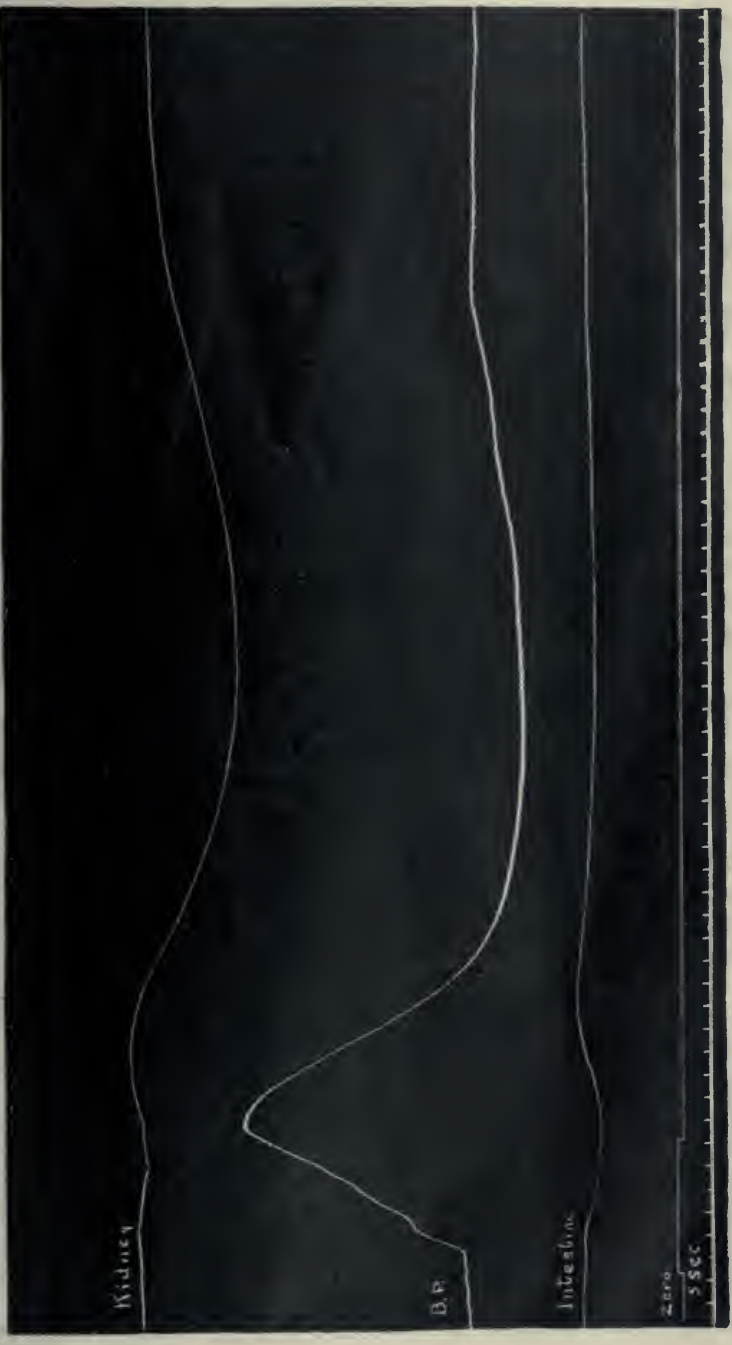

Fig. 9. Reaction of same kidney as in fig. 8, preliminary dilatation and prolonged constriction, after larger dose (0.5 cc., 1 : 10,000) (Reduced ½).

the lever, which only gradually returned to the base line, not before 170 to 180 seconds had elapsed, and thus continued long after the blood pressure had regained its normal level. The cause of the occurrence of this dilatation we were not able to determine.

In conclusion we wish to point out that the reactions of the various organs, though they may be of a similar nature, do not necessarily take place at the same time, nor for the same dose. Thus, for instance, in dog 23, table 2, though both kidney and intestine give constriction in response to small doses and dilatation in response to large ones, nevertheless that dose of adrenalin (0.4 cc., 1:100,000 per kilogram) which is enough to cause transition from a dilatation to a constriction in the intestine, still gives rise to nothing but a constriction in the kidney. Numerous other examples may be found by reference to that table.

That the output of adrenalin, which has been shown by the work of the last few years (3), (4), (5), to be so small during normal quiet life as to have no appreciable effect on blood pressure, is augmented during conditions of mental excitement, as well as by the asphyxia attendant on violent exercise, and by sensory stimulation, has been shown in a series of experiments by Cannon and the workers in his laboratory (6). The exact extent of this increase in secretion has not been determined, nor is it known whether it is sufficient to effect a rise rather than a fall in blood pressure. Elliott (7), in working on the secretion from the adrenal glands which is brought about by stimulation of the splanchnic nerves supplying them, has shown that in this case the quantity secreted is within the range of doses which have a depressor effect on the general blood pressure; whether this is also the case during the reflex stimulation of normal life, we are still ignorant. In any case, as shown by our experiments, the first effect of the outpouring of adrenalin during excitement must be to cause a constriction of the intestine and kidney, and generally, though not always, a similar constriction in the spleen. By this means there is brought about a shifting of the blood from these organs to the muscles, which, as Hoskins, Gunning and Berry (2) have shown, are at the same time actively dilated. If, as may prove to be the case, the output of adrenalin increases till the concentration in the arterial blood is of the order of about one-half or more that necessary to bring about a rise in blood pressure, a dilatation of the intestine, and perhaps also of the kidney, must take place, through the agency of some central mechanism, the location of which, and the source of stimulation to which, are as yet unknown.

TABLE 1

Shortening of the duration of intestinal constriction by the occurrence of dilatation

ANIMAL	WEIGHT	AMOUNT OF ADRENALIN INJECTED, CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT	LATENT PERIOD	MAGNITUDE OF CONSTRICTION	DURATION OF CONSTRICTION WHEN DILATATION IS ABSENT	DURATION OF CONSTRICTION WHEN DILATATION IS PRESENT	LATENT PERIOD	BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY	DURATION OF BLOOD PRESSURE CHANGE
	kgms.		*seconds*		*seconds*	*seconds*	*seconds*		*seconds*
Cat 4.....	3.15	0.06		Marked	160			Fall 136 to 110	160
		0.16		Marked		110		Fall 138 to 110	215
Cat 7.....				Small	97			175 to 184 to 142	97
				Small		13		164 to 218	63
			12	Marked		28		170 to 235 to 151	240+
Dog 14.....	7	0.03	11	Marked	32			102 to 90	33
		0.14	10	Very marked		28		99 to 87	23
		1.26*		Marked		15		96 to 180	105
Dog 20.....	9.5	0.05	12	Marked	33		11	160 to 130	60
		0.31	10	Marked		21	11	150 to 122	37
		2.08	9	Very marked		12	7	52 to 182	210
Dog 21.....	15	0.02	32	Marked	41		14	162 to 146	31
		0.67	11	Marked		29	8	106 to 122 to 86	30
		2.00	9	Marked		17	7	101 to 151	101

* All doses greater than 2 cc. adrenalin 1: 100,000 were actually injected into the animal in a 1: 10,000 concentration, but to make the dose comparable in the tables they are given in terms of 1: 100,000 dilution; 5 cc. adrenalin 1: 100,000 in the table was really 0.5 cc. adrenalin 1: 10,000 in the experiment. This applies to both tables.

TABLE 2

A comparison of the effects of adrenalin on different organs in the same individual

ANIMAL	WEIGHT	AMOUNT OF ADRENALIN INJECTED CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT	EFFECT ON INTESTINE				EFFECT ON SPLEEN		EFFECT ON KIDNEY		EFFECT ON BLOOD PRESSURE	
			Constriction		Dilatation							
			Magnitude of constriction	Duration of constriction	Magnitude of dilatation	Duration of dilatation	Magnitude of change	Duration of change	Magnitude of change	Duration of change	Change in millimeters of mercury	Duration of change
	kgms.			*seconds*		*seconds*		*seconds*		*seconds*		*seconds*
Dog 11	15	0.03	None		Small	80	Small constriction	Persistent			152 to 147	45
		0.27	None		Marked	105	Marked constriction	230			174 to 187 to 142	87
		0.67	Small	15	Very marked	120	Very marked constriction	525			161 to 175 to 150	112
Cat 13	2.9	0.07	Small	83					Marked constriction	80	132 to 140 to 109	67
		0.35	Small	172					Very marked constriction	210	80 to 92 to 68	115
		1.72	Marked	Persistent					Very marked constriction	285	106 to 213	63
Dog 21	15	0.01	No effect				Marked dilatation	50			160 to 148	20
		0.07	None		Small	130	Small dilatation followed by marked constriction				154 to 128	85
		2.00	Marked	17	Marked	210	Marked dilatation	160			102 to 152	125

Dog 22	25	0.02	No effect				Small dilatation	48			132 to 123	44
		0.20	Small	28	None		Marked constriction	Persistent			127 to 90	131
		0.60	None		Marked	285	Marked constriction followed by small dilatation	28			122 to 134 to 110	160
		1.00	Small	18	Very marked	312	Small constriction followed by persistent dilatation					
Dog 23	21.5	0.02	Barely perceptible	13		115			Marked constriction	100	143 to 121	30
		0.47	Slight	12	Small				Marked constriction	102	148 to 131	128
		0.93	None		Marked	Persistent			Marked constriction followed by dilatation	42	144 to 125 to 224	97

In investigating the dilatation occurring in various organs in the body under the influence of different concentrations of adrenalin in the blood circulating through them, we hoped to gain some light on the vexed question of the existence of dilator fibers in the sympathetic nerves to the blood vessels. The existence of these has been denied by many authorities, notably by Brodie and Dixon (8), and by Cannon and Lyman (9). On the other hand, evidence deduced from experiments of widely differing character has been brought forward in support of the theory that dilator fibers are present in the blood vessels, and are sensitive to adrenalin in solutions too dilute to stimulate the endings of the constrictor fibres. Dale's experiments on the reversal of the effect of adrenalin by ergotoxine (10), are interpreted to this effect by him. The pioneer work of Brodie and Dixon (8) on the lung already cited, and the later results of Desbouis and Langlois (11) and especially those of Enid Tribe (12) seem to point in this direction, the dilatation observed by Park (13), Elliott (17), and Cow (15), in coronary vessels, and that in vessels of other organs by Pari (16) and by Ogawa (17), in response to adrenalin offer further proof of the possibility of the existence of vaso-dilator fibres in the sympathetic system. On this subject our experiments have the value only of a negative finding, but as far as they carry us we have found no evidence of a direct stimulation of vaso-dilator endings by adrenalin, in any concentration approaching that which occurs under physiological conditions.

SUMMARY

1. Small doses of adrenalin cause constriction of the vessels of intestine, of kidney, and generally also of spleen.

2. The minimal dose necessary to produce this constriction is in much the same order of magnitude as that required to cause a fall in blood pressure, but it is not necessarily identical with it, nor is it the same for every organ in the same animal.

3. Increase of the dose of adrenalin causes in all cases marked dilatation of the intestine. This dilatation is brought about by doses materially less than those which are necessary to cause a rise in general blood pressure.

4. This dilatation in the intestine is under control of the central nervous system, and is done away with by severing connection with the central nervous system.

5. Adrenalin in the majority of cases has in all doses a constrictor

effect on the spleen; in some, minute doses cause first dilatation, which is the initial change of a series of rhythmical splenic waves.

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AN HYDROLYTIC STUDY OF CHITIN[1]

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Since its discovery in 1823, chitin has aroused a great deal of interest. This substance has a very wide distribution throughout the invertebrate kingdom, particularly in the skeletons of arthropods, but the mode of its formation and its nature are still shrouded in mystery. For the solution of this problem the first and essential thing is a clear understanding of the composition of chitin. Although much attention has been given to this matter by previous investigators neither the constitution of chitin nor indeed its empirical formula may be said to be beyond dispute, and this uncertainty is due to the fact that the subject has not been approached from the proper point of view.

In general it may be said of chitin that it is characterized by extreme resistance to strong reagents. Thus it is soluble in fairly concentrated mineral acids only, and is not affected by boiling with strong alkali, in which respect it resembles glycogen. In most analytical studies of chitin, the substance was dissolved in sulphuric acid. The nature of the chitin has been argued from the products thus obtained. No attempt has ever been made—so far as I am aware—to appraise the decomposition products by a strictly quantitative method, except perhaps for the sugar which is yielded as a glucosamine.

The material for this investigation was prepared from thirty-three lobsters with the total weight of about 11 kilograms. The animals, carefully weighed, were arranged in three groups; the average weight of the lobsters in the first group was 344.1 grams, in the second group 270.2 grams and in the third 337.8 grams. The lobsters were decalcified in 10 per cent nitric acid, which for this purpose was found more effective than the hydrochloric. The decalcification was continued, the acid being frequently changed, until there was no further reaction

[1] Published with the permission of the Commissioner of Fisheries.

for calcium. The material was then washed in running water. After this preliminary treament the shell can be very easily separated from the soft parts. The large bulk of the soft tissues was therefore removed mechanically. The decalcified shells were then boiled with 20 per cent potassium hydroxide which completely digested all fatty stuff as well as every trace of meat. The hydroxide solution was renewed several times and the boiling maintained until the KOH remained colorless. The material, which at this stage is practically white, was again washed in running water. To insure complete renewal of traces of pigment it was left over night in dilute potassium permanganate, washed again in running water and bleached with sodium bisulphite. The chitin thus obtained is of a clear, snow white color. It is now washed with distilled water for several days, until tests for sulphate or chlorides are negative. The chitin is then dehydrated by treating it with several changes of alcohol, followed by several changes of dry ether. When removed from the ether, the substance dries very quickly. The material thus prepared is dazzlingly white, retains every structural detail of the original shell, and is tough as leather. The material is chemically pure chitin, leaving no ash residue on incineration. The purity of the several samples was further tested by determining its nitrogen content, as this has been found to be very constant. The final desiccation of the chitin was accomplished at a temperature below 100°C. until its weight remained unchanged. The total yield of chitin was 358.63 grams. In the first group I found 11.54 grams chitin per lobster; in the second 9.588 grams; and in the third group 11.68 grams.

AVERAGE WEIGHT OF LOBSTER	AVERAGE WEIGHT OF CHITIN	PER CENT OF CHITIN
270.2	9.588	3.08
344.2	11.54	3.35
337.8	11.68	3.45

It is evident, therefore, that the larger animals contain not merely an absolutely greater quantity of chitin, but also a relatively larger amount.

Dry chitin contains 6.39 per cent nitrogen. The elementary analysis[2] yields 6.7 per cent hydrogen and 44.94 per cent carbon. The entire composition of chitin (lobster) may therefore be presented thus:

[2] I avail myself of this opportunity to express my sincerest gratitude to Dr. P. A. Levene for the courtesy of making this analysis for me.

C	H	H	O
44.94	6.70	6.39	41.97

The empirical formula which corresponds to this elementary composition is $C_8H_{15}NO_6$. This formula may represent a monacetylglucosamine of the following constitution:

$$CH_2OH—(CHOH)_3—CHNHCH_3\ CO—CHO$$

From the fact that upon decomposition chitin yields acetic acid, which may even be detected by smell, and glucosamine, the theory has been developed that chitin represents nothing else than a polymerized acetylated glucosamine. The isolation of an acetylglucosamine from a sulphuric acid solution of chitin by Fränkel and Kelly[3] would thus seem to furnish experimental proof of the theory. We shall see later that the substance isolated by these authors probably has nothing to do with chitin, and the correspondence between the empirical formula and that for acetylated glucosamine is entirely fortuitous. The iron chain of logical arguments upon which the theory is based has always seemed to me to possess a weak link; namely, that the solution of chitin in concentrated sulphuric acid may so modify the original molecule as to completely obscure its relation to the chitin.

Following Krawkow's[4] method, I attempted to prepare chemically pure chitin by dissolving the material obtained by the above described procedure in concentrated sulphuric acid, with every precaution to prevent heating of the mixture, and precipitating it with a great excess of water. Although I made numerous trials to get chitin by precipitation—primarily because I wished to have the material in the form of a powder—I succeeded only once in getting a fine white precipitate from an hydrochloric acid solution of chitin. I will subsequently refer to this precipitated material and its probable relation to chitin. I wish merely to mention here that even in concentrated sulphuric chitin goes into solution somewhat slowly, and by the time the substance has dissolved the solution invariably gave a reduction with Fehling's, showing that the chitin was already decomposed. In searching for a method by which the chitin could be dissolved without immediately causing it to break down, it was found that much more dilute acid (60 to 80 per cent) would dissolve it as quickly as concentrated acid with the

[3] Fränkel and Kelly: Sitzungsb. der klin. Akad. d. Wissensch. in Wien, 1901, 110, Abt. 2.

[4] Krawkow: Zeitschr. f. Biol., 1892, xxix, 177.

added advantage that decomposition (as evinced by development of a brownish color) is delayed much longer. It was found still further that by raising the temperature to about 60°C with even 35 to 40 per cent acid large quantities of chitin may be easily dissolved, the solution remaining colorless for a considerable length of time.

The study of the solubility of chitin in various strengths of H_2SO_4 showed that 20 per cent of the acid is still effective and I availed myself of this weak reagent to determine the following points: (1) Will chitin dissolve in it completely if sufficient time be allowed: (2) Will this treatment cause the splitting off of the acetyl groups; (3) Will it hydrolyze the theoretical amount of glucose? With these points in mind a series of experiments was performed and the technique employed will be described here fully inasmuch as it has been followed throughout this work.

A weighed quantity of the dry chitin was put in a large Erlenmeyer flask with 200 cc. of the acid mixture. The flask was closed with a rubber stopper having four holes. Through one hole a tube passed almost to the bottom of the flask, while a short tube passed through another hole and the tubes were bent on the outside. The former was connected with the compressed air, the air bubbling through sulphuric acid and potassium hydroxide before passing through the chitin solution. The second short tube was connected to another tube reaching down to the bottom of a receiving flask containing standard sodium hydroxide, so that any volatile acid produced in the acid-chitin mixture was carried off by the air current and absorbed. Through the other two holes in the stopper were inserted a thermometer and a separatory funnel from which water could be admitted from time to time to maintain a constant volume in the flask. The flask was placed on an electric plate and the heat so regulated that the temperature never varied more than 50° to 60°C.

In the experiment which I shall describe here 1.8828 gram of chitin was used. On the basis of the structural formula quoted above this quantity of chitin should yield 0.5112 gram. CH_3COOH, 1.5336 grams. $C_6H_{12}O_6$ and 0.1193 gram nitrogen. This amount of CH_3COOH is equivalent to 85.2 cc. $\frac{N}{10}$ acetic acid. The hydrolysis was continued uninterrupted for nearly five days. At the end of that period most of the material was still undissolved, and the mixture was very turbid owing to the suspension of fine particles which have been detached mechanically through the agitation. Of the total quantity of standard alkali used 88.5 cc. $\frac{N}{10}$ were neutralized by volatile acid, driven off from

the hydrolysis mixture, as was determined by titrating with $\frac{N}{10}$ HCL, using phenolphthalein as indicator. This represents 0.531 gram. of CH_3COOH. In view of the fact that not all of the chitin had yet been dissolved, the recovery of 104 per cent of the total theoretical amount of acid made the computation on the basis of the structural formula appear erroneous.

The mixture was then filtered, the clear filtrate made up to volume and analyzed for sugar and nitrogen. This contained 0.2210 gram glucose, or 17.6 per cent.

The residue was further hydrolyzed with 40 per cent H_2SO_4. It dissolved completely within a very short time. The hydrolysis was carried on for thirty-six hours, during which time an amount of acid was produced equal to 50.1 cc $\frac{N}{10}$, or 0.3006 gram $\cdot CH_3COOH$. The total amount of acid thus formed was (reckoned as acetic) $0.5310 + 0.3006 = 0.8316$ gram, or practically 63 per cent more than could be expected according to the formula.

In the second hydrolysis there was produced also 1.1650 grams glucose and 0.989 gram nitrogen. The total amount of glucose yielded was, therefore, 1.3860 grams (97 per cent), and that of nitrogen 0.1240 gram (103 per cent).

This and similar experiments gave weight to my skepticism towards the hypothesis that chitin is an acetylated glucosamine. It also strengthened my belief that the acetic acid detected when chitin is treated with violent reagents is not a primary hydrolytic product but rather a by-product resulting from the decomposition of the glucose molecule itself. In view of these results it was desirable to follow the different phases in the hydrolysis to get a more exact understanding, expressed in quantitative terms, of the entire process. After many trials and errors it was found that a concentration of about 35 to 40 per cent sulphuric acid (in 30 per cent the chitin is not completely dissolved) at a temperature of 50° to 60°C. are the most ideal conditions under which it is possible to carry on hydrolysis for days without causing the mixture to become much colored, and the mixture usually lacks the caramel smell. In 50 per cent sulphuric acid it is no longer safe to continue the hydrolysis very long as the mixture not merely turns a dark reddish brown, but an insoluble charred residue is formed and the whole mass smells strongly of caramel. In all such hydrolysis the amount of sugar recovered was invariably smaller than the theoretical, the glucose evidently having undergone decomposition. The production of volatile acid under the circumstances was usually very large. It is there-

fore clear that in hydrolyzing the chitin a great deal of care had to be exercised to guard against secondary decomposition. In this respect the results obtained with 40 per cent sulphuric were as nearly free from objection as is possible under the circumstances, and the account will be confined to these results exclusively.

The hydrolysis was performed by the method already described, the volatile acid being driven off by a slow but continuous current of air and absorbed in standard alkali. The hydrolytic mixture was made up to a definite volume of which aliquot portions were analysed for glucose by the Berthrand permanganate titration method. It was noted already in the early experiments that if the acid mixture was made alkaline, ammonia would distil off. When this was collected in standard acid it was discovered that the amount of ammonia nitrogen thus recovered was less than the theoretical amount of nitrogen in the chitin hydrolyzed. If the mixture was *first digested* with an excess of acid, made alkaline and distilled as is usual in Kjeldahl determinations, the ammonia nitrogen distilled off was equal to the theoretical amount. In other words, some of the nitrogenous groups were easily split off by the weak acid forming ammonium sulphate, while other groups were present in the chitin in a more stable combination and could only be set free by the complete oxidation of the molecule. It was a natural conclusion that the easily detachable nitrogenous group was the NH_2 group of the glucosamine and this was still further corroborated by the observation to be recorded later that the formation of glucose and ammonium sulphate keep pace with each other during hydrolysis. This fact suggested immediately that the empirical formula fails to convey an adequate idea of the complexity of the chitin molecule, which is certainly more intricately constituted than the adherents of the acetylated glucosamine hypothesis suspect.

In view of the significance of the results, the records of the experiments are fully reproduced in what follows:

I. August 18. 2.0339 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.

9.20 a.m. Hydrolysis started. 72.24 cc. $\frac{N}{10}$ NaOH in receiving flask.

9.50 a.m. Chitin entirely dissolved. Solution colorless.

2.00 p.m. Straw yellow color.

August 19.

9.20 a.m. Hydrolysis discontinued. Solution clear, dark yellow. Faint acid smell. Alkali in receiving flask titrated with 16.13 cc. $\frac{N}{10}$ HCL.

72.24 − 16.13 = 56.11 cc. $\frac{N}{10}$ used up.
Duration of hydrolysis, twenty-four hours.
Solution made up to 500 cc.
Two 25 cc. samples carefully neutralized. Glucose determined.
(1) 22.60
(2) 22.70
22.65 cc. $\frac{N}{10}$ KMn 0_4 used.
22.65 × 0.00663 = 0.1502 gram Cu = 0.0826 gram glucose.
0.0826 × 20 = 1.652 grams glucose.
Two 50 cc. samples made aklaline; distilled into standard acid (11.28cc. $\frac{N}{10}$).
(1) 6.42
(2) 6.33
6.38 cc. $\frac{N}{20}$ NaOH required.
11.25
3.19
8.09 cc. $\frac{N}{10}$ HCL used up.
8.09 × 10 × 0.0014 = 0.1133 gram N.

II. August 19. 2.0063 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.

9.40 a.m.	Hydrolysis started.	60.20 cc. $\frac{N}{10}$ NaOH used.
	After twenty-four hours.	10.05 cc. $\frac{N}{10}$ HCL required.
		50.15 cc. $\frac{N}{10}$ NaOH neutralized.

August 20.

9.40 a.m.	Color light yellow.	60.20 cc. $\frac{N}{10}$ NaOH used.
		4.23 cc. $\frac{N}{10}$ HCL required.
		55.97 cc. $\frac{N}{10}$ NaOH neutralized.

August 21.

9.40 a.m.	Color dark yellow.	60.20 cc. $\frac{N}{10}$ NaOH used.
	After twenty-four hours.	17.15 cc. $\frac{N}{10}$ required.
		43.05 cc. $\frac{N}{10}$ NaOH neutralized.

August 22.

9.40 a.m.	Color same.	60.20 cc. $\frac{N}{10}$ NaOH used.
	After twenty-four hours.	20.87 cc. $\frac{N}{10}$ HCL required.
		39.33 cc. $\frac{N}{10}$ NaOH neutralized.

August 23.

9.40 a.m. Hydrolysis discontinued. 188.50 cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, ninety-six hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; glucose determined.
(1) 21.40 cc.
(2) 21.30 cc.
21.35 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 21.35 = 0.1416 gram Cu = 0.0773 gram glucose.
0.0773 × 20 = 1.5460 gram glucose.
Two 50 cc. samples made alkaline; distilled into standard acid (11.28 cc. $\frac{N}{10}$).

(1) 6.60
(2) 6.70

6.65 cc. $\frac{N}{20}$ NaOH required.
11.28
3.33

7.95 cc. $\frac{N}{10}$ HCL used up.
$7.95 \times 10 \times 0.0014 = 0.1113$ gram N.
Two 50 cc. samples, digested and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 4.50
(2) 4.50

4.50 cc. $\frac{N}{20}$ NaOH required.
11.28
2.25

9.03 cc. $\frac{N}{10}$ used up.
$9.03 \times 10 \times 0.0014 = 0.1264$ gram N.

III. August 21. 2.0492 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
9.30 a.m. Hydrolysis started. 60.20 cc. $\frac{N}{10}$ NaOH used.
11.30 a.m. Slight turbidity.
4.00 p.m. Solution complete; very slight color.
9.30 p.m. Hydrolysis discontinued. 26.40 cc. $\frac{N}{10}$ HCL required in titration.
$60.20 - 26.40 = 33.80$ cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, twelve hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; glucose determined.
(1) 22.40
(2) 22.30

22.35 cc. $\frac{N}{10}$ $KMnO_4$ used.
$0.00663 \times 22.35 = 0.1482$ gram Cu = 0.0813 gram glucose.
$0.0813 \times 20 = 1.6260$ grams glucose.
Two 50 cc. samples made aklaline and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 6.35
(2) 6.45

6.40 cc. $\frac{N}{20}$ NaOH required.
11.28
3.20

8.08 cc. $\frac{N}{10}$ HCL used up.
$8.08 \times 10 \times 0.0014 = 0.1131$ gram N.
Two 50 cc. samples digested and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 3.85
(2) 3.90

3.88 cc. $\frac{N}{20}$ NaOH required.

11.28
1.94

9.34 cc. $\frac{N}{10}$ HCL used up.
9.34 × 10 × 0.0014 = 0.1308 gram N.

IV. August 23. 1.9990 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
4.00 p.m. Hydrolysis begun. 30.10 cc. $\frac{N}{10}$ NaOH used.
4.20 p.m. Material completely dissolved.
11.00 p.m. Hydrolysis discontinued. Solution slightly colored. Titrated with 10.20 cc. $\frac{N}{10}$ HCL.
30.10 − 10.20 = 19.90 cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, seven hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; sugar determined.
(1) 20.50
(2) 20.50

20.50 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 20.5 = 0.1359 gram Cu = 0.0738 gram glucose.
0.0738 × 20 = 1.4760 grams glucose.
Two 100 cc. samples made alkaline and distilled into standard acid (22.55 cc. $\frac{N}{10}$).
(1) 22.70
(2) 22.50

22.60 cc. $\frac{N}{20}$ NaOH required.
22.55
11.25

11.25 cc. $\frac{N}{10}$ HCL used up.
11.25 × 5 × 0.0014 = 0.0788 gram N.

V. August 23. 2.2024 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
4.30 p.m. Hydrolysis started. 60.20 cc. $\frac{N}{10}$ NaOH used.

August 24.
11.30 a.m. Titrated with 1.10 cc. $\frac{N}{10}$ HCL.
60.20 $\frac{N}{10}$ NaOH used.
59.10 cc. $\frac{N}{10}$ NaOH used up.

August 25.
8.30 a.m. Hydrolysis discontinued. Titrated with 19.75 cc. $\frac{N}{10}$ HCL.
60.20 − 19.75 = 40.45 cc. $\frac{N}{10}$ NaOH used up.
99.55 cc. $\frac{N}{10}$ used up.
Duration of hydrolysis, forty hours.
Solution clear, dark yellowish. Made up to 500 cc.
Two 25 cc. samples neutralized; sugar determined.
(1) 23.25
(2) 23.25

23.25 cc. $\frac{N}{10}$ $KMnO_4$ used.

0.00663 × 23.25 = 0.1541 gram Cu = 0.0850 gram glucose.
0.0850 × 20 = 1.7000 grams glucose.
Two 50 cc. samples neutralized and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 5.30
(2) 5.34

5.32 cc. $\frac{N}{20}$ NaOH required.
11.28
2.66

8.62 cc. $\frac{N}{10}$ HCL used up.
8.62 × 10 × 0.0014 = 0.1207 gram N.

VI. August 24. 1.8550 dry chitin. 100 cc. of 40 per cent H_2SO_4. Temperature, 60°C.

No aeration, flask stoppered. Material dissolved in about 45 minutes. At the end of one hour solution made up to 500 cc. On standing a slight turbidity appeared.

Two 25 cc. samples neutralized; glucose determined.
(1) 11.05
(2) 10.95

11.00 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 11 = 0.0729 gram Cu = 0.0375 gram glucose.
0.0375 × 20 = 0.7500 gram glucose.
Two 100 cc. samples neutralized and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 8.10
(2) 8.20

8.15 cc. $\frac{N}{20}$ NaOH required.
11.28
4.08

7.20 cc. $\frac{N}{10}$ HCL used up.
7.20 × 10 × 0.0014 = 0.0504 gram N.

The results just presented are summarized in the following table:

CHITIN	DURATION OF HYDROLYSIS	TOTAL NITROGEN	NH_2-N RECOVERED	PER CENT OF NITROGEN RECOVERED AS NH_2-N	PER CENT OF CHITIN RECOVERED AS NH_2-N	UNRECOVERED NITROGEN PER CENT OF CHITIN	GLUCOSE (THEORETICAL)	GLUCOSE RECOVERED	PER CENT OF GLUCOSE RECOVERED	PER CENT OF CHITIN RECOVERED AS GLUCOSE	TOTAL VOLATILE ACID IN CC. $\frac{N}{10}$
gm.	*hours*	*gm.*	*gm.*				*gm.*	*gm.*			
1.8550	1	0.1185	0.0504	42.5	2.72	3.67	1.5044	0.7500	49.9	40.4	?
1.9990	7	0.1277	0.0788	61.7	3.94	2.45	1.6202	1.4760	91.1	73.8	19.90
2.0492	12	0.1309	0.1131	86.4	5.52	0.87	1.6619	1.6260	97.84	79.4	33.80
2.0339	24	0.1300	0.1133	**87.1**	**5.57**	**0.82**	1.6495	1.6520	**100.15**	**81.4**	50.10
2.2024	40	0.1407	0.1207	85.8	5.48	0.91	1.7861	1.7000	95.2	77.2	99.50
2.0063	96	0.1282	0.1113	86.8	5.55	0.84	1.6271	1.5460	95.0	77.1	188.50

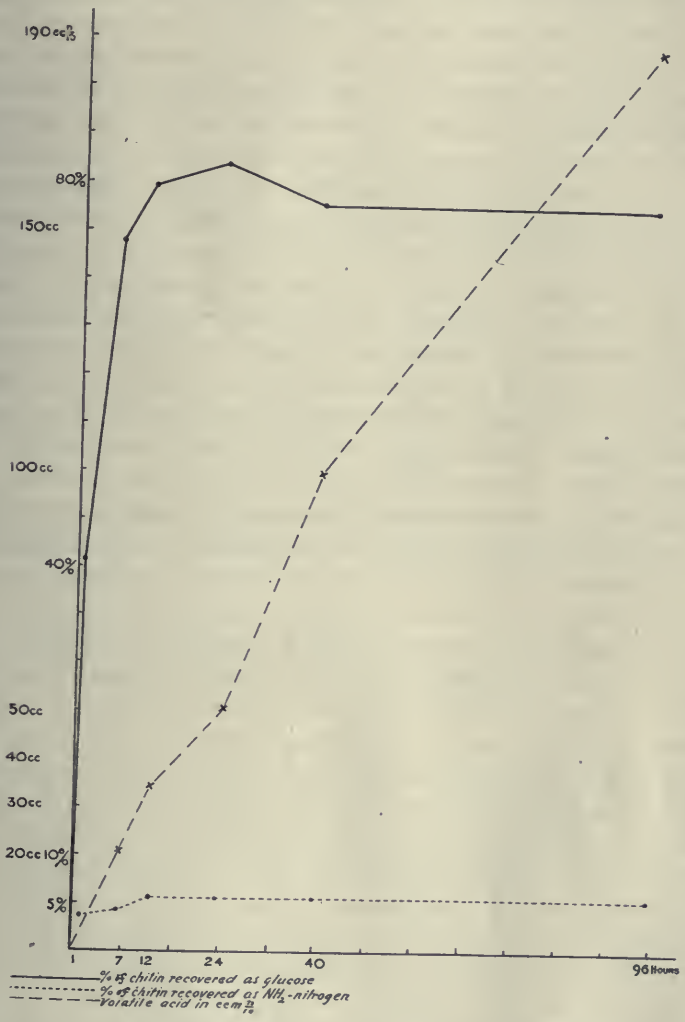
This table brings out the following points: As the duration of the hydrolysis increases, more of the NH_2 groups are split off being converted into ammonium sulphate, so that after twelve hours practically the maximum NH_2-nitrogen is obtained. This maximum represents 5.5 per cent of the chitin, while the stable nitrogen which cannot be cleaved off by the hydrolysis is about 0.8 to 0.9 per cent of the chitin. It was found that merely dissolving the chitin in 40 per cent sulphuric and allowing it to stand for one hour is sufficient to cause 49.9 per cent of the total glucose to be split off. An examination of the table will show that after 24 hours of hydrolysis a maximum yield of glucose was obtained equal to 81.4 per cent of the chitin. This coincides with the largest recovery of NH_2-nitrogen. The amount of glucose found at this stage is practically 100 per cent of the theoretical expectation. Beyond this stage further hydrolysis is accompanied by a loss of glucose and a very rapid increase of the volatile acid fraction.

The data contained in the above table are presented graphically in a series of curves (fig. 1) which bring out the various important points of this study at a glance.

We may say, therefore, that the maximum yield of NH_2-nitrogen coincides with the maximum yield of glucose and is independent of the acid formation but that if the hydrolysis is continued beyond 24 hours the great increase in the acid production is coincident with the oxidation and destruction of glucose.

If the volatile acid is the product of oxidation of glucose—in other words, if it is not a primary constituent of chitin—is it possible that it is represented by acetic acid only? It seemed to me that if the volatile acid produced in the hydrolysis was a mixture of the lower fatty acids such a finding would take away the ground from under the hypothesis that an acetyl group is present in the chitin molecule, for there would be then just as much justification—or just as little—to consider every one of those acids as a constituent part of the chitin. Owing to the difficulty of identifying various volatile acids in small quantities this side of the question has not yet been fully worked out. In one experiment the air current was passed through a solution of $AgNO_3$ and the presence of formic acid was indicated by the reduction of the silver. To verify this observation and to gain some approximate idea as to its relative amount, about 2 grams of chitin were hydrolysed in the usual way for forty-two hours and the distillate was collected in standard alkali. The amount of acid absorbed, as determined by titration, was 60.65 cc $\frac{N}{10}$. To the neutralized solution a mixture of mercuric-chloride and

sodium acetate was added; the solution was then boiled for 6 hours, care being taken to prevent evaporation. A fine crystalline precipitate of mercurous chloride was formed. This was filtered off, dried



and weighed. There was thus obtained 0.0480 gram HgCl which corresponds to 0.0047 gram HCOOH, or approximately to 1 cc. $\frac{N}{10}$. The formic acid thus formed 1.65 per cent of the total acidity produced. While these experiments must be further extended it is evident, none-

theless, that in the hydrolysis of chitin acetic acid is not the only low fatty acid liberated.

The question next to be considered is the relation to chitin of the acetylglucosamine which has been separated from acid solutions of chitin and which has the same empirical formula as the latter. The substance investigated by Fränkel and Kelly was obtained in the following manner: Chitin was dissolved in 70 per cent sulphuric acid and this was left standing for several days. When this solution was poured into an excess of water a fine powder precipitated out which on analysis proved to be monacetylglucosamine. It must be pointed out, however, that acetyldiglucosamine was likewise found (Offer[5]). In one of my experiments, already referred to, I succeeded in getting a fine precipitate by pouring a solution of chitin in hydrochloric acid into ten times its volume of distilled water. The precipitate formed very slowly, a mere turbidity appearing at first, but over night it accumulated in a fair quantity on the bottom of the beaker. It was filtered, washed free of chlorides with distilled water, then with alcohol and ether and dried. Unfortunately the elementary composition of this precipitate was not determined. But its nitrogen content throws much light on its nature especially when this is considered in conjunction with the data gained from the hydrolysis. This substance was found to contain only 5.8 per cent of nitrogen instead of 6.4 per cent which was found in the lobster material. From the hydrolysis experiments we learned already that about 0.8 per cent of chitin is in the form of a stable nitrogenous combination quite different from the remaining 5.5 per cent which represents the amino groups. It would thus seem likely that the substance I got by precipitation from the acid solution of chitin consisted of the latter moiety of the nitrogen in combination with glucose while the other nitrogenous portion remained in solution. In other words, the precipitate which was formed from chitin *was really no longer chitin,* and I believe that the substances which have been studied by other investigators were derived *from* chitin but were *not* chitin. How then is this acetylated aminosugar formed? This, too, it seems to me, is very easy to understand. The fact must be clearly borne in mind—and reference to experiment VI will substantiate this—that leaving chitin in sulphuric acid will lead quickly to its breaking up with the liberation of glucose and ammonia. Acids, too, may readily be formed through the oxidation of the glucose by the sulphuric

[5] Offer: Biochem. Zeitschr., 1907, vii, 117.

acid. The stronger the acid the more extensive and rapid may these changes be. But glucosamine and acetic combine in the presence of a dehydrating agent, and in our mixture all conditions are present (the sulphuric acid acting also as the dehydrating agent) for the recombination of these substances. This synthesis of the hydrolytic and decomposition products of the chitin molecule may account for the circumstances that the new substances thus produced may either be acetylglucosamine, or acetyldiglucosamine or diacetylglucosamine. The acid medium will hold all these in solution, but being insoluble in water they are thrown down when the acid is greatly diluted.

Having conclusively shown the fallacy of the assumption that chitin is a polymere of acetylglucosamine, a different interpretation of its composition may be attempted on the basis of the above hydrolytic studies. Referring again to the data recorded in the table, it will be noted that the two kinds of nitrogen present in chitin bear a definite ratio to each other as 1 to 7. Starting from this fundamental fact, it is clear, therefore, that chitin, whose empirical formula is $C_8H_{15}NO_6$, must be a polymere of no less than eight such groups, and its composition must therefore be $C_{64}H_{120}N_8O_{48}$. If we designate the nitrogenous portion of the chitin, the true character of which is still unknown to us, by the symbol Xn we may represent the cleavage brought about by hydrolysis by the following equations;

$$(1)\quad C_{64}H_{120}N_8O_{48} + 8H_2O =$$
$$(2)\quad 7C_6H_{13}NO_5 + C_6H_{12}O_6 + Xn + 7H_2O =$$
$$(3)\quad 7NH_3 + \underbrace{7C_6H_{12}O_6 + C_6H_{12}O_6}_{8C_6H_{12}O_6} + Xn.$$

According to this chitin should yield 81.1 per cent by weight as glucose; 5.54 per cent of nitrogen in the form of ammonia (which can be distilled off directly without preliminary digestion); 0.8 per cent of nitrogen which is not split off by hydrolysis and can only be converted into ammonium sulphate by digestion. The amino nitrogen, therefore, would constitute 87.5 per cent of the total nitrogen. The agreement of the experimental findings with these theoretical expectations is truly remarkable. It reveals the great complexity of the chitin molecule which consists not merely of glucosamine but also of glucose and a nitrogenous substance of a still unknown nature That the glucose is present in the molecule in two forms is borne out by the study of the relation between the total amount of the sugar and the total NH_2-nitrogen recovered. If all the sugar were in the form of glucosamine

the ratio of N:G would be 1:12.8, but in the experiment with the maximum yield this ratio N:G is equal (0.1133:1.6520) to 1:14.6, thus proving beyond any doubt that some of the sugar does not possess the amino group.

What the nature of the unrecovered nitrogenous residue is, is still a mute point. It may very well be that it forms a nucleus around which the glucose molecules are grouped. Attempts to isolate this substance for purposes of identification and detailed study have thus far been unsuccessful, but further efforts in this direction are continued. I think that we may say with certainty that this residue cannot be of a protein nature because its nitrogen content is too low. If the reactions involved in the hydrolysis have been correctly represented in the preceding scheme—and this would seem to be thoroughly corroborated by the experimental data—this residue should have the formula $C_{16}H_{33}NO_{15}$. This formula is suggested without any intention on my part to forestall what should be based on careful study. It may serve as a guide in the research which is to follow.

SUMMARY

1. The nitrogen of the chitin molecule is partly in the form of the NH_2 group of the glucosamine, which is readily split off in hydrolysis with dilute acid, and partly in the form of a stable combination from which it can be released by digesting with concentrated sulphuric acid only. This latter portion constitutes one-eighth (12.04 to 12.45 per cent) of the total nitrogen.

2. The volatile acid produced in hydrolysis of chitin is probably a mixture of lower fatty acids. Its production is associated with a decomposition of the glucose molecule.

3. The maximum yield of glucose is about 81 per cent by weight of the chitin.

4. The evidence presented in this paper is against the accepted view that chitin is a polymerized acetylglucosamine.

5. An hypothesis is suggested, based upon results of a quantative study of the hydrolytic products, according to which chitin is composed of glucose amine, glucose and a nitrogenous moiety of still unknown nature.

THE FATE OF SUCROSE PARENTERALLY ADMINISTERED

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It has been stated by many investigators that some substances which are introduced into the organism in excessive amounts, whether they are foreign to the organism or not, can be eliminated through other channels than the kidneys, namely through the gastrointestinal tract, mammary glands, etc. Various elements such as iron, copper, strontium, barium or radium are eliminated in part, at least, through the bowel (1), (2), (3). Among organic compounds, the excretion of antipyrine, curarine, diphtheria toxin, egg protein, etc., through the intestine has been reported. (4) Many dyes, e.g., Sudan III, Biebrich scarlet, methylene blue, phenolsulphonephthalein, phenoltetrachlorphthalein, alkali blue, indigo carmine, have been reported to be eliminated into the alimentary canal, either by way of the liver or through the intestinal wall (5), (6), (7), (8), (9).

Kóssa (10) reported that in phlorhizinized hens sugar is eliminated in the intestine, the proportion of the sugar in the urine to that in the feces being as 1 : 0.3. Rösseler (11) also reported that feces of diabetic patients contained a demonstrable quantity of sugar and in an increased quantity after sugar feeding. In experimental diabetes, however, Allen (12) could find no sugar in the feces, even after feeding a large amount of dextrose. No sugar was found in the saliva of his diabetic animals. On the other hand, Naunyn (13), Brauer (7) and Woodyatt (14) were able to find reducing sugar in the bile of the animals, which were made diabetic by pancreas extirpation, piqûre or phlorhizin injection. After injecting a large quantity of sodium chloride solution intravenously into rabbits, MacCallum (15) reported that sugar is eliminated into the alimentary tract. Investigating this problem further, Fischer and Moore (16) demonstrated that rabbits which are made to secrete sugar in the urine by a sugar puncture or by intravenous injection of dextrose or sucrose solution, do not excrete sugar into their gastrointestinal tract. But when a sodium chloride solution is injected intravenously at the same time, sugar is eliminated by the small intestine. They ascribed this phenomenon to an increased permeability of the intestinal mucous membrane resulting from the salt injection. Injecting a dextrose solution alone into rabbits, intravenously, in a sufficient quantity, Kleiner proved that hyperglycemia alone can cause the sugar elimination into the intestine and stomach. The amount of sugar excreted, however, is incomparably smaller than the amount eliminated through the kidneys. A preceding double nephrectomy increases the gastrointestinal elimination of the sugar. After injecting 112 to 162 grams of dextrose in a 25 per cent solution intravenously into dogs, Grigaut and Richet

(17) also confirmed that the hyperglycemia suffices to cause sugar elimination into the gastrointestinal tract. They recovered 1.0, 3.5 and 10.2 grams of glucose, respectively in the intestinal content, while 14.0, 11.0 and 14.6 grams of glucose, respectively were found in the urine. They also demonstrated that sodium chloride and urea can be eliminated in the intestine in the same manner.

According to the reports of Mendel and Kleiner (18) and others, sucrose, introduced into the animal body parenterally, can not be recovered quantitatively in the urine. In my own previous experiments, an average of 75.5 per cent of sucrose injected was recovered in the urine. In trying to explain the fate of the rest, the question of the possible production of invertin, which has been reported by Abderhalden and others to appear in the serum, was taken up by some investigators. My previous experiments failed to demonstrate the presence of invertin in the serum. Recently, Folkmar (19) also has been unsuccessful in finding invertin in the serum after parenteral administration of sucrose. He considered that the damage of renal function, resulting from sugar injection, might have some relation to the retention of the sugar injected. Jappelli's (20) experiments suggested that part of the sucrose injected might be kept in the liver, gradually appearing in the circulation or in the bile afterwards, or might be excreted by the stomach or salivary glands into the alimentary tract.

It has already been briefly indicated that under certain circumstances other organs than the kidneys can eliminate substances which appear abnormally in the organism. At the suggestion of Prof. Lafayette B. Mendel, I have tried to find out whether sucrose parenterally administered, is in part eliminated through the liver or the intestinal mucous membrane.

METHODS

Full-grown dogs were used. In all experiments, a 10 per cent sucrose solution, sterilized by boiling, was injected into a jugular vein, sometimes very slowly (in the course of an hour), sometimes a little more rapidly (in twenty to thirty minutes). The amount of the solution injected was 150 cc. to 480 cc. The bile was collected by a biliary fistula, through a cannula in the common bile duct. After completing the experiment the bile obtained through the fistula was united with that in the gall-bladder, and this mixture was used for sugar determination. It is a very difficult problem to ascertain whether injected sucrose is eliminated through the intestinal membrane or not. The sugar may be eliminated there, but almost immediately be inverted and absorbed. In the present experiments, a loop of the small intestine was isolated between two ligatures, and a large glass cannula inserted

at each end. The upper end of the loop was a few centimeters from the beginning of the duodenum, the length of the loop varying between 22 cm. and 79 cm. The intestine was replaced in the abdominal cavity and the abdominal wall closed, only the rubber tubes connected with the cannulas being kept outside. The animal was kept warm during the experiment. Physiological saline solution, warmed to the body temperature, was introduced into the upper end of the loop, and the solution coming out from the cannula of the lower end of the loop collected. Every precaution was taken not to damage the intestinal mucous membrane and the blood vessels supplying the intestinal tract. Before the sucrose injection, the intestinal loop was washed out very gently with the saline solution (about 300 cc.), so that there was no reducing substance in the last portion. After sucrose injection, the saline solution was introduced drop by drop and the fluid coming out from the lower end of the loop analyzed for sugar. The saline solution was not permitted to be contaminated with blood. In some experiments, the blood vessels of both kidneys were ligated before sucrose injection.

For analysis sucrose in the bile or in the saline solution which was used to irrigate the intestinal loop, was first hydrolyzed by invertin. The reducing sugar, obtained, was then determined by Allihn's gravimetric method, colloidal iron being used to remove the protein and other disturbing substances. Invertin was prepared from compressed yeast, following the suggestions of Hudson. The reaction of the preparation was very slightly acid.

Concerning the occurrence of reducing sugar in the normal bile, there are many contradictory reports. In discussing those which claim the absence of sugar in normal bile, Naunyn (13) stated that a minimal amount of sugar is a quite common occurrence in the normal bile, obtained from a fistula in the common bile duct. Brauer, (7) however, demonstrated, that normal human or dog bile does not contain any reducing sugar. Woodyatt (14) reported that in his dog experiments no reducing substance was found in the bile before phlorhizin injection.

Okada (21) reported that the hydrogen ion concentration of normal dog's bile is variable within the ranges of P_H 5.34 to 6.97 in the case of bladder bile and 7.49 to 8.15 and 7.54 to 8.01 in liver bile during fasting and digestion respectively. On the other hand, the activity of the invertin is very sensitive to the hydrogen ion concentration of the medium. Following Michaelis and Davidsohn's (22) report, the optimal zone for invertin is P_H 3.67 to 5.25, the medium between P_H 3.16 and 8.30 still allowing invertin to be active. Sörensen (23)

The fate of sucrose parenterally administered

Dog	Number		I ♂	II* ♂	III ♂	IV ♂	V ♀
	Body weight		10.0 kgm.	12.5 kgm.	32.0 kg.	13.0 kgm.	8.0 kgm.
Anesthetics			Morphine (0.1 gram) + ether	Urethane (7.0 gram) + ether	Chloretone (13.0 gram + ether	Chloretone (6.0 gram) + ether	Urethane (6.0 gram) + ether
Duration of experiment			2 hrs. 20 min.	4 hrs.	3 hrs.	1 hr. 15 min.	4 hrs.
Operation			Biliary fistula	Biliary fistula + ligation of renal vessels.	Biliary fistula + intestinal loop	Biliary fistula + intestinal loop + ligation of renal vessels.	Biliary fistula + intestinal loop + ligation of renal vessels.
Sucrose injection	Amount injected		15.0 grams	22.5 grams	48.0 grams	25.0 grams	20.0 grams
	Duration of injection		20 min.	30 min.	1 hour	30 min.	30 min.
Bile	Volume	From fistula	11.4 cc.	16.0 cc.	24.5 cc.	15 cc.	4.7 cc.
		From gall bladder	10.0 cc.	9.5 cc.	36.5 cc.	16.5 cc.	5.3 cc.
	Reducing sugar, calculated as invert sugar	Before inversion	8.6 mgm.	0 mgm.	10.7 mgm.	14.8 mgm.	0 mgm.
		After inversion	24.1 mgm.	64.3 mgm.	29.3 mgm.	47.8 mgm.	29.3 mgm.

Irrigation of intestinal loop	Length of intestinal loop				22 cm.	63 cm.	79 cm.
	Volume of saline solution collected				630 cc.	310 cc.	680 cc.
	Reducing sugar, calculated as invert sugar	Before inversion			0 mgm.	33.4 mgm.	418.2 mgm.
		After inversion			45.0 mgm.	73.5 mgm.	522.0 mgm.
Urine	Volume		134 cc.	2.5 cc.	247 cc.	2.0 cc.	1.0 cc.
	Reduction	Before inversion	+	−	+	−	−
		After inversion	+++	−	+++	−	−
	Rotation	Before inversion	V° + 16.8		+ 10.9		
		After inversion	V° − 4.3		− 3.1		

* At the end of the experiment, the blood sugar content was examined by the Lewis-Benedict method. The sugar content, calculated as dextrose, was 0.08 per cent before inversion, increasing to 0.49 per cent after inversion.

ascribed P_H 4.4 to 4.6 as the optimal point for invertin, the medium between P_H 2.55 and 7.30 still allowed some activity of the ferment.

As preliminary experiments, therefore, the reducing-power of the normal bile and the activity of the invertin preparation added to the bile were examined. The bile, obtained from a normal dog, a cat and two pigs never showed any reducing power. Sucrose added to those samples of the bile was easily hydrolyzed by the invertin preparation under the experimental conditions employed. When the invertin preparation was previously boiled, or the active invertin preparation alone was added, no reducing substance was produced in the bile.

The procedure for sugar determination in the bile was as follows: After adding 5 cc. of the invertin preparation and 0.1 cc. of toluene to 10 cc. of bile, the mixture was kept $2\frac{1}{2}$ hours at 38° to 40°C. Sixty cubic centimeters of distilled water and 25 cc. of colloidal iron solution were then added and the mixture was shaken vigorously for a few minutes. Half an hour later, about 0.5 gm. of sodium sulphate was added. Seventy-five cubic centimeters of the clear and slightly yellow filtrate were used for sugar determination by Allihn's method. For control, the same procedure was always carried through with boiled invertin. When the amount of bile was not enough, less than 10 cc. of it was used for one procedure, the added substances being lessened in the same proportion. Determination of sucrose in the saline solution, which was used to irrigate the intestinal loop, was performed in the same manner. Twenty cubic centimeters of the invertin preparation were added to 100 cc. of the saline solution. Five cubic centimeters of colloidal iron solution were enough to remove protein, The control with boiled invertin was also performed. The amount of reducing sugar, both before and after inversion, was always calculated as invert sugar.

After completing the experiment, the small intestine and the ligature of the renal vessels were examined. The mucous membrane of the intestinal loop showed a slight edema in some cases. Otherwise no noteworthy changes were observed, either in the loop or in the rest of the intestinal tract. The urine obtained from the bladder at the end of the experiment was tested for sugar. When both kidneys were shut out from the circulation, the urine obtained was only a few cubic centimeters and contained no sugar either before or after inversion. When the kidney vessels were left intact the urine contained a large amount of sucrose and only a small amount of reducing sugar. When the sucrose was hydrolyzed with invertin, the urine acquired a marked levorotatory and strong reducing power. The results of the experiments are shown in the preceding table.

CONCLUSION

From the results of the present experiments, it will be seen that sucrose, injected intravenously into dogs, is eliminated very quickly through the kidneys, but at the same time, a minimal amount of it can be eliminated through the liver and the intestinal mucous membrane. The ligation of the renal vessels does not necessarily facilitate its excretion through the liver and the intestinal mucous membrane.

The permeability of the kidneys and intestinal mucous membrane for dextrose is said to be increased by parenteral administration of sodium chloride (24), (16). Phlorhizin glycosuria and glycocholia are also considered to be due to the change of the permeability of the kidneys and liver respectively.

It is not improbable that sucrose may behave similarly. Parenteral administration of sucrose, in sufficient quantities, may increase the permeability of various organs, not only for sucrose itself, but also for dextrose in the blood. This may explain why a small amount of reducing sugar preëxisted in the bile and the urine together with sucrose. In my previous work (25) already, it was noticed that reducing sugar sometimes appears in the urine after parenteral administration of sucrose, (no anesthetics were used). At that time, it was suggested that part of sucrose might be inverted somewhere in the body, so that the reducing sugar, preformed, appeared in the urine. The change of renal permeability for normal blood sugar offers another explanation. The preëxisting sugar in the saline solution collected from the intestinal loop may also be explained by change of the permeability for dextrose. At the same time, however, in this case, the activity of invertin in the intestinal juice must be considered. It can not be asserted that the irrigation of the intestinal loop, however carefully it may be performed, is absolutely harmless to the mucous membrane. It is very unlikely, however, that this is the only cause of the appearance of sucrose in the intestinal canal. On the other hand, part of the sucrose, which appeared in the intestinal loop, might be absorbed immediately, notwithstanding the constant irrigation. If such absorption did not exist, the amount of the sucrose, recovered in the loop, might be much larger.

When sucrose is injected parenterally, 20 to 30 per cent or more of the amount injected, fails to be recovered in the urine. As the amount of sucrose excreted in the alimentary tract is extremely small, this channel for sucrose excretion seems to be insufficient to explain the fate of the missing part of the sucrose injected. The question as to

whether sucrose is rendered utilizable by being inverted in its passage through the intestinal wall remains unanswered.

I desire to express my thanks to Prof. Lafayette B. Mendel, to whom I am greatly indebted for his suggestions, help and criticism; also to Prof. F. P. Underhill for his advice.

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THE PERFUSION OF THE MAMMALIAN MEDULLA: THE EFFECT OF CARBON DIOXIDE AND OTHER SUBSTANCES ON THE RESPIRATORY AND CARDIO-VASCULAR CENTERS

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INTRODUCTION

It is generally accepted at the present time that the hydrogen ion concentration of the blood is alone concerned in the chemical regulation of respiration. Haldane (1) and his co-workers laid the foundations for this conception of the respiratory function but Winterstein (2) apparently was the first to present the facts in the form of a concrete hypothesis. Winterstein reported experiments on the perfusion of new-born rabbits which led him to the conclusion that neither oxygen-want nor carbonic acid-excess as such stimulated respiratory activity; rather the hydrogen ion concentration of the perfusate was the determining factor in the stimulation. This hypothesis has received fundamental support in the work of Hasselbalch (3), Barcroft (4) and others, and has been successfully employed as a basis for the treatment of disease and for the explanation of numerous physiological processes.

While this hypothesis has received wide application and has stimulated a renewed interest in respiratory problems, it is nevertheless open to criticism chiefly as regards its exact experimental basis, a basis which has not been strengthened by Winterstein's subsequent contribution (5). Laqueur and Verzár (6) using Winterstein's method of perfusing new-born rabbits, believed they were able to show that carbonic acid acts as a specific respiratory stimulant. The evidence which they offer, however, is not convincing. Rona and Neukirch (7), studying the behavior of isolated loops of rabbit's intestine in different solutions, found that the addition of sodium bicarbonate materially improved the rhythmic contractions of the preparation. Furthermore, they were able to show that the effect thus produced was

apparently in no wise associated with the concomitant hydrogen ion concentration of the solution. We have here an instance, therefore, in which the HCO_3 ions apparently act specifically. Consequently it has seemed advisable to put the original hypothesis as advanced by Winterstein to a new experimental test. This test was to perfuse the mammalian medulla by a method previously described (8) using blood perfusates of which the hydrogen ion concentration and carbon dioxide tension were known, and to study the effects produced by these two factors upon the activity of the respiratory center. Our results would indicate that an adequate statement of the regulation of respiratory activity cannot be fully summarized in the brief statement of Winterstein's hypothesis. We have obtained evidence that carbonic acid exerts a specific influence upon the respiratory center independent of its effect upon the hydrogen ion concentration of the blood perfusate. From these results the conclusion seems justifiable that either carbon dioxide acts as a specific stimulus or alters the irritability of the center to the normal stimulus.

EXPERIMENTAL

A number of preliminary experiments were performed which demonstrated that the method was adequate to the problem in hand. Reference to table 1 will make it clear that the respiratory center responds to changes in the blood perfusate in accordance with current ideas on this subject. These experiments show the general nature of the response. Sodium bicarbonate and sodium hydroxide depress respiratory activity; carbonic acid, hydrochloric acid, lactic acid and oxygen-want increase respiratory activity.[1] The effect upon the cardiac and vasomotor centers appears to be of the same order as that upon the respiratory center. That is to say, those substances which increase respiratory activity tend to increase the arterial blood pressure and heart-rate, and those substances which depress respiratory activity tend to decrease the arterial pressure and heart-rate. The response of the latter centers is what we should expect from our knowledge of the functional interaction of the respiratory and cardio-

[1] In the single experiment that we have carried out increased respiratory activity was very apparent when blood poorer in oxygen was perfused. The rapidity of response precludes the possibility of the formation of acid metabolic products by decreased oxidation and suggests that oxygen-want may cause increased irritability of the respiratory center. Further work along this line is contemplated.

Condensed results of preliminary experiments

DATE	TEST MATERIAL	BLOOD p_H =		RESPIRATION						BLOOD PRESSURE	HEART RATE
		Control	Experiment	Amplitude in millimeters		Rate per minute		Rate × amplitude		Per cent change		
				Before	Result.	Before	Result.	Before	Result.			
June 16, 1915.....	NaOH			10	0	33	0	330	0	−100	>	Stopped
	HCl			5	15	15	24	75	360	380	<	⇳
				10	30	3	15	30	450	1,400	⇳	87 to 72
November 6, 1915.....	$NaHCO_3$			10	12	30	24	300	288	−4	>	⇳
				7	10	33	24	231	240	4	>	⇳
December 17, 1915.....	CO_2 5 per cent	7.6	7.4−	40	38	6	8	240	304	27	⇳	>
		7.6	7.4−	40	37	6	10	240	370	54	>	>
	CO_2 10 per cent	7.6	7.2	10	65	39	57	390	3,585	819	<	81 to 120
		7.6	7.2	10	25	27	30	270	750	178	⇳	⇳
December 21, 1915..	HC.	7.6	7.2	7	15	27	37	189	555	178	⇳	⇳
		7.6	7.2	20	20	21	21	420	420	0	⇳	⇳
	CO_2 10 per cent	7.6	7.2	15	30	27	30	405	900	122	⇳	⇳
	HCl	7.6	6.9−	20	20	18	33	360	660	83	⇳	⇳
	HCl	7.4	7.25	10	25	57	54	570	1,350	137		
		7.4	7.25	15	35	90	75	1,350	2,625	94		
	Lactic acid	7.4	7.3	10	25	78	75	780	1,875	140		
		7.4	7.3	10	20	78	66	780	1,320	69		
		7.4	7.3	7	20	75	66	525	1,320	151		
January 18, 1916....		7.4	7.1	10	20	87	78	870	1,560	79		
		7.4	7.1	10	20	84	69	840	1,380	64		
	HCl	7.4	7.0	5	15	81	75	405	1,125	178		
		7.4	7.0	10	15	82	75	820	1,125	37		
	Lactic acid .	7.4	7.3	10	15	81	81	810	1,215	50		
		7.4	7.1	5	15	63	63	315	945	201		
	O_2—want, weak	7.5	7.5?	25	45	54	48	1,350	2,160	60	<	108 to 144
		7.5	7.5?	20	35	57	57	1,140	1,995	75	<	126 to 144
January 31, 1916....		7.5	7.5?	35	35	51	42	1,785	1,470	−18	<	129 to 132
	O_2—want, strong	7.5	7.5?	20	45	54	54	1,080	2,430	125	<	⇳
		7.5	7.5?	20	35	57	51	1,140	1,785	57	<	⇳

vascular centers. Nevertheless, the action of these several substances on the cardio-vascular centers has not been discussed and the results are, therefore, of interest.

It will be noted that the response of the cardiac and vasomotor centers is not so definite as that of the respiratory center. This may be due in part to a lesser sensitiveness of the centers themselves, but it seems more probable that the difference is primarily due to the condition of the peripheral mechanisms. In these experiments circulation is maintained in the trunk to preserve the diaphragm as a recording mechanism for respiratory activity and no effort is made to retain normal conditions as to temperature, etc., for the peripheral vasomotor system. The trunk, therefore, may be regarded as being in "shock." The cardio-motor fibers are, on the other hand, almost certain to suffer some injury in the technical procedure of inserting the perfusion cannula in the common carotid artery close to its origin on the aortic arch. It is not surprising, therefore, that cardio-motor responses should be weak and often entirely absent.

In both table 1 and table 2 the figures in the column under percentage change in respiration represent the change in the factor rate $\times$ amplitude of respiration expressed in percentage of the same factor before the experimental blood was tested. The minus sign ($-$) before the figure indicates a decrease in the factor; its absence indicates an increase in the factor. This factor is used to give an approximate indication of pulmonary ventilation. It is of significance because, as will be noted, variations of rate and amplitude do not run parallel; sometimes the one and sometimes the other determines the value rate $\times$ amplitude and sometimes one is increased while the other is decreased. But their product gives quite consistent results throughout the table. To this there are but two exceptions, namely, in the experiment of November 6, 1915 and of January 31, 1916, and in these cases the deviation from the control values is relatively small.

The necessity of using this factor (rate $\times$ amplitude) as a basis of comparing the results obtained, raises the interesting question as to whether or not sensory impulses from the lungs alone control the rate of respiration. Scott (9) has presented evidence to show that when carbon dioxide is inspired the increase in rate of respiration is wholly dependent upon vagus function; that when the vagi are sectioned and carbon dioxide is administered, the only result is an increase in depth of respiration. In the experiments here reported, the rate as well as the amplitude of respiration is variously affected. The vagi are in-

tact but the thorax is open and the lungs are being regularly expanded with artificial respiration. Vagal action cannot, therefore, be of influence in determining the respiratory rate, and the conclusion appears inevitable that variations in the blood supplying the respiratory center may determine not only the amplitude but also the rate of respiratory discharge.

In one of these preliminary experiments we used lactic acid to render the blood more acid to see if its effect differed from that of hydrochloric acid yielding the same hydrogen ion concentration. The experiment is pertinent in that lactic acid is a normal product of intermediary metab-

TABLE 2

Condensed results in comparison of CO_2 *with* HCl

DATE	TEST MATERIAL	BLOOD p_H=		RESPIRATION							BLOOD PRESSURE IN MILLIMETERS
				Amplitude in millimeters		Rate		Amplitude × rate		Per cent change	
		Control	Experiment	Before	During	Before	During	Before	During		
June 15, 1916	CO_2 5 per cent	7.6	7.31	3	35	69	87	207	3,045	1,366	40
	HCl	7.6	7.31	45	40	48	75	2,160	3,000	39	5
	CO_2 5 per cent	7.6	7.31	35	55	39	84	1,365	4,620	165	25
June 21, 1916	CO_2 5 per cent	7.55	7.25	18	50	75	93	1,350	4,650	244	−8
	HCl	7.55	7.25	19	25	78	96	1,482	2,400	62	0
	CO_2 5 per cent	7.55	7.25	25	25	45	72	1,125	1,800	60	15
June 22, 1916	HCl	7.6	7.23	3	5	75	78	225	390	73	6
	HCl	7.6	7.23	5	9	78	99	390	891	128	−9
	CO_2 5 per cent	7.6	7.23	5	100	72	114	360	11,400	3,069	3
	CO_2 5 per cent	7.6	7.23	55	70	18	48	990	3,360	239	5

olism and is formed in excessive quantities under certain conditions which lead to lowering of the carbonic acid content of the blood. Under these conditions it might presumably be as efficient as carbonic acid in calling forth a respiratory response and more efficient than other acids, for example, hydrochloric. The results of this experiment indicate that there is no marked difference in the action of the two acids and since hydrochloric acid was somewhat more convenient, we used it in the subsequent experiments designed to investigate the specificity of carbonic acid as a respiratory stimulant.

Our experiments were directed primarily to a comparison of the respiratory response elicited by blood, the hydrogen ion concentration

of which was altered to the same degree by the addition of carbon dioxide and hydrochloric acid. To this end we used as a control, blood which had been shaken to remove the excess of carbon dioxide and observed the physiological reaction produced by the substitution of bloods with higher hydrogen ion concentration.

In this procedure a number of precautions had to be observed. (1) *General character of the several perfusates.* To insure the utmost similarity in regard to possible unknown factors, the defibrinated blood was collected, mixed and aerated in a porcelain-lined vessel after which it was subdivided in clean stoppered flasks. These samples were then submitted to the appropriate change of hydrogen ion concentration and placed in a constant temperature water-bath until used. (2) *Temperature.* Variations in temperature doubtless influence the behavior of the medullary centers. To guard against possible errors due to this factor, the sample, the response to which was next to be investigated, was placed in the substitution reservoir of the apparatus some ten minutes before being used so that its temperature, if not actually that of the control in circulation, was at least constant as compared with any other similar sample investigated in the same experiment. (3) *Change in irritability of the respiratory center.* The medullary centers undoubtedly lose in functional capacity in the course of perfusion and this rate of loss is not comparable in different experiments. It is evident, therefore, that the comparison of the response produced by two stimuli so closely alike as, for example, carbon dioxide and hydrochloric acid bloods, is unsafe unless they are both tested in the same experiment. And even then the progressive loss of irritability may establish conditions such that the test of the second specimen of blood is, for purposes of comparison, invalid. If, however, the perfusion of carbon dioxide blood produces the greater increase of respiratory activity when tested *after* as well as *before* hydrochloric acid, we may believe that the carbon dioxide blood is the more effective stimulus. This we were able to show in the present experiments.

PREPARATION OF PERFUSATES

The defibrinated blood used as control was agitated in air until its hydrogen ion concentration had reached a minimum. One portion of this blood was placed in a flask and agitated while being exposed to an atmosphere of 5 per cent carbon dioxide in oxygen. The hydrogen ion concentration of this sample was determined independently by two

of us using the indicator and electrical methods. A second portion was brought to the same reaction by treating with hydrochloric acid and shaking to remove the excess of carbon dioxide liberated. In this way two specimens of blood were prepared; one in equilibrium with a high tension of CO_2 (5 per cent of an atmosphere) and therefore containing a considerable quantity of free carbonic acid and a relatively high concentration of total carbonate; the other in equilibrium with a low tension of CO_2 (0.04 per cent of an atmosphere) and containing relatively less carbonic acid and total carbonate but with the same hydrogen ion concentration as the former.

The reactions of both specimens were determined by the colorimetric and the electrical methods. The former could be used advantageously to obtain approximate results in a short time, while the latter method furnished the more trustworthy results especially with the bloods containing carbonic acid. These determinations were carried out at room temperature, using the improved Hasselbalch hydrogen electrode, McClendon potentiometer and a recently standardized Weston cell. A portion of the blood specimen was placed in the electrode to establish CO_2-equilibrium with the hydrogen gas and then replaced with a second portion on which the determination was made in the usual manner.

Three experiments comparing the efficacy of carbonic acid and hydrochloric acid bloods were performed. The results are presented in table 2. The headings used in this table are the same as those used in table 1 and require no further consideration. We wish, however, to draw particular attention to the figures in the column under percentage change of respiration. These figures bring out forcefully the difference in response to the two experimental bloods. Carbonic acid blood when used before as well as after hydrochloric acid blood produces much the greater effect. The apparent exception to this statement in the second test of carbonic acid blood in the experiment of June 21 may, we believe, be properly explained by a progressive deterioration of functional activity in the center. In this case the response is at least as great as it was in the preceding test with hydrochloric acid blood. The influence of such a progressive loss of function was considered earlier in the paper and we do not believe that our conclusions are weakened by one instance in which carbonic acid blood failed to elicit a greater response than that produced by hydrochloric acid blood tested previously.

Portions of the graphic record of one of these experiments are pro-

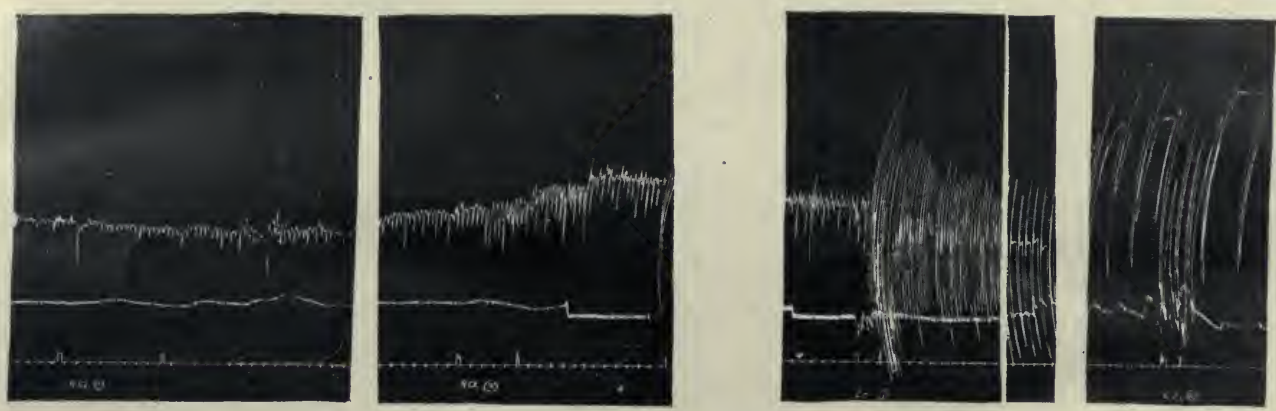

Fig. 1. Portion of graphic record obtained in experiment of June 22, 1916. Upper record, respiratory movements of epigastrium. Middle record, blood pressure recorded with mercury manometer. Lower record, zero blood pressure with time in two second periods. The two breaks in this line indicate in each section of the record the beginning and completion of the perfusion with the experimental blood. The reaction of the normal blood was $P_H = 7.55$; that of the hydrochloric acid blood $P_H = 7.25$ and that of the carbon dioxide blood $P_H = 7.25$. Note the relatively slight effect produced by the hydrochloric acid blood, although it was allowed to act for a longer time when the medullary centers were presumably more irritable. Note also the shorter latency in the reaction with carbon dioxide blood.

duced in figure 1, the protocol of which may be given as an example of all.

Experiment of June 22, 1916. Comparison of effects of blood brought to the same hydrogen ion concentration with CO_2 and HCl on dog's medullary centers. Large dog bled several times after intravenous injections of warm Ringer's solution. Total volume of defibrinated blood thus diluted about 3000 cc. While still warm the blood was aerated by rapid stirring in a large vessel for half an hour to liberate excess of CO_2. Four hundred cubic centimeters were submitted to an atmosphere of CO_2 5 per cent + O_2 95 per cent by bubbling the gas through a trapped flask containing the blood for about fifteen minutes. Two hundred cubic centimeters were treated with M/7 HCl in 0.4 per cent NaCl and the blood shaken until the hydrogen ion concentration ceased to diminish, i.e., until the excess of free carbon dioxide was removed. The reactions of the three specimens were:

	INDICATOR METHOD	ELECTRICAL METHOD
Normal blood	p_H = 7.6+	p_H = 7.55
CO_2 blood	7.25	7.22
HCl blood	7.25	7.22
Normal blood after repeated perfusion		7.51

Blood in stoppered flasks placed in water bath at 36°C.

The experimental animal was operated under chloretone anesthesia. The perfusion was at 80 mm. Hg, and 37°C. The venous outflow was about 180 cc. per minute (30 cc. in 8.5 seconds). Probably the normal blood will tend toward an acid reaction as the rapidity of flow made it technically difficult to segregate the experimental blood. During perfusion of the experimental bloods they were of necessity partly exposed to room atmosphere. This will not affect the HCl blood but may result in lowering the hydrogen ion concentration of the CO_2 blood by diffusion of the gas.

Inspection of the figure shows, furthermore, as indicated in the elapsed time between the breaks on the time record that the hydrochloric acid blood was perfused for periods of eighteen and ten seconds without eliciting a sharp reaction in either case, while the perfusion of CO_2 blood for two periods of four seconds each produced not only a much more decided reaction but one which exhibited itself very much more promptly. The fact is significant as further indicating the greater efficacy of carbon dioxide. The other experiments in this group showed this sharp response to carbonic acid blood to a lesser degree probably because the rate of perfusion was slower.

DISCUSSION

The experiments thus outlined appear to prove conclusively that blood with a comparatively high tension of carbon dioxide causes a greater stimulation of the respiratory center than does blood with a lower tension of carbon dioxide but with the same hydrogen ion concentration. The method of stimulation is unknown though several theoretical considerations are of interest.

The reactions of the bloods were within physiological limits. As the bloods with higher hydrogen ion concentration were still alkaline, the diffusion of free acids other than carbonic may be considered negligible. The similarity in the results obtained by the use of bloods to which either hydrochloric or lactic acids were added suggests that there is no appreciable difference in the diffusion of the two anions. As it would seem that lactic acid ions could easily penetrate the respiratory center on account of their great diffusibility the above observation is opposed to the conception that hydrochloric acid ions elicit a relatively small response because of their failure to readily diffuse into the same cells. The variations in the concentration of the salts in the perfusates, incident to the alteration in the reaction of the blood do not have an appreciable inhibiting action on the respiratory center, as unpublished experiments have shown. The cause for the smaller stimulation by the bloods to which hydrochloric acid was added (and the excess of carbon dioxide shaken out) must therefore be due to the decreased concentration of carbon dioxide or carbonic acid.

As the tendency for diffusion of carbon dioxide from the cells into the blood depends on the difference in the tension of carbon dioxide of the two systems, the higher the carbon dioxide content of the circulating blood, the slower the diffusion from the cells and the higher the concentration in the cells when a new equilibrium is established. The tension of carbon dioxide in the respiratory center would presumably, therefore, be higher in our experiments in which blood with a high tension of carbon dioxide was employed and, vice versa, low when blood with low carbon dioxide concentration was used. It would seem that the hydrogen ion concentration of the two systems would likewise maintain an equilibrium. If this is true, the hydrogen ion concentration in the center would be the same in both series of experiments because the reaction of the two bloods was the same. The greater activity produced by the bloods containing the high tension of carbon dioxide must therefore be due to some specific action of the carbon dioxide.

Whether carbon dioxide as such stimulates the respiratory center or whether variations in the carbon dioxide concentration alter the irritability of the respiratory center can hardly be demonstrated. If we are to adopt an explanation for the results obtained in these experiments, the most satisfactory point of view would seem to be that the hydrogen ion concentration of the environment of the respiratory center is its effective stimulus but that the irritability of the center to this stimulus may vary and be influenced by many factors. The normal irritability of the respiratory center is doubtless the summation of a number of effects including those produced by carbonic acid, oxygen and various ions as well as changes in metabolic activity, the nature of which is not understood.

CONCLUSIONS

1. An increase in alkalinity of the perfusing blood (produced by adding sodium bicarbonate or sodium hydroxide) tends to depress the medullary centers.

2. A decrease in alkalinity of the perfusing blood (produced by adding hydrochloric acid, lactic acid or carbon dioxide) tends to stimulate these centers.

3. A specimen of blood containing a high tension of carbon dioxide causes greater activity of the respiratory center than another specimen of the same hydrogen ion concentration but with a low tension of carbon dioxide. Carbonic acid thus acts specifically upon the respiratory center.

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THE RETURN OF UREA FROM THE KIDNEY TO THE BLOOD

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During unsuccessful attempts to measure the rate of flow of blood through the kidney, we found that the concentration of urea may be higher in the renal vein than in the renal artery. The rate of flow of blood through the kidney can be calculated if the urea concentration in the blood of the renal artery and vein is determined over a period during which the rate of flow of urine and the rate of urea excretion is estimated. But we found that we could not obtain these data without adopting measures designed to prevent or lessen vasoconstriction of the renal arteries. For after tying off one kidney and exposing the other, the manipulations required for the removal of blood from the renal vein were always attended by a cessation of the flow of urine which appeared to be due to vasoconstriction, since we were able in some instances to observe that the renal artery grew smaller. Renal vein blood obtained under these conditions contained more urea than the blood entering the kidney. The experiments cited in this paper extend and confirm this initial observation.

This additional urea found in the renal vein must have had its origin in some accumulation of urea within the kidney. The kidney contains more urea than other organs and is an exception to the rule of the approximately even distribution of urea throughout the tissues and fluids of the body (1). The microchemical work of Leschke (2) and of Oliver (3) demonstrates that the reason for this high urea content is the special concentration of urea in two separate situations in the kidney, in the cortex within the cells of the proximal convoluted tubules

and in the medulla in the urine lying within the collecting tubules. It is not possible to determine which of these stores of urea is the source of the urea returned to the renal blood, nor which contributes the greater part if the returned urea comes from both. We found that the medullary portion of the kidney contained somewhat more urea than the cortex, but on the other hand the cellular store in the cortex is in direct contact with the blood, while the urinary accumulation in the medulla is separated from the blood by a layer of renal cells.

But whatever the exact source of the urea added to the renal blood may be, the fact of its return from the kidney whenever the secretion of urine stops, throws some light on the mode of action of that force which enables the kidney to prepare a concentrated solution of urea such as the urine from a dilute solution of urea such as the blood. The accumulation of urea at certain locations within the kidney in much higher concentration than exists elsewhere in that organ must be accomplished by a force which is able to annul or overrule the physical laws governing the diffusion of urea. Such a force might act through the medium of some physical or chemical configuration which would remain passively operative even when the active functions of the kidney were in abeyance, just as the valve of a machine will continue to prevent the reflux of fluid when the power is shut off. But the immediate return of urea from the kidney to the blood indicates that this force has to be in continual operation to maintain the high urea concentration. When the kidney stops working this force relaxes its hold upon the heaped up urea, so that it again becomes subject to the laws of diffusion, and falls from the site of high concentration in the kidney to the lower levels of the blood, just as a weight held in the hand will fall to the ground when the grasp is relaxed.

THE RELIABILITY OF THE METHOD USED FOR DETERMINING THE UREA CONTENT OF THE BLOOD

Triplicate determinations were made on each blood except in a few instances in which only duplicates were obtained because of accident to one of the samples or failure to obtain enough blood.

From such material an expression of the probable error in the measurements might have been obtained by finding the standard deviation for each set of triplicate or duplicate results, and multiplying the average of these standard deviations by 0.67. This procedure however is not only cumbersome but has been shown by Otis (4) to give a value

less than the true probable error. He has demonstrated that the median of all the differences between triplicate or duplicate measurements divided by the square root of 2 gives the probable error of a single determination. The probable error of the average of three determinations is this probable error divided by the square root of 3.

The differences between repeated determinations on a blood with a high urea content were no greater than those found when the urea content of the blood was low. All such differences are therefore directly comparable. There were in all 168 differences. The median of this series was 0.9 mgm. Applying the formula we obtain a probable error of 0.64 mgm. for single determinations and 0.37 mgm. for the averages of three determinations such as are given in our tables. In other words, in half of our figures the quantity recorded, e.g., 100 mgm., might have been any value between 100.37 mgm. and 99.63 mgm. In the other half of our figures, the error is greater than 0.37 mgm. Mr. Otis pointed out to us that if the frequency distribution of errors may be assumed in this case to be "normal," that is, in accordance with the law of the distribution of errors, then theoretically the errors will be less than twice the probable error in 82 per cent of the cases, less than three times the probable error in 95 per cent, and less than four times the probable error in slightly over 99 per cent of cases. In only one case in a hundred, therefore, will the error reach 1.5 mgm.

The urease method of Marshall was used, carried out in much the same way as is recommended by Van Slyke and Cullen. The quantity of blood taken for each determination was 1 cc. measured with an Ostwald pipette. Care was taken that the bloods whose urea content was to be compared were treated in an exactly similar manner as regards the amount of soy bean extract added, and the length of time of incubation and aeration. The acid was measured with an automatic pipette. In titration those refinements were used which were introduced by Barnett (5) in connection with his method for determining small quantities of ammonia.

An increase in the urea content of blood from the renal vein at a time when the secretion of urine had stopped, was observed in a number of the unsuccessful attempts to measure the rate of flow of blood through the kidney referred to at the commencement of this paper. Those figures are not given since the determinations were carried out on such small quantities of blood that the reliability of the method

was considerably less than when the larger quantities available in the experiments cited here were used.

The animals used were rabbits. In some cases urea was given by stomach tube before operation in order to increase the urea content of the blood.

THE DECREASE IN THE UREA CONTENT OF BLOOD FROM THE RENAL VEIN WHEN PRECAUTIONS ARE TAKEN TO DISTURB THE FUNCTION OF THE KIDNEY AS LITTLE AS POSSIBLE

As soon as the animal was fully under the influence of ether, the left kidney was fully exposed through an incision in the flank, the renal vein cut with scissors and the blood collected in a vessel containing a little powdered oxalate.

In two rabbits a direct comparison was made between the urea content of the renal vein blood and the blood of the renal artery. Two ligatures were placed in position round the renal vein, the one next the vena cava tied, the swollen vein snipped with scissors, and after enough blood had collected the one next the hilus of the kidney was tied. Immediately afterwards the renal artery was cut. A decrease was found in the venous blood, 12 and 39 mgm., as against 15 and 41 mgm. in the arterial. The venous blood was slightly more concentrated as judged by the relative volumes occupied by red blood cells and plasma, but not to such a degree as appreciably to affect the urea content.

Such direct comparisons between blood from the renal vein and artery have the disdavantage of involving a cessation of the flow of blood through the kidney and therefore carry with them a tendency to interference with kidney function. Further, even such brief manipulations as are required in clamping or ligaturing the renal vein in two places are apt to induce a constriction of the renal artery. In our other experiments we have therefore taken the blood of the jugular vein as representing blood from the renal artery so far as its urea content is concerned. In a few experiments blood from the femoral artery was used. That this is justifiable is shown by the result of comparisons of the urea content of blood from the renal artery with the urea content of blood from the jugular and carotid. Such differences as are recorded are not significant. (table 1.)

In twelve rabbits the renal vein blood was compared with the jugular. The jugular was first bared, the kidney quickly exposed and the

renal vein snipped with scissors. Immediately thereafter, without waiting to tie the renal vein, the jugular was cut. The whole procedure did not take more than one or two minutes, and there was no mechanical interference with the circulation through the kidney. The results are given in table 2.

TABLE 1

Comparison of the urea content of blood from the renal artery, jugular vein and carotid artery

RENAL ARTERY	JUGULAR VEIN	CAROTID ARTERY
mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*
40.47	40.00	
21.00	22.37	
24.50		24.47
83.35	83.90	
19.57	19.74	
	24.75	25.47

TABLE 2

Comparison of the urea content of blood from the renal vein and the jugular vein when the blood was taken quickly

RENAL VEIN	JUGULAR VEIN	LESS IN RENAL VEIN	MORE IN RENAL VEIN
mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
47	57	10	
27	27		
180	189	9	
97	102	5	
22	23	1	
42	53	12	
184	196	12	
212	223	11	
197	196		1
38	38		
139	149	10	
211	216	5	

There can be no question from these figures that in some of these cases the kidney must still have continued to remove urea from the blood passing through it. In seven out of the twelve experiments the decrease in the urea content of the venous blood is considerably greater than could be accounted for on the basis of technical error.

A DECREASE IN THE UREA CONTENT OF THE BLOOD OF THE LEFT RENAL VEIN WHEN THE BLOOD IS TAKEN QUICKLY FOLLOWED BY AN INCREASE IN THE UREA CONTENT OF THE BLOOD OF THE RIGHT RENAL VEIN OBSERVED IN THE SAME ANIMAL AFTER A PERIOD OF OPERATIVE MANIPULATION DURING WHICH THE SECRETION OF URINE CEASED

In seven rabbits, blood was obtained quickly in the manner already described from the left renal and jugular veins. The vessels of the left kidney were then clamped and ligatured. The bladder was opened and a catheter inserted into the right ureter. This was done in order to make sure that the secretion of urine had stopped. We did not

TABLE 3

Comparison of the urea content of blood from the left renal vein and the jugular vein when the blood was taken quickly and comparison of the urea content of blood from the right renal vein and the jugular vein when the secretion of urine had stopped

RABBIT NO.	BLOOD TAKEN QUICKLY				BLOOD TAKEN WHEN THE SECRETION OF URINE HAD STOPPED			
	Left renal vein	Jugular vein	Difference		Right renal vein	Jugular vein	Difference	
			More	Less			More	Less
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
11	169	178		9	194	188	6	
15	88	93		5	100	100		
38	92	96		4	105	105		
39	180	187		7	201	189	12	
40	130	129		1	136	131	5	
41	130	140		10	145	139	6	
42	77	78	1		87	87		

obtain urine from the catheter in any of these animals. The right kidney was then exposed and blood collected from the right renal vein and immediately afterwards from the jugular. In a few cases the comparison was made with blood from the femoral artery, as enough blood was not obtainable from the jugular.

The urea content of the renal vein could thus be compared with that of blood corresponding in urea content to the blood sent to the kidney in the renal artery under two conditions, first at a time and under circumstances in which the kidney might still be functioning, and secondly at a time when we had evidence that kidney function had ceased. The results are given in table 3.

It will be noted that there is in general a decrease in the urea content of the renal vein when the blood is taken quickly, and an increase when the blood is taken at a time when no urine is being secreted. That there should be considerable variation in the amount of the decrease or increase is to be expected, since neither the degree of kidney activity nor the time at which that activity ceased was known or exactly controlled. But that there should be in any of these cases a clear decrease in the urea content of the renal vein blood can only be accounted for by the passage of urea from the blood into the kidney. Similarly a definite increase under other conditions is only to be explained by the return of urea from the kidney to the blood.

SUMMARY

When blood is taken from the renal vein so as to disturb the function of the kidney as little as possible, it usually contains less urea than the blood sent to the kidney in the renal artery. This is explained by the passage of urea from the blood to the kidney.

When blood is taken from the renal vein at a time when the secretion of urine has stopped, it usually contains more urea than the blood sent to the kidney in the renal artery. This is explained by the passage of urea from the kidney to the blood.

The return of urea from the kidney to the blood whenever the kidney ceases to secrete urine is taken as indicating that the force or forces which store urea in high concentration within the kidney must remain in continued operation to hold that accumulated urea, and do not act through the medium of any physical or chemical mechanism which would passively maintain its hold upon the urea even when the active concentration of new urea from the blood had ceased.

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ADDENDUM

Some time after we had sent our manuscript for publication a paper by Cushny (Journ. Physiol., 1917, li, 36) reached us, which contains data demonstrating in another way the return of urea from the kidney to the blood after the activity

of the organ ceases. One kidney was removed while urine was being secreted, and its urea content per gram of tissue determined. At the same time the cord was cut in the cervical region so that a pronounced drop in blood pressure was produced and urine secretion stopped. After an interval of one to one and one-half hours the remaining kidney was removed. It was found to contain less urea than the kidney removed while still active.

A COMPARISON OF THE EFFECTS OF BREAKFAST, OF NO BREAKFAST AND OF CAFFEINE ON WORK IN AN ATHLETE AND A NON-ATHLETE

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INTRODUCTION

The results pertaining to this investigation were obtained in 1912 and 1913, and are reported under two sections. The first section deals with experiments secured with a modified Lombard type of ergograph; the second section deals with tests obtained with an ergometer.

The experiments were conducted on two men in perfect health but of very different physical training.

Subject "B," the athlete, was 5 feet, 8 inches in height, weighed 196 pounds, was 29 years of age and an instructor in physical education. During the two years preceding these tests, in addition to his duties, he had been accustomed to take daily exercise, especially of the arms, chest and back muscles.

Subject "A," the non-athlete, was 5 feet in height, weighed 140 pounds and was 26 years of age. Before beginning these tests he had taken no special physical exercise.

The object of the experiment was to compare the pulse rate, blood pressure, ergographic and ergometer work in both men under the following conditions: of certain doses of caffeine without breakfast, breakfast without caffeine, neither breakfast nor caffeine, and of different intervals of time following the partaking of caffeine or breakfast.

The literature bearing directly on this problem is limited. The references will be confined to reports that may aid to a better understanding of the subject. The ergographic work, calculated from the cyclometer that recorded the sum of the heights of the contractions multiplied by the weight lifted, was performed by the flexor muscles of the second finger of the right hand, lifting a weight of 5 kgm. every two seconds. To prevent the neighboring flexors from participating, the first and third fingers were placed in an adapted clamp, and the

hand held securely in pronation to the ergographic support by means of a narrow non-elastic bandage, placed back of the metacarpal-phalangeal joints. With this arrangement the subjects were able to work more than an hour without discomfort or interference with the circulation.

The experiments were performed in the morning between 8 and 10 o'clock, and under similar conditions. Breakfast at 7.15 a.m., for each man consisted of one soft boiled egg, 2 ounces wheat bread and $\frac{3}{4}$ ounce of butter. For the experiments with caffeine, no breakfast was taken, instead 7 ounces of coca-cola,[1] containing a total of 1.42 grains of caffeine, being the average amount in a strong cup of coffee or of tea. The experiments with caffeine alternated with those obtained either with or without breakfast. The athlete did not drink coffee and the non-athlete had taken it for breakfast, but several weeks before and during the time that the experiments were in progress, of course, both of the subjects gave up the use of articles of diet that had caffeine in them.

Records were kept of the hours and conditions of sleep, of the pulse and systolic blood pressure on rising, before and after exercise, and the intervals elapsing between exercise and partaking of food or of caffeine. The pulse and blood pressure were secured with a Tycos sphygmomanometer while the subject was seated.

SECTION I. ERGOGRAPHIC WORK WITH FLEXOR MUSCLES

Preliminary to the main experiments, the flexor muscles in both subjects were daily exercised on the ergograph under like conditions. After six weeks of training the results became more constant, and for practical purposes the muscles were considered in training. Before that time, therefore, the muscles were considered untrained. Part 1 deals with the average results of the untrained; part 2, with those of the more trained flexors.

Part 1. For purposes of comparison, only the average of all the results observed were tabulated in table 1. A study of this table reveals that at the beginning and after eating breakfast, "A" could contract the untrained flexors two hundred and seventy-nine times until utterly fatigued in nine minutes and eighteen seconds, doing 12.65

[1] Analysis of coca-cola syrup: Sugar 53 per cent, caffeine 1.42 per cent, water 44 per cent, citric and phosphoric glycerine and alcohol qualitative test. One ounce of syrup equals about 7 fluid ounces of coca-cola.

kgm. of work; but by the end of the month he actually trebled these figures. On the other hand, it is seen that from the first "B" did one and one-half times as much work: 18.75 kgm. of work in eight minutes, fifty-five seconds, and in less time than "A" did his, and that he more than trebled his power for work during the month's training. Now instead of doing one and one-half times as much, he did one and two-thirds times as much as did "A," in one and one-fifth the time.

TABLE 1

Average results of experiments with untrained flexors on the ergograph. (a) The first and twenty-fourth test. (b) Average of eight experiments after eating breakfast. (c) Without breakfast. (d) After taking caffeine.

	DATE 1912	CONDITION	SUBJECT	DURATION OF WORK IN MINUTES	NUMBER OF CONTRACTIONS	DISTANCE LIFTED IN CENTIMETERS	WORK IN KILOGRAM METERS
(a)	November 19	Breakfast	A	9′ 18″	279	253	12.65
	December 20	Breakfast	A	26′ 54″	798	726	36.30
(a)	November 19	Breakfast	B	8′ 55″	273	375	18.75
	December 20	Breakfast	B	30′ 18″	918	1,205	60.25
(b)		Breakfast	A	14′ 17″	402	377	18.85
(b)		Breakfast	B	15′ 24″	462	733	36.65
(c)		No breakfast	A	12′ 00	360	357	17.75
(c)		No breakfast	B	15′ 03″	398	665	35.25
(d)		Caffeine	A	25′ 18	759	939	46.95
(d)		Caffeine	B	38′ 43″	1,154	1,421	71.05

The average hours sleep for both subjects = 7½.

The lapse of time between breakfast and caffeine and exercise = 1 hr.

The average room temperature = 59° F.

The number of contractions until fatigued = until unable to lift the 5 kgm. weight.

These experiments were begun November 19 and ended December 20, 1912.

Comparing the amount of work done, without breakfast, one hour after breakfast, and also after a dose of caffeine, it was noticed that both subjects did almost as much without, and in practically the same time, as after having eaten breakfast. "B" moreover, felt better than when working after having eaten the meal.

When both subjects took a dose of 1.42 grains of caffeine, "A" was able to do 46.97 kgm. of work which was two and one-half times as much work, and "B" 71.05 kgm., which was twice as much work

as when working one hour after eating breakfast. The after effect for both, but more observable in "B," was a heightened sense of irritability and weariness not noticed except when working after taking caffeine. The reason that the same dose of caffeine stimulated the working power of the athlete less than it did the non-athlete was probably because the athlete is the larger and heavier man. The dose per kilo weight was less, therefore, for him than it was for "A."

A consideration of this set of experiments demonstrates that the flexors and probably other untrained muscles in an athlete, are more efficient and more readily trained, than are the untrained muscles in a non-athlete.

Part 2. Ergographic results with flexor muscles in training. After six weeks of training the muscles, the results became more constant, and the second set of experiments was begun, and continued for two months. The object of this set of experiments was to compare the effects of no breakfast, of breakfast one hour and one and one-half hours before work. The average results are tabulated in table 2. It is there shown that when working without having eaten breakfast, "B," the athlete, continued as before (table 1) to do twice as much work in one and one-half the time, as did "A." But when working one hour or one and one-half hours, after having eaten breakfast, although both of the men increased their power for work enormously, they did more after eating than when not eating breakfast, and more in one hour, and in less time, than in one and one-half hours after eating breakfast. Nevertheless "B" no longer did twice as much work as "A" but only one and one-half as much. It may be that the greater increase in power in "A" is due to the fact that he attained his maximum power more gradually than "B" did his. The ergographic work had practically no effect either on the pulse or blood pressure. The normal blood pressure was much higher in "B" than it was in "A" but there was little difference in their pulse rate. It appears, and this agrees with Lombard's (1) results, that exercise of this character can be performed better one hour than one and one-half hours after eating breakfast.

SECTION II

The influence of neither breakfast nor caffeine, breakfast without caffeine, and caffeine on ergometer muscle work in a trained athlete and a non-athlete. The ergometer consists of an adjustable grip bar, connected through two pulleys to a weight of 25 kgm. The recorder,

similar to the one attached to the ergograph consists of an endless tape, 1.5 cm. wide and 500 cm. in length that passes around two pulleys, each 50 cm. in circumference. A cyclometer attached to one of the pulley records, therefore, 50 cm. for each revolution. These are noted, and thus the whole height of contraction can be directly ascertained, and the work in kilograms determined.

In conducting the experiment, the subject stands on a line, a definite distance from the bar. The bar has been properly adjusted to the height of the subject, so that with arms fully extended he is able to grip it firm-

TABLE 2

Average results of flexor muscles in training on ergograph, no breakfast (A_1 B_1) *one hour* (A_2 B_2), *and one and one-half hours* (A_3 B_3) *after eating breakfast*

SUBJECT	CONDITION	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	CENTIMETERS LIFTED	NUMBER OF CONTRACTIONS	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			hours								
A_1	No breakfast	67		33′ 29″	1,194	994	59.7	69	71	108	109
A_2	Breakfast	72	1	49′ 00″	1,990	1,470	99.5	71	70	109	109
A_3	Breakfast	74	1½	40′	1,754	1,186	87.7	71	70	107	107
B_1	No breakfast	67		50′	2,487	1,494	124.3	70	71	129	130
B_2	Breakfast	72	1	60′	3,150	1,800	157.5	75	73	128	130
B_3	Breakfast	73	1½	65′	2,571	1,950	128.5	75	72	128	128

The experiments were begun January 15 and continued until March 15, 1912, the different tests alternating with each other during that time.

ly. The bar is lowered by the contraction of the muscles of the chest and arms as far as possible without stooping or raising the heels. In this way the 25 kgm. weight is raised a definite distance, and this distance is read from the recorder. The contractions were repeated every three seconds to the beat of a metronome and in every case were the result of maximum effort, and continued until the weight could no longer be lifted. The three seconds time was adopted because, after the muscles were in training, it proved to be the least time in which the rested muscles could complete a full contraction. In view of the fact that the experiments with the flexor muscles of the middle finger which, when worked on the ergograph until utterly fatigued, had, if any, but a slight effect on the pulse rate and blood pressure, it was

decided to repeat the experiments discussed in Section I with a set of muscles employed under ordinary conditions in hard labor. For this purpose the ergometer is admirably suited.

The experiments were begun March 15, 1913, and continued until June 6, 1913. Records of the first month's preliminary work were not kept. Both subjects exercised their muscles daily by repeating on the ergometer the order of the work that was to be followed, as soon as the muscles in both subjects were given equal training and had attained a certain degree of efficiency and uniformity of results. The tests were made as nearly as possible under similar conditions, and by alternating those with no breakfast, with those performed after eating breakfast or after taking caffeine.

Effect of no breakfast. Table 3 shows the average of ten experiments performed by each subject without eating breakfast.

TABLE 3

An average of ten ergometer records without breakfast

SUBJECT	NUMBER OF OBSERVATIONS	WEATHER CONDITIONS	ROOM TEMPERATURE	DURATION OF WORK	NUMBER OF CONTRACTIONS	CENTIMETERS LIFTED	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
A	10	Cold, stormy	66	2′ 18″	46	3,824	956	70	88	107	130
B	10	Cold, stormy	68	3′ 31″	70	8,305	2,074.2	73	102	128	150

From a study of the tabulated results it is learned that "B" did 2074 kgm. of work in three minutes, thirty-one seconds, and "A" 956 kgm. in two minutes, eighteen seconds. Therefore, "B" did more than twice as much work and in one and one-half the time, that "A" was able to accomplish his, but his blood pressure rose no higher above the level, while his pulse was one and one-half times more rapid than that of "A."

Effect of length of time after eating breakfast on work. The object of this series of experiments was to ascertain if the length of the interval following breakfast and the beginning of exercise influenced the ergometer work, the pulse and blood pressure.

Owing to the lack of time, only the one, one and one-half, two, and two and one-half hour intervals were tested.

Table 4 records four average tests of the sixteen carried out by each subject. It shows that the duration and power for muscular work in both men gradually increased as the interval following the meal lengthened from one up to two and one-half hours. At that time "B" did one and five-eighths as much work in less than one and one-half the time that "A" did it; also, that "B" accomplished as much without as when working one and one-half hours after eating breakfast, and that "A" did more work after eating than without breakfast.

TABLE 4

Ergometer records showing the effect of different intervals between breakfast and work

SUBJECT	NUMBER OF OBSERVATIONS	WEATHER CONDITIONS	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	CENTIMETERS LIFTED	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
				hours								
A	4	Generally stormy with snow, the temperature ranged from 25° to 66° F. with the lower temperature predominating.	76	1	2′ 40″	53	4,651	1,162.7	72	88	110	130
	5		74	1½	3′ 05″	62	5,508	1,377.0	72	84	100	128
	4		71	2	3′ 08″	63	5,599	1,399.0	68	78	106	126
	3		75	2½	3′ 20″	67	6,670	1,667.5	76	88	108	128
B	4		68	1	3′ 10″	63	7,986	1,996.5	76	116	128	158
	5		62	1½	3′ 35″	72	8,078	2,019.5	68	108	132	152
	4		60	2	3′ 55″	78	9,547	2,386.7	68	100	128	148
	3		60	2½	4′ 50″	96	10,710	2,677.5	72	92	134	154

The increase in pulse rate in "B" above normal was, as a rule in every case, more than double the increase in "A." In both subjects, however, the acceleration was less two and one-half hours after doing the greatest amount of work, than one hour following the meal.

The rise in blood pressure above the normal level was practically the same in both men, notwithstanding the athlete did far more work.

It was interesting to learn that with the ergograph the maximum

work was done one hour after, while with the ergometer the power for muscular work was greater two and one-half hours after eating breakfast.

The gradual effect of different doses of caffeine without either breakfast or work on the pulse rate and the blood pressure. The average of eight

TABLE 5

Average effect of different doses of caffeine without either breakfast or work on pulse and blood pressure, (A_1 B_1) dose = 1.42 grains, (A_2 B_2) dose = 2.24 grains of caffeine

SUBJECT	CONDITION		LAPSE OF TIME	PULSE	BLOOD PRESSURE
A_1	No breakfast, record taken	8.05		72	110
	Drank 1.42 grains caffeine	8.10			
	Record taken at	8.30	20′	71	119
	Record taken at	8.40	30′	73	122
	Record taken at	8.55	45′	76	124
	Record taken at	9.10	60′	76	126
	Record taken at	9.40	90′	75	125
	Record taken at	11.10	3 hr.	72	118
A_2	No breakfast, record taken	8.05		72	110
	Drank 2.24 grains caffeine	8.10			
	Record taken at	8.30	20′	80	124
	Record taken at	8.40	30′	68	128
B_1	No breakfast, record taken	8.05		68	128
	Drank 1.42 grains caffeine	8.10			
	Record taken at	8.30	20′	71	144
	Record taken at	8.40	30′	75	148
	Record taken at	8.55	45′	73	146
	Record taken at	9.10	60′	74	144
	Record taken at	9.40	90′		
	Record taken at	11.10	3 hr.	68	141
B_2	No breakfast, record taken	8.05		68	128
	Drank 2.24 grains caffeine	8.10			
	Record taken at	8.30	20′	71	144
	Record taken at	8.40	30′	84	151

tests, showing the effects of 1.42 and 2.24 grains of caffeine on the heart rate and the blood pressure are recorded in table 5. As will be shown later, the 2.24 grain dose for the athlete is equal per kilo body weight to 1.42 grain for the non-athlete. The first effect of 1.42 grain in "A" was a slowing of the pulse of twenty minutes duration, then a

gradual acceleration of four beats per minute during the next hour, and a return to the normal rate within three hours. With the larger dose there was no initial fall, but a rapid acceleration of ten beats per minute during the first twenty minutes and a slowing below the normal rate in thirty minutes.

In "B" the initial fall was absent, but both of the doses caused a gradual acceleration in the heart rate that appeared more promptly after taking the stronger dose, but after taking the weaker dose persisted for three hours, before the normal rate was again attained.

TABLE 6

Effect of taking caffeine at different intervals before work

SUBJECT	NUMBER OF OBSERVATIONS	AMOUNT OF CAFFEINE	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
		grains	°F									
	8	1.42	66	20′	4′ 27″	87	7,486	1,871	72	106	118	135
A	12		66	30′	3′ 33″	71	8,511	2,138	72	110	118	138
	8		70	45′	4′ 23″	88	10,204	2,538	76	110	124	138
	8		70	1 hr.	5′ 34″	111	12,101	3,025	76	117	126	139
	8		66	3 hr.	9′ 10″	183	19,380	4,827	72	128	118	148
	8		66	20′	5′ 27″	109	13,287	3,321	71	109	144	161
	10		70	30′	4′ 53″	97	13,906	3,476	72	112	142	160
B	8		70	45′	6′ 40″	133	14,586	3,646	70	110	146	164
	8		72	1 hr.	5′ 42″	114	13,127	3,381	74	112	143	163
	8		68	3 hr.	5′ 38″	112	12,209	3,052	78	104	142	158

The blood pressure rose about 20 mm. Hg above the level within one hour in both men, after taking either one of the doses, but after the weaker dose was taken, the normal level was not reached in either of the men within three hours.

The effect of taking caffeine from twenty to one hundred and eighty minutes before beginning ergometer work. From an inspection of table 6 where the average results are tabulated, it is seen, that in "A" the power for work steadily increased as the interval between taking the drug and beginning work increased, from twenty minutes up to three hours. Three hours after taking the 1.42 grains of caffeine, he was able to do 4827 kgm. of work in nine minutes, ten seconds, which was two and one-half times the work he was able to do twenty minutes

after having taken the drug. In fact this dose seemed to exert its greatest effect in three hours after it was taken by "A," while in "B," the athlete, its optimum influence was manifested three-quarters of an hour after the drug was taken, but he did only one-eleventh more work at that time than he did twenty minutes after taking the drug. In both men the blood pressure did not rise much higher than was usual after work without the drug, except that in "A," at the three-hour interval, it rose 13 mm. higher than it did twenty minutes after the dose was taken, and his pulse rate also increased with those intervals from thirty-four to forty-six beats per minute.

In comparing the results obtained at the optimum period that is two and one-half hours after eating breakfast (table 3), with those secured at the optimum interval, or three hours for "A" and three-quarter hours for "B," after taking 1.42 grain of caffeine (table 6), the following important facts are brought to our notice: that at those specified periods "A" did three times as much work, his pulse was almost five times as much accelerated, and his blood pressure rose one and one-half as high above the normal level after taking the 1.42 grain, as was possible two and one-half hours after eating breakfast. "B" did not do quite one and one-half as much work, his pulse increased twice the rate and his blood pressure was practically the same three-quarters of an hour after taking 1.42 grain of caffeine as two and one-half hours after eating his breakfast. Consequently this dose had a more prolonged and much greater effect three hours after taking the drug on the force, rate and power of muscular contraction, and upon the cardiac tissue in "A," the lighter weight subject, than it did at any time in "B," the heavier man, for whom it consequently was a weaker dose per kilo of his body weight.

The effect of different doses of caffeine on work. It became of interest to ascertain the effect of larger doses of caffeine, and for that purpose a series of experiments was conducted with 1.42 grain, 2.84 grains and 3.58 grains of caffeine, allowing thirty minutes in each case before the work was begun. This interval was decided upon in order to economize time and because the results for this interval had been quite constant for both subjects. The average results of these experiments are recorded in table 7. They show that endurance and power for work do not keep pace with increase of dosage, but that both subjects did their maximum work after taking the medium dose of 2.24 grains at the thirty minute interval chosen for comparison for these tests. With that dose they could lift the weight a greater number of times,

with greater force, and work longer before fatigued than they could at that interval after taking either of the other doses. "B" did 5429 kgm. work in nine minutes, fifty-four seconds, lifting the 25 kgm. one hundred and ninety-eight times. "A" did 2680 kgm. work in five minutes, fourteen seconds, lifting the 25 kgm. one hundred and four times. With the strongest dose of 3.58 grains, both subjects did less work than they did with the medium dose. In fact "A" did even less after taking the strongest and "B" only one-seventh more than after taking the weakest dose of 1.42 grain. It is evident that the strongest dose proved more depressing to both men than did the medium, and far more so to "A," the lighter weight man, than to "B" the heavier weight man.

TABLE 7

Average effect of different doses of caffeine on work

SUBJECT	NUMBER OF OBSERVATIONS	ROOM TEMPERATURE	AMOUNT OF CAFFEINE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
		°F	*grains*									
A	12	66	1.42	30′	3′ 33″	71	8,511	2,138	72	110	118	138
B	10	66	1.42	30′	4′ 53″	97	13,906	3,476	72	112	142	160
A	8	70	2.24	30′	5′ 14″	104	10,700	2,680	69	119	125	137
B	8	70	2.24	30′	9′ 54″	198	21,716	5,429	71	116	145	165
A	6	70	3.58	30′	3′ 27″	69	7,109	1,777	71	118	124	131
B	3	70	3.58	30′	7′ 20″	147	15,728	3,932	78	116	136	156

The strongest dose of 3.58 grains greatly accelerated the pulse rate, but depressed the blood pressure below the normal level after work in "A," while it had only a little more influence on the pulse and practically no more on the blood pressure in "B" than the medium dose had on these activities.

The effect of caffeine when the dose is taken per kilo body weight. Two sets of experiments were undertaken for the purpose of ascertaining the effects of caffeine thirty minutes after each subject received equal amounts of caffeine per kilo body weight. In the first set of experiments "A," who weighed 66.6 kilos, took 1.42 grains, and "B," whose weight was 93.3 kilos took 2.24 grains of the drug. Each subject was then receiving practically 0.21 grain per 9.3 kilo of his weight. In the

second tests, subject "A" took 2.24 grains and "B" 3.58 grains. The results obtained from these experiments, and which are summarized in table 8 add another viewpoint to the facts obtained from the experiments that dealt with equal doses of caffeine for each subject, without respect to their weight.

At the thirty minute interval after each subject received the weaker dose of 0.21 grain of caffeine per kilo of his body weight, "B" was able to do two and one-half times as much work, and work almost three times as long before becoming fatigued, as was "A." His heart rate at the same time was only seven more beats per minute than was that in

TABLE 8

Effect of caffeine when given per kilo body weight

	SUBJECT	NUMBER OF OBSERVATIONS	ROOM TEMPERATURE	AMOUNT OF CAFFEINE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
				grains									
1	A	12	66	1.42	30′	3′ 33″	71	8,511	2,138	72	110	118	138
	B	8	70	2.24	30′	9′ 54″	198	21,716	5,429	71	116	145	165
2	A	8	70	2.24	30′	5′ 14″	104	10,700	2,680	69	119	125	137
	B	3	70	3.58	30′	7′ 20″	147	15,728	3,932	78	116	136	156

1 = 0.2 grains of caffeine per 9.3 kilo body weight = A 1.42 grains, B 2.24 grains.

2 = 0.2 grains of caffeine per 5.9 kilo body weight = A 2.24 grains, B 3.58 grains.

These experiments were begun March 28 and ended June 6, 1913.

"A," while the increase in blood pressure above the normal level was the same in both. Thirty minutes after each subject received the stronger dose of 0.21 grain per 5.9 kilo of their body weight, "A" did one-fourth more work, and worked two-thirds longer before becoming fatigued than he did with the weaker dose. "B," however, was able to do only about two-thirds as much work, and work about two-thirds as long as he could with the weaker dose. Therefore "B" now did only one and one-half as much work and worked only one and two-thirds as long as "A" before becoming fatigued. His pulse rate was

accelerated thirty-eight and "A's" fifty beats per minute. On the other hand his blood pressure was 20, and "A's" only 12 mm. Hg above the normal level.

Comparing the results obtained from these two doses of caffeine, the fact is brought out that in "B," the heavier subject, the weaker dose of 0.21 grain per 9.3 kilo body weight was more stimulating to the muscular and cardiac activity than was the stronger dose of 0.21 grain per 5.9 kilo per body weight. On the other hand in "A" the stronger dose of 0.21 grain per 5.9 kilo body weight proved the more stimulating. Therefore we conclude that doses given per kilo weight exert for each individual a specific effect, and that at the same interval after taking the drug the effect may be different for different individuals.

The after-effect of caffeine. It became of interest to ascertain whether caffeine exerted a prolonged influence on the system. For this purpose twelve experiments were performed consisting of three sets of four each; with 1.42 grain, 2.84 grains caffeine, and control tests respectively. For the control tests no breakfast was eaten. Each experiment involved five observations on the effect of ergometer work, namely, at 8.20 and 9.20 a. m., and at 4.20 and 8.20 p.m., and after twenty-four hours at 8.20 a.m. Between these intervals the subjects rested or did light routine work. The average results for the control tests are recorded in table 9. They show first, that when working without breakfast, both of the subjects worked longer, with greater force, pulse rate and blood pressure the first period, than they did one hour later, indicating that they had not fully recovered from the previous hour's fatigue. Second, that both subjects worked longer, did more work, and their pulse and blood pressure increased more after the 4.20 and 8.20 p.m. periods; that is, four and two hours following the meal, than they did the first hour in the morning. This indicates that they had recovered from the fatigue of the previous work and were benefitted by the preceding meal. Third, repeating the tests the following morning at 8.20 it was observed that practically all the conditions and data were the same as they were the previous morning. Fourth, "B" did one and one-third more work, had a higher pulse and blood pressure than "A" at the beginning of work, but at 8.20 p.m. his power had lessened, although his pulse and blood pressure had not. Since at this period "A" did his best work, it happened that at this hour his power for work practically equaled that of "B."

A study of the data secured on the duration of the effect of 1.42 grain of caffeine, brings out the interesting results that now both

TABLE 9

Effect of caffeine after one to twenty-four hours

SUBJECT	CONDITION	ORDER OF WORK	DOSE	LAPSE OF TIME AFTER DOSE	TIME BEGAN WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAM-METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			grains	*hours*									
Ia Control	No breakfast. Began work	1			8.20 p.m.	3′ 25″	68	6,579	1,644.7	76	100	108	120
	Rested 1 hour. Began work	2		1	9.20 a.m.	2′ 57″	59	5,630	1,407.5	72	92	114	120
	Lunch at 12.30. Routine work of day. Began work	3		8	4.20 p.m.	3′ 53″	78	7,864	1,966.0	76	112	116	130
	Routine work of day till 6 p.m. Dinner at 6.30. Light reading. Began work	4		12	8.20 p.m.	4′ 30″	90	8,935	2,233.7	76	108	116	138
	Next morning 7½ hours sleep. No breakfast. Began work	5		24	8.20 a.m.	3′ 24″	68	6,576	1,644.2	76	100	108	120
Ib Control	No breakfast. Began work at	1			8.20 a.m.	4′ 05″	82	8,904	2,223.5	76	108	128	148
	Rested 1 hour. Began work at	2		1	9.20 a.m.	3′ 25″	68	6,732	1,683.0	64	92	142	152
	Routine work of day. Lunch at 12.30 p.m. Began at	3		8	4.20 p.m.	4′ 25″	88	9,634	2,408.5	82	116	132	164

Ib Control	Routine work of day until 6 p.m. Dinner 6.30 p.m. Light reading	4		12	8.20 p.m.	4′ 20″	87	9,888	2,272.0	72	109	128	162
	Eight hours sleep. Next morning no breakfast. Work	5		24	8.20 a.m.	4′ 08″	83	8,936	2,234.0	74	106	128	146
IIa	No breakfast	1			8.20 a.m.	3′ 20″	66	6,827	1,706.7	72	108	110	144
	Rested until 9. Took 1.42 grains caffeine. Began work 20 minutes later	2	1.42	½	9.20 a.m.	5′ 05″	102	10,342	2,588.5	84	124	110	144
	Routine work of day. Lunch at 12.30 p.m.	3		7	4.20 p.m.	4′ 18″	86	8,935	2,233.7	80	124	110	140
	Routine work of day until 6 p.m. Dinner, light reading. Work	4		11	8.20 p.m.	7′ 07″	142	11,291	2,822.7	72	130	110	136
	Seven and one-half hours sleep. Next morning no breakfast. Work	5		23	8.20 p.m.	4′ 02″	81	8,415	2,103.7	72	120	112	132
IIB	No breakfast. Began work at	1			8.20 a.m.	3′ 55″	78	8,629	2,157.2	76	104	128	164
	Rested till 9. Took 1.42 grains caffeine. Began work 20 minutes later	2	1.42	½	9.20 a.m.	5′ 35″	112	12,025	3,006.2	84	116	138	162
	Routine work of day. Lunch at 12.30 p.m. Work at	3		7	4.20 p.m.	5′ 20″	107	12,178	3,044.5	76	116	138	152

TABLE 9—*Continued*

SUBJECT	CONDITION	ORDER OF WORK	DOSE	LAPSE OF TIME AFTER DOSE	TIME BEGAN WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			grams	*hours*									
IIB	Routine work of day till 6 p.m. Dinner 6.30 p.m. Light reading. Work at....	4		11	8.20 p.m.	4′ 35″	92	9,180	2,295.0	80	120	140	168
	Eight hours sleep, work. Next morning no breakfast.....	5		23	8.20 a.m.	4′ 20″	67	8,976	2,244.0	76	108	128	144
IIIA	No breakfast.....				7.00 a.m.	4′ 10″	83	7,466	1,866.5	72	108	112	128
	No breakfast. Took 2.84 grains caffeine at 8 a.m.....	1	2.84	½	8.30 a.m.	4′ 38″	92	10,189	2,541.2	68	120	120	140
	Next morning, no breakfast. Work at	5		24	8.30 a.m.	4′ 13″	84	8,904	2,223.5	80	116	114	132
IIIB	No breakfast.....			½	7.00 a.m.	5′ 25″	107	11,444	2,861.0	78	106	134	148
	No breakfast. At 8.15 took 2.84 grains caffeine. Began work	1	2.84		8.45 a.m.	9′ 55″	198	22,338	5,584.5	88	120	140	168
	Next morning. No breakfast. Work at...	5		24	8.30 a.m.	7′ 30″	150	15,330	3,837.5	60	104	128	158

subjects did more work the second period than they did the first; that is, one hour after working without breakfast and twenty minutes after taking caffeine, and there was no indication of fatigue due to the previous hour's work but rather signs of stimulation, or inhibition of fatigue. Their pulse rate was much greater, but their pressure was little changed. Moreover, even after twenty-four hours, they still did more work than they did at the same hour of the previous day, and their pressure and pulse rate were no higher than at that time. As *without*, so now *with* caffeine for the periods chosen for observation "A" displayed his greatest power for work at 8.20 p.m. He did now one and one half times as much work as was possible without the drug, and what is remarkable, did at least as much work at that particular time as "B" could perform.

Concomitant with the great amount of work on "A's" part, there was an enormous acceleration of fifty-eight heart beats per minute and an increase of 26 mm. Hg in blood pressure which was a fall of 8 mm. Hg from the increase in pressure due to work without breakfast.

"B" now did his best work, as before, without eating breakfast with the 1.42 grain of caffeine at 4.20 p.m., or seven hours after taking the dose and four hours after luncheon. He did more work than at any of the other periods tested. His heart rate increased forty beats per minute, but his blood pressure increased only 14 mm. Hg which was much less and his heart beats considerably more than when working without eating breakfast.

With the stronger dose of 2.84 grains of caffeine, "A" did one and one-third as much work as was possible without the drug, but did no more than he was able to do twenty minutes after taking the weaker dose of 1.42 grain. "B" did twice as much as he did without the drug, almost twice as much as he did at his best with the weaker dose, and twice as much as "A" was able to do with the stronger dose. "B's" pulse and blood pressure were increased, the former more so than with the weaker dose, while "A's" pulse rate fell slightly after taking the drug but was almost doubled after the work, and his blood pressure showed less rise than it did with the weaker dose.

After twenty-four hours, however, both subjects did much more work than they did at their best without eating breakfast, showing that the after effect of the strong dose not only persisted twenty-four hours, but that it still had a very powerful effect that would probably continue considerably longer. The strong dose, after twenty-four hours, caused a great depression of "B's" pulse rate far below normal

and an increased rate above normal in "A's". In both subjects the pulse was greatly accelerated by the work, indicating a heightened irritability to stimuli produced by the work at that time. Therefore the stimulating influence of the caffeine persisted at least twenty-four hours after the doses were taken. There seems also to be an optimum period when the effects were more pronounced, and a maximum dose limit beyond which the power for muscular work is lowered. This period and limit varies in different individuals.

When these investigations were undertaken, "B" complained of weakness of his eyes, and on November 3, 1912, was fitted with glasses. On June 3, 1913, during the time he had been experimenting with the larger doses of caffeine and after he had taken one of the strongest doses, the external rectus muscle of his left eye became paralysed. This may have been a weak muscle and readily affected by the drug. The paralysis of the muscle incapacitated him for further work. At about this time "A" also complained of being very irritable and feared that the caffeine was detrimental to his health. It seemed to him that he could work indefinitely without fatigue yet his muscles failed to contract and his heart beat at a tremendous rate. The experiments were therefore discontinued and the study of the after effects of caffeine, which had been planned and just begun, had to be abruptly abandoned. The indications were that there were after effects that interfered with efficiency of physical and mental activities. "B's" friends, among them two physicians, charge it to the influence of the caffeine that "B" failed in an athletic exhibition in which he took part in the spring during the time that he was conducting the caffeine experiments. Before he began the experiments he had trained himself so that he was able to hit the punching bag with his head, feet and hands alternately on its rebound. It required speed, accuracy and control of muscles, and concentration of thought. He had become an expert in this feat. But his power of concentration, accuracy and precision in his muscles had been greatly impaired so that he was unable to repeat the athletic demonstration with any credit during the time he was taking the strong doses of caffeine.

DISCUSSION

In the two subjects on which the tests were conducted, the pulse rate, blood pressure and power and duration of muscular contraction, in the trained as well as the untrained muscles, were more or less differently affected by the following conditions: the same doses of caffeine as well as the doses of caffeine per kilo body weight; no breakfast; breakfast without caffeine; and the interval following either breakfast or a dose of caffeine.

Lombard (1), by repeating his ergographic tests several times daily, quadrupled the power of his untrained flexor in twenty-two days. Food increased his power for muscular contraction, and he accomplished

more work one hour after than one and one-half hour after a meal. He found that the usual amount of coffee did not affect his power for muscular contraction. The results obtained by the athlete and the non-athlete corroborate those found by Lombard, but do not agree with his conclusions in reference to the effect of the usual amount of a cup of coffee. The difference may possibly be due to the fact that the effects of caffeine as a rule do not disappear in twenty-four hours, and therefore drinking this daily would keep the subject constantly under its influence and he could not compare its effect with those obtained with no caffeine. The athlete had seldom in his life, and the non-athlete only moderately, partaken of coffee, which facts may explain why the athlete was affected more strongly and more rapidly by the drug, than was the non-athlete or Lombard. The athlete felt generally better when working without breakfast, and both subjects did more ergometer work two and one-half hours after than one hour after eating. The pulse rate was much more accelerated in the athlete, in fact often more than double that of the non-athlete, and this may account for the greater fatigue of the athlete from work after eating breakfast.

The conclusion of previous workers that an optimum dose of caffeine increases the capacity for muscular work and inhibits the sense of fatigue, and that a larger dose decreases the power for muscular contraction was confirmed. It was also found that an optimum dose of caffeine may double the capacity for work over that produced at any time after eating breakfast; that it inhibits the sense of fatigue for many hours after the most exhaustive work with the ergometer; and that when the optimum dose, which was different per kilo weight in the two subjects was increased the working power and blood pressure were decreased, the heart rate accelerated, and with a very large dose was inhibited. Rivers and Webber's (2) results are in harmony with these. They found that 1 to 3 grains of caffeine stimulate and 4 to 6 retard the speed of typewriting, and that the relation between blood pressure and pulse rate is not constant, and that strong doses accelerate the pulse but depress the blood pressure. These results, according to Sollmann and Pilcher (3) and other investigators, are held to be due to the stimulating effect of the optimum dose on the muscle and cardiac tissue, and the depressing effect on the nervous system that controls the sense of fatigue. The large doses stimulate the muscle and cardiac tissue more, and if increased above a certain amount produce a progressive depression of these tissues. The large dose also inhibits the peripheral vasomotor mechanism, causing dilation of the blood vessels,

and at the same time stimulates the vasomotor center. These two effects neutralize each other more or less, and thus produce changes in the blood pressure that are proportional to the extent of neutralization.

Sollmann and Pilcher (3) also observed that large doses may be followed by paralytic phenomena, causing fatigue and depression of muscular contractions.

It was interesting to find that the effect of the same dose varies considerably with the interval of time after the drug is taken. In the athlete, the effect of the weak dose of 1.42 grain of caffeine gradually increased from twenty to forty-five minutes, and in the non-athlete from twenty minutes to three hours after the drug was taken—that is, this dose was only five-eighths as strong per kilo body weight for the athlete as it was for the non-athlete, but had its maximum effect in the athlete in two-eighths of the time that it did in the non-athlete. It was of shorter duration, less stimulating to muscular contraction and less effective in inhibiting fatigue; but it had a greater stimulating effect on the pulse rate of the athlete, and but little more on his blood pressure forty-five minutes after it was taken, than it did at the same interval of time on the non-athlete. Therefore, for each indivdual, different doses of caffeine may exert their optimum influence at definite intervals after the dose is administered.

When taken per kilo body weight, the effect of doses of caffeine taken at definite intervals before beginning work was different in the two subjects. Of the two doses, 0.2 grain per 9.3 kilo body weight, and 0.2 grain per 5.9 kilo body weight, and thirty minutes after the drug was taken, the weaker dose proved more stimulating to the working power of the athlete, and the stronger dose to that of the non-athlete. At the same time, the pulse rate was enormously increased in both subjects, while the increase in blood pressure was the same in the athlete as with the stronger dose, and less in the non-athlete than with the weaker dose.

It was shown throughout the experiments that the athlete was more sensitive to the effects of the caffeine than was the non-athlete, and therefore, a dose that would prove stimulating to the athlete's power of muscular contraction and heart action might prove less so for these functions in the non-athlete at that particular time.

For comparative work it therefore seems advisable to study the effects of the dose irrespective of body weight as well as of definite doses taken per kilo body weight of the subject.

Another factor that deserves consideration is that the observations ought to be made at certain periods of the day. Lombard and other investigators found that there were diurnal variations that had an influence on the power for work and recovery from fatigue. Lombard observed that his power for muscular contractions was greater from 5.30 to 6.30 p.m. than from 3.30 to 4.30 p.m., and greater at 4.30 p.m. than 11.30 a.m. Maggiora (4) held that recovery from fatigue was more rapid before 10 a.m. than at 11 a.m. These observations are strengthened by those obtained in studying the influence of caffeine and no breakfast at definite periods of the day. It was found that without eating breakfast, and also with the weak dose of caffeine, the power for muscular contractions in the athlete was greater at 4.20 p.m. than at 8.30 a.m. or 8.20 p.m. and in the non-athlete under the same conditions at 8.20 p.m. than at any of the other periods of the day tested. In both subjects, moreover, the effects of the caffeine continued at least twenty-four hours after the drug was taken. In Hollingsworth's (5) subject the stimulating effect of caffeine was noticeable even after three days. Kraeplin also observed that strong doses of caffeine retarded the transformation of intellectual conceptions into actual movements. These after effects of caffeine are explained by Cushny (6) as the results of stimulation of the central nervous system that is associated with psychical activity. After a strong dose of the drug connected thought is rendered more difficult and impressions follow each other so rapidly that attention is destroyed, and it requires more effort to limit it to a single object. These views would explain why the athlete, during the time he was taking strong doses of caffeine, was unable to perform the athletic feats in which he had excelled before conducting these experiments.

SUMMARY

The general results of the experiments recorded in this paper are as follows:

The working power of the untrained flexor muscles in a trained athlete may be increased at least three and one-half times, and the same muscle in a non-athlete three times in one month of daily training. From the first the athlete did one and one-half, in less time, and later at the same stage of training, twice as much work as was done by the same muscles in the non-athlete.

The lack of breakfast had at first a slightly less favorable effect upon the amount of work done, although the athlete always felt less fatigued

working without breakfast than when working one hour after the meal. Both subjects were able to do more work on the ergograph when the muscles were in training after eating breakfast, and more one hour than one and one-half hour following the meal. After taking 1.42 grain of caffeine, both subjects did more than twice as much work than they were able to do after eating breakfast. The after effect, however, was a heightened degree of irritability especially noticeable in the athlete. The ergographic work had practically no effect on blood pressure and only a slight effect, if any, on the pulse rate, when working either without or after eating breakfast. The normal pulse rate was practically the same, but the normal blood pressure was higher at all times in the athlete than in the non-athlete.

After both subjects had had equal preliminary training for one month of the arm and trunk muscles on the ergometer, the athlete did more than twice the work done by the non-athlete in one and one-half the time without, and more than one and one-half as much work, after eating breakfast. The efficiency of both subjects grew in proportion as the interval between the meal and beginning of the work increased from one to two and one-half hours. The non-athlete did one-half and the athlete one-third more work two and one-half hours after than they were able to do one hour after the meal.

The increase above their normal blood pressure after working either with or without breakfast was the same for both subjects, notwithstanding that the athlete did more work. But under the same conditions the pulse rate in the athlete was practically double that in the non-athlete. The increase in heart rate was least in both subjects when working two and one-half hours after eating breakfast, that is, the time when the greatest amount of work was accomplished.

A weak dose of 1.42 grain of caffeine, without work or breakfast, gradually increased the pulse rate during the first hour, but in the non-athlete as a rule only after a slight initial fall. In both subjects the pulse returned to the normal rate within three hours. With the larger dose, 2.24 grains, under the same conditions, the increase in pulse appeared more promptly, but in thirty minutes was depressed below normal in the non-athlete, and accelerated above the normal rate in the athlete. The blood pressure rose above the normal level in one hour and frequently had not returned to the level in three hours after taking either of the doses of caffeine.

The effects of caffeine taken at different intervals before work, varied with the dose and the individual. In the athlete the maximum influ-

ence of a dose of 1.42 grain was manifested in three-quarters of an hour, and in the non-athlete three hours after the dose was taken. The athlete did but little more work forty-five minutes after than he did twenty minutes after taking the drug. But the non-athlete did two and one-half times as much work three hours after as he did twenty minutes after taking the dose.

Power and endurance for work, and cardiac activity and increase in blood pressure do not keep pace with increase of dosage. The maximum power for work in both subjects was attained with the dose of 2.24 grains of caffeine. With this dose both subjects did two and a half times as much work as they were able to do one hour after eating breakfast. In the athlete with this optimum or with the weaker dose of caffeine, the blood pressure was no greater than after the maximum work done either with or without breakfast, and the heart rate was only slightly more accelerated. In the non-athlete the pulse rate was increased almost three times as much, but the blood pressure was no higher than it was after the maximum work following the meal. A stronger dose of 3.58 grains depressed the muscular power for work in both men, but very markedly so, as well as the blood pressure and pulse rate in the non-athlete. In the athlete the blood pressure was no different, but the heart rate was less after the work following the weaker dose. When the dose was taken in proportion to the body weight, e.g., 0.2 grain of caffeine per 9.3 kilo body weight, or a stronger dose of 0.2 grain per 5.9 kilo weight, the results presented another viewpoint to those obtained when the dose was taken irrespective of body weight. The facts showed that of these two doses thirty minutes before beginning work, the weaker dose and not the stronger stimulated the working power in the athlete most. But in the non-athlete the reverse was the case. With the stronger dose the athlete did one-fourth less and the non-athlete one-fourth more work than with the weaker dose. At the same time the pulse rate was enormously increased in the non-athlete and less so in the athlete who did double the work done by the non-athlete. On the other hand, the blood pressure fell slightly in the non-athlete, and fell also or remained unaltered in the athlete after work and after taking the stronger dose. Therefore, for each subject there was a definite optimum dose which, when increased, proved depressing for muscular work, blood pressure and pulse rate.

One hour's rest did not remove the sense of fatigue produced by the ergometer work, but when caffeine was taken the fatigue of the previous

hour's work was inhibited and both subjects did more work then, and even twenty-four hours after taking caffeine, than they did before taking the drug. With the same dose of caffeine and also without eating breakfast, the power for muscular work in the athlete was greater at 4.20 p.m. and in the non-athlete at 8.20 p.m. than at 8.20 a.m. That is, the athlete did his best work eight hours after taking the caffeine and four hours after luncheon, and the non-athlete did his best work twelve hours after taking the dose, and two hours after dinner. At these respective periods, the pulse rate and blood pressure increased greatly in the non-athlete, and the pulse but not the pressure in the athlete. The after effect of the larger dose was a heightened condition of irritability that persisted many hours after the drug was taken. The power and endurance for work were increased, and the cardiac activity greatly affected, but the blood pressure less so than with the stronger dose. It was not possible to state how long the after effect would endure, because the experiments were suddenly interrupted by the paralysis of the rectus muscle of the left eye in the athlete, and the nervous condition of the non-athlete.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

IV. Effect of Massive Doses on the Outflow from Muscle

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That intravenous injections of adrenin in all dosages cause dilatation of the vessels of the muscles of the limb was recently reported from this laboratory (1). The largest dosage used in that investigation was 5 cc. of a 1–25,000 solution. Inasmuch as such dosages as these are probably many times greater than any single discharge from the normal adrenal glands, dosages of higher concentration were not studied. Also we saw no reason to believe that dosages of higher concentrations than those used would alter the reaction.

Recently Cannon and Gruber (2) published some muscle contraction graphs of animals under the influence of large doses of adrenin. Following the injections the contractions were diminished in height—a fact which suggests that vasoconstriction in the muscle might be occurring. Indeed the authors postulate this condition. Later we were informed by Dr. W. J. Meek that he had observed vasoconstriction in the muscles due to very large doses of adrenin. Gruber (3) reported that in perfused muscles, with the nerve supply cut, adrenin in *small doses* produces vasoconstriction. Dilatation to be sure could not be expected with the vessels in a state of extreme relaxation due to the loss of the tonic effect of the constrictor nerves when the vessels are supposedly dilated to their limit. Cannon and Lyman (4) have observed that after the blood pressure has reached a certain low level, intravenous injections of adrenin will no longer exert a depressor effect. In view of the foregoing observations it was decided that further experimentation on the effect of adrenin on the circulation in the muscles was desirable by way of supplementing our former communication.

METHOD

The same methods of investigation were used as were described in the first paper of the series (1). As before, dogs were used for experimentation. Volume curves were not recorded. The observations were all made on venous outflow.

RESULTS

In investigating this phase of the problem, small doses were used at first and the amount gradually increased. When the dosage reached

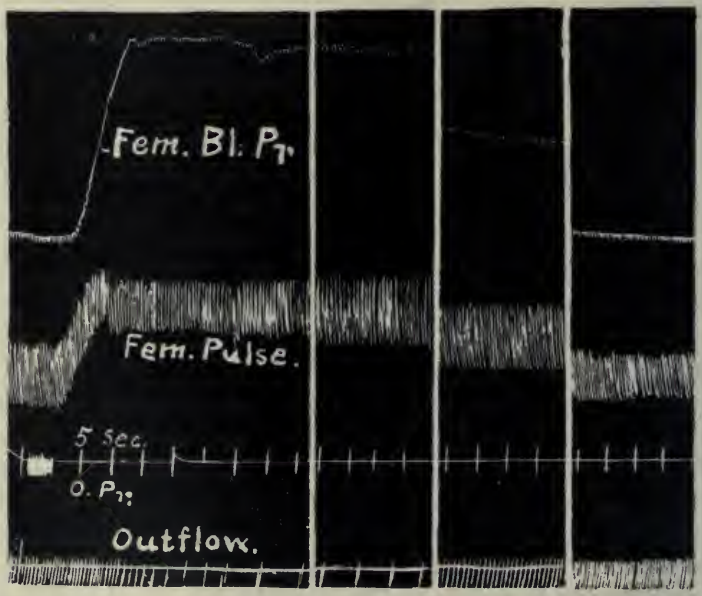

Fig. 1. Successive segments of graph showing effect of massive dose of adrenin on blood pressure, femoral pulse and venous outflow from muscle. Interval omitted in each case two minutes. Dose 1 cc. 1: 1000 "Adrenalin." Dog; weight, 11 kilos. No reduction.

1 to 2 cc. of a 1–1000 solution the reaction of pure dilatation, such as was previously reported, changes as is shown in the accompanying figure. There is a short preliminary increase in the outflow following the injections which lasts about ten seconds and occurs during the rise in blood pressure. This is probably due to the vasoconstriction forcing the blood out of the vascular spaces into the veins. At the time,

or shortly after the blood pressure reaches its crest, an active vasoconstriction takes place in the muscle circulation. This is evidenced by a diminished outflow which lasts, when the injections do not produce a too long maintained blood pressure reaction, until some time after the blood pressure has again returned to normal. In the long maintained blood pressure reactions the diminished outflow begins its return to normal several seconds after the blood pressure commences to drop. The outflow, after returning to normal, invariably showed a tendency to "over recovery" by a second increase in the rate of outflow. This secondary increase might be due to the release of blood dammed back by constriction in the veins of the muscles as was observed in the mesenteric circulation by Henderson (5) from mechanical stimulation of the intestines. It was observed, however, that there was no recovery from this secondary dilatation and also that the tendency to it was more marked toward the end of an experiment. This "over recovery" then is due most probably to a fatigue of the vascular musculature.

That the vasoconstriction was not taking place in fatigued or overdilated vessels was proven by the facts that the blood pressure was maintained, that the vasoconstriction would take place early in an experiment and that small dosages of adrenin would produce pure dilatations at any time during the experiment.

The threshold for the vasoconstrictor effect was found to vary both in the same dog and in different dogs. It was found to be slightly lower with the prolongation of an experiment and with an increase in the concentration of the injection. It was observed that 5 cc. of 1–5000 solution might produce in a given animal pure local dilatation without a marked general blood pressure reaction, whereas 1 cc. of a 1–1000 solution would produce the vasoconstriction reaction as described. The quantity of adrenin in both cases was the same. These results are probably due to the fact that the concentrated solutions reach the tissues still in more concentrated form. The solutions of lower concentration, since they are greater in volume, amount in fact to a short lasting infusion.

DISCUSSION

These results hardly have a bearing on our main thesis which is concerned with suprarenal physiology. The high dosages necessary to produce vasoconstriction in the muscles are not physiologic. They are rather to be considered as pathologic. A study of the blood pres-

sure curves, induced by these massive doses, gives the impression of a tetanus. The fact that a maintained "over recovery" occurs suggests a toxic effect which has injured some part of the vasomotor apparatus. It is a well known fact that adrenin in very large doses does produce toxic effects. It is extremely unlikely that the normal adrenal glands could at any one time pour such quantities as these into the circulation. The normal discharge, judging by all data now available, is similar to an experimental infusion of low concentration.

SUMMARY

1. Adrenin in massive dosages produces a diminished circulation in the skeletal muscles.

2. There is a preliminary increase in the outflow due supposedly to the blood present in the vascular spaces being forced into the veins by the vasoconstriction.

3. There is a maintained secondary dilatation due probably to a fatigue of the vascular musculature.

4. The vasoconstrictor threshold was found to be lowered by an increase in the concentration of the adrenin and by the repetition of injections.

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A correction. In the first paper of this series (1) the statement was made that hydremic plethora serves to convert a vasoconstrictor effect of adrenin in a limb to a vasodilator effect. Subsequent investigation has failed to corroborate our earlier observations on this point.

EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

V. Volume Changes and Venous Discharge in the Intestine

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Although a number of investigators have studied the effects of adrenin on the activity of the intestines relatively little attention, apparently, has been given to the effects on vascular conditions. Oliver and Schaefer (1) made no direct determinations but concluded from inspection that vasoconstriction in the gut wall follows the administration of adrenin. The generalization was offered that this substance, or, more specifically, suprarenal extracts, cause vasoconstriction throughout the splanchnic area. Elliott (2) has reported a single instance in which he painted adrenin on the wall of the intestines of a fowl and also gave an intravenous injection. The result as determined by inspection was an intense vasoconstriction.

Froelich (3) in a brief Centralblatt article has reported that both *d*- and *l*-suprarenin as well as "adrenalin" caused long lasting contraction of a segment of gut enclosed in a plethysmograph. He used both cats and dogs. Few details as to the experiments were given. Vincent (4) states that sometimes the intestine expands under the influence of adrenin and publishes a plethysmograph curve that shows a slight degree of expansion that might be interpreted as passive.

Brodie and Dixon (5) noted that the addition of adrenin to a perfusate materially decreased the flow through the vessels of the isolated intestines.

Ogawa (6) included the gut in a series of perfusion studies on the vasomotor effects of adrenin. Rabbits, cats and dogs were utilized as experimental animals. It was reported that *l*-adrenin in greater concentration than 1:5,000,000 caused a clean-cut constriction in the intestinal vessels, as was shown by a decreased venous outflow. With the higher concentrations occasionally a secondary dilatation was ob-

served. In higher dilutions, e.g., 1: 50,000,000, the effect was primary dilatation. It was found that *d*-adrenin had a similar effect but larger doses were required.

Technique. In our investigations both plethysmograph studies and determinations of the outflow from the opened intestinal veins have been made. Ether anesthesia was used in all cases. The adrenin solutions were made with Parke, Davis "Adrenalin" in distilled water. The technique employed has been described in sufficient detail in the first two papers of this series (7). It need only be added that in the plethysmograph work segments of small intestine about 20 cm. in length were used. These were coiled or folded into the box with due care, of course, to prevent occlusion of the blood vessels. The prompt volume changes and frequently the appearance in the tracings of vascular pulsations showed that success in this respect had been achieved. In all some three hundred and twenty-five determinations were made on forty-five dogs. In the infusion experiments the duration varied from one-half to nine minutes.

Results. The results of the experiments are summarized in the accompanying table. In many instances a brief inconsequential preliminary dilatation was observed but as this is a common feature in all the organs studied and probably is merely a passive effect, it is ignored in the table and subsequent discussion. The most prominent features in the results as a whole were augmentation of the gut volume and of the discharge from the opened veins. Often, however, the dilatation phase was preceded by a more or less pronounced contraction and in some instances contractions only were obtained. The only possible combination of these two conditions that was not encountered was dilatation followed by contraction. In case of the venous outflow the results were somewhat more consistent an augmentation always having occurred but this was not infrequently preceded by a decrease during the first part of the reaction.

There was no very definite correlation between the dosage and the reaction in the gut. In some animals smaller quantities caused contraction which was succeeded by dilatation when the amount of the adrenin was increased. In other animals, however, the reaction remained constant except as to degree whatever quantity was injected. The doses varied in the injection experiments from 0.25 to 8 cc. of 1: 100,000 solution. The effects of massive doses were not investigated but the upper range actually employed very materially transcended physiological limits so far as these can be determined from data now

available. The data in the accompanying table are reduced roughly to a quantitative basis. Since the dogs used were not greatly dissimilar in size (averaging about 12 to 13 kilos), while the variations in reactions among various animals were much greater, nothing would

Effects of adrenin in the small intestine

	NORMAL		AFTER ECK FISTULA	
	Number of cases	Average dose	Number of cases	Average dose
		mgm.		*mgm.*
1. On volume				
a. Injections				
Pure contraction	9	0.013	0	
Pure dilatation	52	0.021	7	0.034
Contraction followed by dilatation	90	0.023	52	0.026
Dilatation followed by contraction	0		0	
		mgm. per minute		*mgm. per minute*
b. Infusions				
Pure contractions	2	0.011	0	
Pure dilatations	15	0.030*	8	0.043
Contraction followed by dilatation	14	0.036	11	0.046
Dilatation followed by contraction	0		0	
		dose in mgm.		*dose in mgm.*
2. On venous outflow				
a. Injections				
Pure augmentation	9	0.020	0	
Diminution followed by augmentation	31	0.025	2	0.020
		mgm. per minute		*mgm. per minute*
b. Infusions				
Pure augmentation	4	0.030*	0	
Diminution followed by augmentation	6	0.030	2	0.050

* Excluding one case of very low irritability.

be gained by expressing the dosages more definitely as milligrams per kilo. The average of all doses giving each effect is stated. Such averages are of restricted value but so far as they go they indicate in gen-

eral a tendency toward constriction with smaller doses and a dilatation with larger. It may be reiterated, however, that in individual cases this statement often does not hold. As previously mentioned, Ogawa (6) obtained dilatations with smaller rather than larger doses.

Unlike the other organs studied, the gut frequently gave reactions with injections different from those obtained with infusions. In various instances with injections a definite contraction phase was noted whereas in the same animals infusions gave pure dilatations. These dilatations were observed both with dosages which caused little change in blood pressure and with those causing a sustained rise. This is a feature worthy of note in consideration of the adaptive significance of the suprarenal glands. If, as there is considerable reason to believe, adrenin discharge plays a significant part in integrating the body for muscular exertion, dilatation of the intestinal vessels would seem to be a detriment. It is quite possible, however, that the exposure of the splanchnic organs and the incidental trauma may be a determining factor in the reactions noted and thus account for the discrepancy. The dilatation is probably correlated with the well known fact that adrenin under ordinary experimental conditions causes flaccidity of the gut. This in itself would supposedly have a material tendency to augment the caliber of the intestinal vessels and might even mask a primary vasoconstrictor effect. This is borne out by the fact that in two animals small doses produced sustained enterocontraction.

The threshhold of reaction in the gut was found to be in general about the same as that of blood pressure. The enteric reaction, however, usually persisted for some time after blood pressure returned to normal. This lag in the volume reactions has been observed very generally in all the organs studied. Often in infusion experiments the blood pressure is briefly shifted but returns essentially to normal whereas the volume change persists throughout the time the drug is being administered. This fact would seem to prove that there is a series of surprisingly delicate and efficient reflex interconnections among the various organs whereby a change in the vascular conditions of one is compensated by a change of an opposite character in another so that the general blood pressure and hence the circulation in the brain and various "neutral" organs remains practically undisturbed.

In case of both injections and infusions there was frequently observed an after dilatation. In cases in which the primary effect was enterodilatation this appeared merely to a prolongation of the reaction but where the reaction proper was enterocontraction it amounted to a dis-

tinct over-recovery. This after-effect usually persisted two or three minutes.

In such cases as those shown in figures 1 and 3 in which enterocontraction occurred, the gut reaction frequently synchronized with a vascular hypertension and supposedly played a considerable rôle in its production. In many cases, however, the gut dilated while the general blood pressure either remained essentially unchanged or even, as in figure 2, considerably increased. In some instances when enterocontraction occurred it outlasted by a considerable period the hypertension. These facts show that although vasoconstriction in the intestines may play a considerable part in augmenting systemic blood pressure there are other structures which have enough greater influence even to cause hypertension synchronous with enterodilatation. These other organs are probably the liver, kidneys, spleen and skin.

In view of the fact that the blood from the intestines has to pass through the liver, an organ which contracts under the influence of adrenin, (8) the question arises: To what extent are volume reactions in the intestine dependent upon back pressure from the liver? To answer the question several dogs were tested before and after the production of Eck fistulae. These were made by the well known method of stitching together the portal vein and the vena cava so as to hold in hermetically tight apposition an elipsoid area of their external walls. Then by traction and sawing with a cutting suture previously placed the walls were ruptured so as to establish a free connection between the veins. When the portal vein was ligated between the liver and the fistula one could make certain not only that the opening was patent, but also, if no evidence of back pressure developed, that the fistula was of adequate size. Having performed all the operation up to the last stage the adrenin reactions were determined. Then the fistula was quickly made and the portal vein ligated and the adrenin administration repeated. In one case the result of shunting the portal blood directly into the vena cava was to convert an enterodilatation to an enterocontraction but in the other instances the reactions were essentially unchanged. (See tabulated results.)

The effect of adrenin on the outflow from an open intestinal vein was purely or predominantly an augmentation, amounting at times to 300 per cent (fig. 3). In cases where the volume reaction was dilatation this result would of course be the expected one but it was observed consistently throughout the series irrespective of whether the gut dilated or contracted or whether arterial pressure was raised

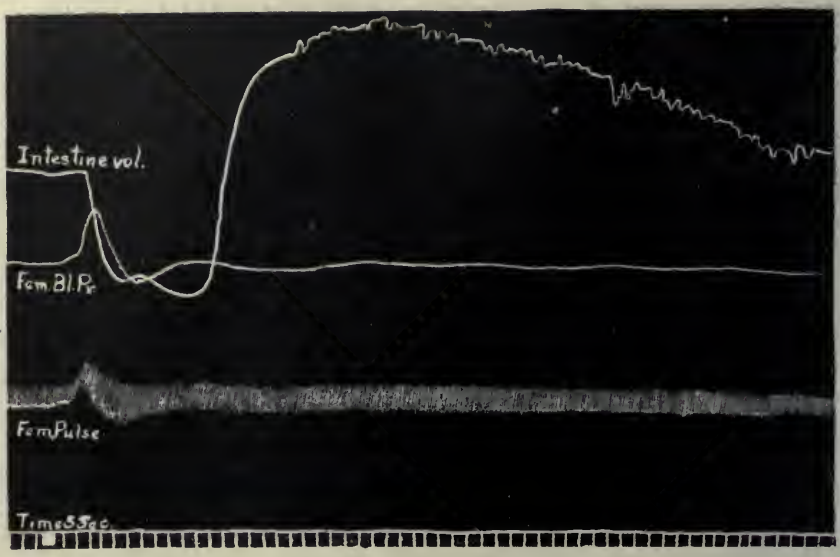

Fig. 1. Graph showing effects of adrenin injection on gut volume, femoral blood pressure and femoral pulse. Dose, 1 cc. 1: 100,000. Dog weight, 16 kilos. Time, five seconds. Reduced to one-half.

Fig. 2. Graph showing effects of adrenin infusion on gut volume and femoral blood pressure. Adrenin 15 cc. 1: 200,000 in ninety seconds, from *a* to *b*. Dog weight, 16 kilos. Time, five seconds. Reduced to one-third.

or lowered. Since any vein large enough to cannulate is connected through anastomoses fairly directly with the portal vein the rate of outflow would depend rather more, probably, upon portal pressure than directly upon capillary changes in the gut wall. Since the outflow was consistently augmented by adrenin, one would accordingly postulate

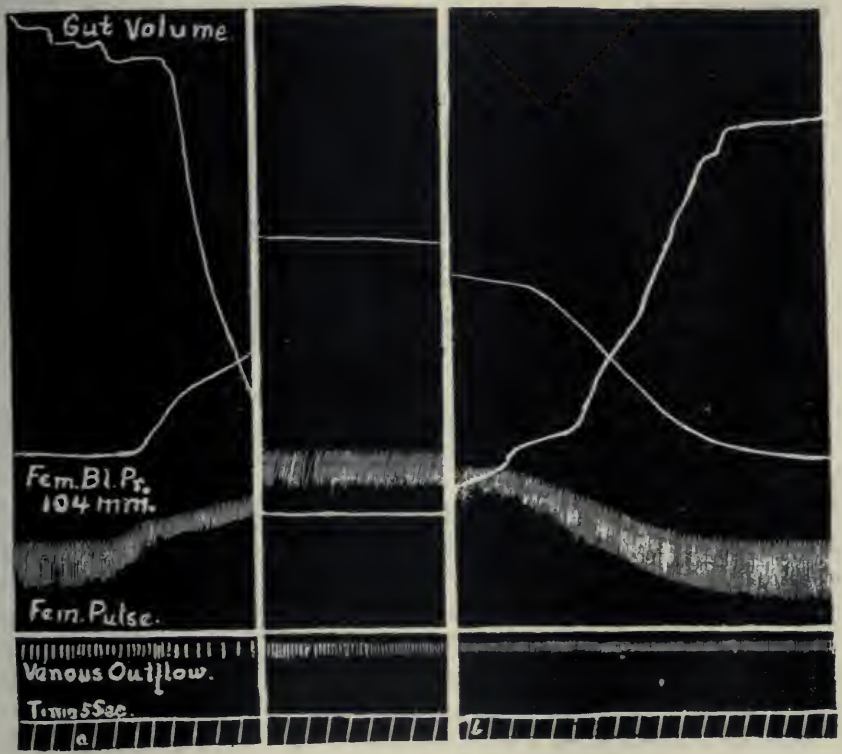

Fig. 3. Graph showing effects of adrenin infusion on gut volume, femoral blood pressure, femoral pulse and venous outflow. Twenty-two and one-half cubic centimeters adrenin, 1: 200,000 in two hundred and twenty-five seconds, from *a* to *b*. Dog weight, 16 kilos. Time, five seconds. Ninety seconds omitted between each segment of the graph. No reduction.

an increase in portal pressure with all effective doses. But that this is not an essential feature in the explanation is shown by the fact that an exactly similar reaction occurs in dogs with Eck fistulae. According to the observations of Capps and Mathews (9) adrenin in the quantities used in these experiments produces little or no effect upon the

pressure in the vena cava, hence the augmented outflow from the intestinal veins is not to be ascribed to back pressure from the large veins. The only explanation that has occurred to us is that the adrenin may produce sufficient contraction in the proximal mesenteric veins to cause considerable back pressure such as Henderson (10) has observed in surgical shock. Whether this actually is the cause was not determined.

SUMMARY

1. The effects of adrenin in physiologic doses were investigated as regards gut volume and venous outflow in anesthetized dogs.

2. There was no definite correlation between dosage and volume changes but dilatation predominated, particularly with larger doses.

3. Often the dilatation was preceded by contraction. In some instances contraction alone occurred.

4. Infusions frequently gave only enterodilatation in animals in which injections gave a preliminary contraction.

5. The thresholds for changes in blood pressure and gut volume were approximately the same.

6. In some cases enterocontraction coincided with vascular hypertension but there was no constant relation between blood pressure and gut volume changes.

7. The volume changes usually persisted after blood pressure returned to normal, indicating reflex compensatory adjustments among various organs.

8. An after dilatation of the gut was frequently noted when the primary adrenin effect had worn off.

9. In most cases Eck fistulae made no difference in the gut reactions hence the liver played no essential part.

10. The outflow from a small cannulated gut vein was augmented by adrenin in all effective doses irrespective of changes of blood pressure or gut volume. This augmentation was not due to back pressure from the portal vein or vena cava. In most cases the augmentation was preceded by brief diminution in the outflow.

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THE VASCULARITY OF THE ADRENAL BODIES

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Our knowledge regarding the innervation of the adrenal bodies is based chiefly upon Biedl's (1) determinations of the flow from the suprarenal vein during stimulation of the greater splanchnic nerve. The procedure practiced in these experiments was as follows: Having excluded the kidneys from the circulation, the inferior vena cava was ligated distally to the entrance of the renal veins. A loose ligature was then placed around this blood-vessel centrally to the orifices of the suprarenal veins. By tightening this ligature the blood of the suprarenal veins could be collected in this pouch and could be directed at any moment through a cannula into a bottle containing a solution of magnesium sulphate. The quantity of fluid displaced from the bottle was measured with the aid of an ordinary drop recorder.

In his experiments proving that the activity of the adrenal glands is controlled by secretory fibers, Dreyer (2) collected the blood from a cannula which was inserted in the left suprarenal vein distally to the gland. Stewart (3) has recently employed a method very similar to that of Biedl with this modification, however, that one of the iliac veins was tied near the cava and the other close to its point of origin. In this way it was possible to place the latter vertical, and to permit it to serve, so to speak, as the neck of a measuring flask, the body of which was formed by the trunk of the inferior cava. Having first emptied this pocket, the moment could easily be determined when the blood from the suprarenal vein first entered it and again when it reached the proximal end of the iliac vein. The quantity of blood required to fill this pouch could easily be measured by simply emptying its contents into a graduated cylinder. No statements, however, are made regarding the volume of the bloodflow in any of the papers cited.

The present experiments were originally undertaken with the intention of obtaining a more exact proof of the existence of vasomotor

fibers in the adrenal bodies than has been presented by Biedl. Certain technical difficulties, however, have prevented us from carrying them to completion. In the succeeding pages we shall confine ourselves, therefore, to a brief discussion of certain data pertaining to the vascularity of the suprarenal gland.

The experiments were performed upon five large dogs during ether narcosis. Having opened the abdomen by a median and left transverse incision, a loose ligature was placed upon the left suprarenal vein centrally to the gland. The left greater splanchnic nerve was then isolated directly below the diaphragm and placed in shielded electrodes. The suprarenal vein having been ligated at a distance of about 1 cm. distally to the gland, a stromuhr[1] was then inserted in this blood-vessel centrally to the ligature and in the immediate vicinity of the gland. Thus, by quickly changing the clip from the distal end of the suprarenal vein to its central end at the site of the loose ligature, the blood could be diverted at any moment into the stromuhr without interrupting the circulation. From the stromuhr the blood was returned to the inferior cava by way of the left renal vein. To accomplish this end it is not always necessary to ligate the renal blood-vessels because in many cases the renal vein arises from two large radicles, only one of which need be closed for the insertion of the distal cannula of the stromuhr. At the completion of the experiment the clip was again placed upon the suprarenal vein distally to the gland and the ligature unloosened on its central side. The blood then followed its usual course into the inferior cava. In all these experiments the general arterial pressure was recorded by a mercurial manometer which was connected with the left femoral artery and the venous pressure by a membrane manometer which communicated with the central cannula of the stromuhr.

The results of these experiments are compiled in tables 1 and 2. It should be mentioned, however, that only those periods of the stromuhr have been included in this calculation during which no special experimental procedure has been resorted to. It will be seen that the weight of these dogs varied between 15 and 21 kilos and that the weight of the left adrenal body varied between 0.85 and 2.60 grams. The blood-flow fluctuated between 0.069 and 0.217 cc. in a second, the pressure between 90.5 and 122.5 mm. Hg. Thus, a gland weighing 1.72 grams receives on the average 0.142 cc. of blood in a second or

[1] We have made use of the recording stromuhr described by Burton-Opitz, Pflüger's Arch., 1908, cxxi, 150.

TABLE 1

The blood supply of the suprarenal gland

NUMBER OF EXPERIMENT	WEIGHT OF		DURATION OF PHASE	TOTAL QUANTITY OF BLOOD	QUANTITY PER SECOND	BLOOD PRESSURE IN MM. HG	
	Dog	Gland				Arterial	Venous
	kg.	*grams*	*min.*	*cc.*	*cc.*		
1	20	1.38	4.0	15.2	0.063	112.0	8.5
			3.7	16.0	0.071		
			3.5	15.4	0.073		
			3.5	15.0	0.070		
Average					0.069	112.0	8.0
2	21	1.59	2.2	18.0	0.133	114.0	10.0
			2.2	17.0	0.129		
			2.5	17.6	0.116		
			2.5	18.0	0.120		
Average					0.124	114.0	10.0
3	18	2.60	1.6	18.2	0.190	110.0	10.0
			1.8	18.0	0.167		
			1.8	18.0	0.167		
			1.4	18.5	0.220		
			1.5	18.2	0.201		
			1.8	18.5	0.166		
Average					0.185	110.0	10.0
4	19	2.21	1.5	18.2	0.201	122.5	11.0
			1.2	17.0	0.236		
			1.2	18.0	0.250		
			1.2	18.0	0.250		
			1.5	18.0	0.200		
			1.6	18.5	0.193		
			1.6	18.5	0.193		
Average					0.217	122.5	11.0
5	15	0.85	2.7	18.5	0.114	90.5	8.5
			2.5	18.0	0.120		
			2.6	18.0	0.115		
Average					0.116	90.5	8.5

TABLE 2

Average values of flow and pressure

NUMBER OF EXPERIMENT	WEIGHT OF		BLOOD FLOW PER SECOND	BLOOD PRESSURE IN MM. HG	
	Dog	Gland		Arterial	Venous
	kg.	*grams*	*cc.*		
1	20	1.38	0.069	112.0	8.0
2	21	1.59	0.124	114.0	10.0
3	18	2.60	0.185	110.0	10.0
4	19	2.21	0.217	122.5	11.0
5	15	0.85	0.116	90.5	8.5
Average.....	18.6	1.72	0.142	109.8	9.5

8.5 cc. in a minute. At a pressure of about 100 mm. Hg, 100 grams of adrenal tissue are supplied with about 490 cc. of blood in a minute.

If this value is now compared with the succeeding values of the blood-flow through other organs (4) it will be evident that the adrenal gland is a very vascular organ; its blood-supply being exceeded only by that of the thyroid body.

	cc. in a minute
Post. extremity	5.0
Skeletal muscle	12.0
Head	20.0
Stomach	21.0
Liver (art.)	25.0
Portal organs, comb	30.0
Intestine	31.0
Spleen	58.0
Liver (ven.)	59.0
Liver (total)	84.0
Brain	136.0
Kidney	150.0
Suprarenal	490.0
Thyroid	560.0

In this connection it is interesting to note that Neuman (5) has found an oxygen-consumption of 0.045 cc. per gram of substance and per minute. These tests were made upon the suprarenal of the cat, the Barcroft-Roberts differential blood-gas apparatus being used. This value indicates a flow of about 6 cc. per gram per minute.[2]

[2] The current number of the Proc. of the Soc. for Exper. Biol. and Med. which has been issued since the preparation of this manuscript, contains a preliminary notice by Stewart and Rogoff in which it is stated that the caval segment fills at the rate of about 8 cc. per minute, in a dog weighing 10 kilos.

By employing the method previously described, Biedl has found that the number of drops escaping from the reservoir may be considerably increased by stimulation of the splanchnic nerve. This acceleration, however, does not set in immediately, but about ten seconds after the beginning of the stimulation and lasts for some time after its close. Moreover, while it pursues a course which is practically parallel to the rise in blood-pressure, the fact remains that it outlasts the stimulus as well as the increase in pressure and this seems to show that it is dependent upon an active enlargement of the suprarenal blood-vessels.

These alterations in the blood-supply of this gland we have succeeded in registering a number of times, but as these curves present only quantitative and not qualitative differences, it may suffice to illustrate them by a single example taken from experiment 3. The second and third phases of this experiment are reproduced in the accompanying figure. The blood-flow (*St*) showed in this case the normal value of 0.155 cc. in a second. The arterial pressure (*A*) amounted to 100.2 mm. Hg and the venous pressure (*V*) to 9.8 mm. Hg. Shortly after the beginning of the excitation of the greater splanchnic nerve at *S*, the blood-flow increased gradually until it assumed its maximal value of 0.244 cc. in a second at about point *T*. The latter coincides with the cessation of the stimulation and also with the maximal rise in the arterial pressure. Subsequent to *T* the arterial pressure decreases gradually and assumes its normal level early in the course of the third phase of the stromuhr, about fifty seconds after the cessation of the stimulation. The venous pressure exhibits a slight rise during the period of the increased flow.

The blood-flow pursues a very similar course. It is true, however, that the flow does not always return to normal with the pressure, but remains augmented for some time after the stimulation. In the present case, for example, the normal minute-volume for 100 grams of suprarenal substance amounts to 260 cc., while the minute-volume during the stimulation measures 560 cc. It appears, therefore, that the excitation of the splanchnic nerve has resulted in an increase of flow of 200 cc. in a minute for 100 grams of substance. This fact in itself, however, does not seem to us to justify the deduction that the suprarenal blood-vessels are equipped with a dilator mechanism, because the dissociation between the flow and the pressure here noted, could also be caused by the rather slow return to normal of passively distended blood-vessels. It also seems singular that the excitation of

Fig. 1. The blood flow through the suprarenal gland during stimulation of the greater splanchnic nerve (15 cm., twenty seconds).

the splanchnic nerve with currents of medium strength and high frequency should dilate the blood-vessels of the suprarenal gland and constrict those of the other organs innervated by this nerve.

The possibility that the splanchnic rise in blood-pressure is the essential factor in the increased blood-supply of the suprarenal gland we have attempted to test by the simultaneous stimulation of the distal and central ends of the splanchnic nerve, the former with a current of ordinary strength and frequency and the latter with a current of low frequency. This procedure necessitated the division of the left thoracic sympathetic nerve a short distance above the diaphragm and the application of two shielded electrodes. The inductoriums were then adjusted in such a way that the fall in blood-pressure obtained in consequence of the excitation of the central end, was about balanced by the rise resulting from the stimulation of the distal end.

In this way, we succeeded in retaining the blood-pressure practically at its normal level, because the rise in pressure resulting from the constriction of the blood-vessels in the different splanchnic organs, was counterbalanced by the vasodilatation produced elsewhere. We have, however, not been able to satisfy ourselves that under this condition the blood-flow through the suprarenal gland is markedly increased. But naturally, this result cannot justly be regarded as proving the absence of vasodilator fibers, because the stimulation of the central end of the splanchnic nerve may have destroyed this effect reflexly through possible nervous connections between the suprarenal gland and the solar ganglia. If the central end of the thoracic nerve alone was stimulated, the resulting fall in blood-pressure was invariably associated with a diminution in the blood supply of this organ.

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THE INFLUENCE OF SECRETIN ON THE NUMBER OF ERYTHROCYTES IN THE CIRCULATING BLOOD

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In 1895 Dolinski (1) showed that acids brought into contact with the mucous membrane of the duodenum set up a secretion of pancreatic juice. Pawlow and his co-workers (2) further decided that the acid acts reflexly through a nerve center. Later Popielski (3), working under the direction of Pawlow, showed that if acids are introduced into the duodenum the pancreatic secretion appears after resection of both vagus and splanchnic nerves, after extirpation of the solar plexus and even after destruction of the spinal cord. He concluded that the secretion arose from a peripheral reflex through scattered ganglia of the pancreas, situated mostly near the duodenum. The same results and conclusions were reached by Wertheimer and Lepage (4). At this point Bayliss and Starling (5) demonstrated the true explanation of the phenomenon. They showed that the acid acts on a substance in the duodenal mucous membrane, prosecretin, and changes it into another substance, secretin. This is carried by the blood and activates the pancreatic cells.

Bayliss and Starling (5) also showed that secretin increases the secretion of bile. We have confirmed this by noting the rate of flow of bile incidentally in the course of other experiments. Sir Edward A. Schäfer (6) states that secretin increases the flow of bile and of succus entericus but to a less extent than it affects the flow of pancreatic juice. He also states that intravenous injection of duodenal extract (evidently secretin from the context) has been shown by Cow (7) to cause the appearance of the pituitary autacoids in the cerebro-spinal fluid.

Beveridge and Williams (8) in their very ingenious exposition of what they call the proteomorphic theory of immunity claim to have the records of over two hundred cases of diabetes and exophthalmic goiter in which the number of red corpuscles per cubic millimeter of

blood was increased by the administration of secretin. Their theory of the production of immunity depends greatly on the power to hydrolyze proteins which they attribute to the red blood corpuscles. If we grant that these premises are correct, then any agent capable of bringing about a sufficient increase in the number of the red corpuscles becomes of therapeutic value. We have been unable to obtain details of the records to which reference has been made. If secretin is to exert any influence as an immunizing agent by increasing the number of red corpuscles in the circulating blood, it is obvious that a single dose must be capable of causing a great and fairly prolonged rise in the red corpuscle count. As a means of ordinary treatment, hypodermic medication is preferable to intravenous, and if it can be shown that secretin administered hypodermically is able to increase the number of red corpuscles, then again, in order to be of service, a single dose, or at most three or four successive doses, should produce and maintain a largely increased erythrocyte count.

Acting in accordance with the ideas thus suggested we determined to try first, the effect of a certain arbitrarily fixed dose of secretin given intravenously and second, the effect of the same dose when introduced hypodermatically.

In our selection of the animal to be used we were guided by the recent work of Lamson (9) on acute polycythemia in which he has shown that adrenalin, fright, pain, etc., cause sudden and very marked elevation of the red corpuscle count in the dog and cat but that these agents are without effect on the erythrocyte count of the rabbit. Therefore, that we might avoid the use of an anaesthetic, especially in those experiments which were to be continued over several days, in order that the attending conditions might be as uniform as possible, rabbits were employed in all of the experiments recorded in this paper.

The secretin was in all cases prepared from the intestine of the dog. The animal was anaesthetized by ether alone and the upper half of the small intestine removed. This intestine was carefully washed in running water and the mucous membrane scraped off with a dull knife. The scrapings were rubbed up in a mortar with sand, covered with 50 cc. of 0.4 per cent hydrochloric acid and allowed to stand for an hour or more. The mixture was then boiled actively for several minutes, neutralized with strong potassium hydroxide while boiling and again rendered faintly acid with glacial acetic acid. Finally the preparation was strained through muslin and filtered.

We found that this preparation when kept in the dark retained its

potency for about five days; but if glacial acetic acid were added to the filtrate in sufficient quantity to make this 2 per cent acid by volume and the solution evaporated to dryness, the residue was found to retain its potency for months at least. When required, a weighed quantity could be dissolved in distilled water and neutralized, thus giving a preparation of the same effectiveness as the original solution.

Over two hundred determinations of the red corpuscles per cubic millimeter of blood were made in the course of these experiments, the blood being obtained from the ear of the rabbit and the count made in the usual manner with the Thoma-Zeiss apparatus.

The dose of secretin solution selected for the first experiments was 1 cc. per kilogram of body weight. Five rabbits were taken, the erythrocytes per cubic millimeter of blood counted, and the proper dose of secretin injected into the femoral vein. The results of these experiments are recorded in table 1.

TABLE 1

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
1	5,320,000	7,510,000	2,190,000	41.16	30	60
2	4,560,000	6,990,000	2,430,000	53.29	15	70
3	4,450,000	6,350,000	1,900,000	42.69	40	65
4	5,610,000	8,210,000	2,600,000	46.35	45	90
5	4,830,000	5,630,000	800,000	16.56	25	45
Averages....	4,954,000	6,938,000	1,984,000	40.04	31	66

As a type of this series of experiments the first one is given in detail:

Experiment 1, November 6, 1916

10.05 a.m. Red blood corpuscles, 5,320,000 per cubic millimeter
10.10 a.m. 1 cc. secretin per kilogram of body weight given intravenously
10.25 a.m. Red blood corpuscles, 6,940,000 per cubic millimeter
10.40 a.m. Red blood corpuscles, 7,510,000 per cubic millimeter
10.55 a.m. Red blood corpuscles, 7,120,000 per cubic millimeter
11.10 a.m. Red blood corpuscles, 5,350,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 5,290,000 per cubic millimeter

The next thing to be determined was the effect of the same dose upon the number of red corpuscles when it was introduced beneath

the skin. A tabulated report of the results obtained in this way will be found in table 2.

TABLE 2

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
6	5,120,000	6,305,000	1,185,000	23.1	60	90
7	4,548,000	5,844,000	1,296,000	28.4	30	30
8	4,536,000	5,545,000	1,009,000	22.2	30	90
9	4,906,000	5,619,000	713,000	14.5	55	95
10	5,840,000	7,197,000	1,357,000	23.2	60	90
Averages....	4,990,000	6,102,000	1,112,000	22.2	47	79

As typical of this series of experiments the first one is here presented in detail:

Experiment 6, November 20, 1916

1.30 p.m. Red blood corpuscles, 5,120,000 per cubic millimeter
1.35 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.05 p.m. Red blood corpuscles, 5,336,000 per cubic millimeter
2.35 p.m. Red blood corpuscles, 6,305,000 per cubic millimeter
3.35 p.m. Red blood corpuscles, 5,601,000 per cubic millimeter
4.35 p.m. Red blood corpuscles, 5,006,000 per cubic millimeter

A comparison of the results obtained in these two groups of experiments shows certain features in favor of the intravenous method of administration. The most striking difference is in the percentage increase—an average of 40 per cent when the secretin is given intravenously and 22.2 per cent when given hypodermatically. However, if we note the average actual increase in number of red corpuscles the difference is only slightly over one-half million in favor of the intravenous method—1,984,000 in the former case and 1,112,000 in the latter. As might be expected the intravenous method gives the effect in a shorter time than the subcutaneous,—the maximum effect obtained in thirty-one minutes in one instance and in forty-seven minutes in the other; but when we compare the duration of the effect we find it almost identical in the average of the two groups—sixty-six minutes was the average time that the increase lasted when the secretin was given intravenously and seventy-nine minutes when it was given hypodermatically.

The second group of experiments had convinced us that secretin injected subcutaneously was capable of exerting an influence, at least so far as affecting the number of red corpuscles in circulation was concerned. Moreover, the greater effect obtained by giving the secretin intravenously was not sufficiently pronounced to make it the method of choice so far as any therapeutic application was concerned. Therefore, we decided to adhere to the method of hypodermic administration in the remainder of our experiments, particularly as these two series of observations appeared to furnish sufficient data from which to deduce the probable action of any particular dose of secretin when given intravenously if we had determined its effect when given hypodermatically.

To determine the most effective dose we made several series of experiments using the following doses per kilogram of body weight: 0.75 cc., 0.5 cc., 0.25 cc., 1.5 cc., 2 cc. In this way we tested the effect of amounts of secretin less than and greater than the original and arbitrary dose of 1 cc. per kilogram of body weight. Each group table gives the summarized results of each experiment in the group and the averages for that particular series. Following each group summary is a report giving the details of one experiment in the group, serving as an example of all.

TABLE 3

Dose: 0.75 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
13	5,327,000	6,224,000	897,000	16.8	60	90
14	6,334,000	7,168,000	834,000	13.1	30	30
20	6,384,000	7,098,000	714,000	11.0	60	60
21	5,324,000	6,961,000	1,637,000	30.7	90	90
Averages....	5,842,250	6,862,750	1,020,500	17.9	60	67.5

Experiment 13, January 12, 1917

9.30 a.m. Red blood corpuscles, 5,327,000 per cubic millimeter
10.20 a.m. 0.75 cc. secretin per kilogram of body weight given hypodermatically
10.50 a.m. Red blood corpuscles, 5,206,000 per cubic millimeter
11.20 a.m. Red blood corpuscles, 6,224,000 per cubic millimeter
12.20 p.m. Red blood corpuscles, 6,042,000 per cubic millimeter
1.20 p.m. Red blood corpuscles, 5,100,000 per cubic millimeter

TABLE 4

Dose: 0.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
16	6,251,000	6,837,000	586,000	9.3	30	30
19	5,741,000	7,875,000	2,134,000	37.1	90	90
24	6,048,000	No effect				
30	5,804,000	No effect				
31	6,760,000	7,046,000	286,000	4.2	60	60
Averages....	6,120,800	6,722,000	601,200	10.1	36	36

Experiment 16, January 22, 1917

12.55 p.m. Red blood corpuscles, 6,251,000 per cubic millimeter
1.00 p.m. 0.5 cc. secretin per kilogram of body weight given hypodermatically
1.30 p.m. Red blood corpuscles, 6,837,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 6,079,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 6,426,000 per cubic millimeter

TABLE 5

Dose: 0.25 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
11	5,888,000	6,810,000	922,000	15.6	30	30
17	5,754,000	No effect				
18	5,280,000	6,144,000	864,000	16.3	30	30
25	4,075,000	5,015,000	940,000	23.1	30	30
27	5,970,000	No effect				
Averages....	5,395,000	5,940,200	545,200	11.0	18	18

Experiment 11, November 28, 1916

1.10 p.m. Red blood corpuscles, 5,888,000 per cubic millimeter
1.15 p.m. 0.25 cc. secretin per kilogram of body weight given hypodermatically
1.45 p.m. Red blood corpuscles, 6,810,000 per cubic millimeter
2.15 p.m. Red blood corpuscles, 5,988,000 per cubic millimeter

TABLE 6

Dose: 1.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
12	6,376,000	7,440,000	1,064,000	15.1	60	90
22	7,188,000	No effect				
28	4,959,000	7,162,000	2,203,000	44.8	120	120
32	5,936,000	7,416,000	1,480,000	24.9	60	90
Averages....	6,114,750	7,301,500	1,186,750	21.2	58	60

Experiment 12, December 20, 1916

10.00 a.m. Red blood corpuscles, 6,376,000 per cubic millimeter
10.30 a.m. 1.5 cc. secretin per kilogram of body weight given hypodermatically
11.00 a.m. Red blood corpuscles, 6,937,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 7,440,000 per cubic millimeter
12.30 p.m. Red blood corpuscles, 6,014,000 per cubic millimeter

TABLE 7

Dose: 2 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
15	4,112,000	5,188,000	1,076,000	26.1	30	60
23	5,928,000	6,935,000	1,007,000	16.9	30	90
26	5,858,000	6,471,000	613,000	10.4	30	90
29	5,400,000	7,502,000	2,102,000	38.9	60	120
Averages....	5,324,500	6,524,000	1,199,500	22.4	37.5	90

Experiment 15, January 12, 1917

9.20 a.m. Red blood corpuscles, 4,112,000 per cubic millimeter
9.30 a.m. 2 cc. secretin per kilogram of body weight given hypodermatically
10.00 a.m. Red blood corpuscles, 5,188,000 per cubic millimeter
10.30 a.m. Red blood corpuscles, 4,507,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 4,207,000 per cubic millimeter

As we proceeded with our observations the results pointed to a dose of 1 cc. per kilogram of body weight as being the most efficient. In order to assure ourselves on this point we made seven more experiments in which the dose was 1 cc. per kilogram of body weight. Taken to-

gether with the five original experiments recorded in table 2 we have a total of twelve such determinations. For the purpose of computing an average with as large a number of experiments as possible these have been brought together in table 8.

TABLE 8

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
6	5,120,000	6,305,000	1,185,000	23.1	60	90
7	4,548,000	5,844,000	1,296,000	28.4	30	30
8	4,536,000	5,545,000	1,009,000	22.2	30	90
9	4,906,000	5,619,000	713,000	14.5	55	95
10	5,840,000	7,197,000	1,357,000	23.2	60	90
33	5,778,000	6,894,000	1,116,000	19.3	30	90
34	5,872,000	6,579,000	707,000	12.0	50	70
35	6,200,000	6,850,000	650,000	10.4	50	90
36	5,456,000	5,955,000	499,000	9.1	70	80
37	5,200,000	7,240,000	2,040,000	39.2	25	105
38	4,720,000	7,264,000	2,544,000	53.8	75	30
39	5,684,000	6,752,000	1,068,000	18.7	35	30
Averages....	5,321,666	6,503,666	1,182,000	22.2	47.5	73.3

Reviewing the average percentage increase with each dose we find the results to have been as follows: 0.25 cc. secretin per kilogram of body weight, 11.0 per cent; 0.5 cc. secretin per kilogram of body weight, 10.1 per cent; 0.75 cc. secretin per kilogram of body weight, 17.9 per cent; 1 cc. secretin per kilogram of body weight, 22.2 per cent; 1.5 cc. secretin per kilogram of body weight, 21.2 per cent; 2 cc. secretin per kilogram of body weight, 22.4 per cent. These records indicate a dose of 1 cc. per kilogram of body weight as the most efficient dose of secretin. We also find that the longest average time the effect lasted was ninety minutes—where the dose was 2 cc. secretin per kilogram of body weight. With a dose of 1 cc. per kilogram of body weight the average duration was 73.3 minutes. It seemed worth while to test the effect of repeated doses of secretin on the increase in the number of erythrocytes per cubic millimeter of blood, both as to the amount of increase and the duration of the increase. Is it possible to produce a summation effect? To answer this question the following experiments were performed: One in which a dose of 1 cc. secretin per kilogram of

body weight was followed in two hours by a second dose of 1 cc. per kilogram of body weight; two experiments in each of which four successive doses of 1 cc. secretin per kilogram of body weight were administered at intervals of one hour; one experiment in which five successive doses of 1 cc. secretin per kilogram of body weight were given at intervals of one hour; one experiment in which five successive doses of 1 cc. secretin per kilogram of body weight were given at intervals of twenty-four hours. The results of these observations are appended.

Experiment 40, February 14, 1917

9.45 a.m. Red blood corpuscles, 4,548,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.30 a.m. Red blood corpuscles, 5,844,000 per cubic millimeter
11.00 a.m. Red blood corpuscles, 5,338,000 per cubic millimeter
12.00 noon Red blood corpuscles, 5,025,000 per cubic millimeter
12.30 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
1.00 p.m. Red blood corpuscles, 5,042,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 5,600,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 5,787,000 per cubic millimeter

February 15, 1917

10.00 a.m. Red blood corpuscles, 4,286,000 per cubic millimeter

February 16, 1917

10.00 a.m. Red blood corpuscles, 4,457,000 per cubic millimeter

Experiment 41, February 19, 1917

9.55 a.m. Red blood corpuscles, 5,765,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.55 a.m. Red blood corpuscles, 7,094,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.55 a.m. Red blood corpuscles, 6,756,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
12.55 p.m. Red blood corpuscles, 7,459,000 per cubic millimeter
1.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.00 p.m. Red blood corpuscles, 8,327,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 6,749,000 per cubic millimeter
4.00 p.m. Red blood corpuscles, 6,195,000 per cubic millimeter

February 20, 1917

9.00 a.m. Red blood corpuscles, 6,156,000 per cubic millimeter

February 21, 1917

9.00 a.m. Red blood corpuscles, 5,832,000 per cubic millimeter

Experiment 42, February 20, 1917

8.55 a.m. Red blood corpuscles, 5,245,000 per cubic millimeter
9.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
9.55 a.m. Red blood corpuscles, 5,645,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.55 a.m. Red blood corpuscles, 6,723,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.55 a.m. Red blood corpuscles, 6,659,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
1.00 p.m. Red blood corpuscles, 6,384,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 5,553,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 5,284,000 per cubic millimeter

Experiment 43, February 21, 1917

10.00 a.m. Red blood corpuscles, 5,825,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.30 a.m. Red blood corpuscles, 6,522,000 per cubic millimeter
10.55 a.m. Red blood corpuscles, 6,764,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.30 a.m. Red blood corpuscles, 6,004,000 per cubic millimeter
11.55 a.m. Red blood corpuscles, 7,402,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
12.30 p.m. Red blood corpuscles, 8,883,000 per cubic millimeter
12.55 p.m. Red blood corpuscles, 6,111,000 per cubic millimeter
1.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
1.30 p.m. Red blood corpuscles, 5,472,000 per cubic millimeter
1.55 p.m. Red blood corpuscles, 7,016,000 per cubic millimeter
2.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.30 p.m. Red blood corpuscles, 7,094,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 8,089,000 per cubic millimeter
4.00 p.m. Red blood corpuscles, 6,912,000 per cubic millimeter

February 22, 1917

10.00 a.m. Red blood corpuscles, 5,446,000 per cubic millimeter

February 23, 1917

10.00 a.m. Red blood corpuscles, 5,296,000 per cubic millimeter

Experiment 44, February 22, 1917

10.00 a.m. Red blood corpuscles, 5,829,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 23, 1917

10.00 a.m. Red blood corpuscles, 5,245,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 24, 1917

10.00 a.m. Red blood corpuscles, 7,710,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 25, 1917

10.00 a.m. Red blood corpuscles, 6,961,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 26, 1917

10.00 a.m. Red blood corpuscles, 5,523,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

March 1, 1917

10.00 a.m. Red blood corpuscles, 5,975,000 per cubic millimeter

March 4, 1917

10.00 a.m. Red blood corpuscles, 6,218,000 per cubic millimeter

These experiments, numbers 40 to 44 inclusive, show that successive doses of secretin at short intervals are capable of causing a progressive increase in the number of red corpuscles per cubic millimeter of blood, but the increase is not maintained from one dose to the next, so that between the doses there is a diminution from the maximum count resulting from that dose before the next dose exerts an effect. In other words, two doses do not give twice the effect of one dose or three doses three times the effect of one dose. Moreover, when the administration of the secretin is stopped the number of red corpuscles in the circulating blood reverts to normal almost as quickly as after a single dose. In the case of the rabbit which received a daily dose of secretin for five days the increase in the number of erythrocytes per unit volume of blood on the eighth day as compared with the initial count was 146,000, showing that secretin has no ability to produce a permanent increase in the red corpuscle content of the circulating blood of the normal animal.

Three main conclusions are inevitable from the observations that have been recorded: first, secretin, even when injected subcutaneously, is capable of producing an increase in the number of red corpuscles in the circulating blood; second, the increase thus effected is not great as compared with the increase that may be obtained by the action of other agents; third, the length of time that this larger number of red corpuscles persists is comparatively short.

Let us consider briefly the possible therapeutic benefits that may be derived from the exhibition of secretin. If we grant the correctness of the theory of Beveridge and Williams (10) that the red corpuscles constitute one of the chief defensive agencies of the animal organism against the invasion of pathogenic bacteria or the products of such bacteria, then, in order that aid may be given to the establishment of immunity by this means, we must be able in some way to bring about a marked augmentation in the number of red corpuscles and an augmentation that will continue for a time sufficiently long to be of service. We have shown that the most efficient dose of secretin is in the proportion of 1 cc. per kilogram of body weight, which means for the average man 70 cc. of secretin subcutaneously or 38.5 cc. intravenously. Furthermore the effect of this dose disappeared on an average 73.3 minutes after it was given. Moreover, it was not possible to produce a lasting increase in the number of red corpuscles by giving successive doses, either at intervals of one hour, two hours or twenty-four hours. The facts that have been adduced militate against any therapeutic value for secretin but do not detract in the slightest from its physiological significance. On the contrary, they appear to give secretin added importance in the normal organism. It is possible that one of the means by which the normal number of red corpuscles is maintained in the blood stream is the action of secretin.

Naturally the next question that presents itself is: How does secretin produce this increase in the number of the red corpuscles in the circulating blood? This is a question which we are not as yet prepared to answer, but several suggestions can be offered. The first and simplest explanation that presents itself is that secretin exerts a direct stimulating influence upon the red marrow of the bones thus leading to the formation of new cells. Such a conclusion is entirely in accord with the known activities of secretin. If this substance is capable of promoting the activity of the pancreatic cells, of the hepatic cells, and of the cells of the pituitary body, it is entirely reasonable to assume that it may also have the ability to increase the formation of red blood corpuscles by the red marrow of the bones.

A second way in which secretin might bring about an increase in the number of circulating erythrocytes is by causing variations in their unequal distribution. This might be effected by a direct constricting action on the capillaries of some large area, such as the liver, or by an indirect action through stimulation of the adrenals. Lamson (9) has shown that in the cat and dog an increase in the number of red cor-

puscles per unit volume of blood may be obtained by the administration of adrenalin. In the same animals fright raises the number of red corpuscles per unit volume of blood an average of 80 per cent. In both cases there is no increase in the number of erythrocytes if the hepatic artery be ligated. It has been shown by Cannon (11) that fright stimulates the adrenals, and Lamson attributes the presence of a greater number of red corpuscles in the blood stream to a constriction of the capillaries of the liver caused by adrenalin. Mautner and Pick (12) inform us of the presence of an extremely sensitive nervous mechanism in the liver of the dog, reacting to epinephrin by constriction of the capillaries, and the absence of such in the liver of the rabbit, or the presence in this animal of a much less sensitive mechanism. Lamson (13) has shown also that excitement or the intravenous injection of adrenalin causes no polycythemia in rabbits. Therefore, it seems that we can rule out the suggestion that secretin increases the number of red corpuscles in the circulating blood of the rabbit by stimulating the adrenals. As to whether the secretin acts directly to promote capillary constriction or not we have no evidence and do not know of any work that has been reported on the subject.

One other explanation that should be mentioned is the possibility that secretin causes a decrease in plasma volume and thus gives rise to a higher erythrocyte count per cubic millimeter of blood.

The means by which secretin acts to produce an increase in the number of red corpuscles in a unit volume of blood is a question outside of the scope of the present investigation. In undertaking these experiments we were actuated by the desire to know whether secretin increased the number of erythrocytes in the blood stream or not; and, if so, how much of an increase could be hoped for, and how long it would be possible to maintain this increase. These questions have been answered, we believe, and the solution of the mode of action will be found later.

CONCLUSIONS

1. It is possible to produce a considerable increase in the red corpuscle count per cubic millimeter of blood by the administration of secretin even in small doses and by subcutaneous injection.

2. The most efficient dose is 1 cc. of secretin per kilogram of body weight.

3. The increase in the count appears quickly and is very transient.

4. By repeating the dose of secretin at short intervals the increase in the erythrocyte count can be kept up for several hours but drops promptly after the administration of the last dose.

5. The administration of secretin over a period of five days, in daily doses of 1 cc. per kilogram of body weight, has very slight, if any, lasting effect on the red corpuscle count in the normal animal.

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THE ACTION OF ULTRA-VIOLET RADIATION IN KILLING LIVING CELLS SUCH AS BACTERIA

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Several theories have been advanced in an attempt to explain the mode of action of ultra-violet radiation in killing living cells. One theory is that the short wave lengths of the spectrum act by destroying the intracellular enzymes. So far as I have been able to find, the only basis for this theory is the fact that intracellular enzymes in common with other enzymes are destroyed by exposure to ultra-violet radiation. The object of this investigation is to show that the destruction of living cells, such as bacteria, by ultra-violet radiation is not due to the destruction of the intracellular enzymes but to the coagulation of the protoplasm of the cells by the radiation.

The bacteria used were B. liquefaciens, B. prodigiosus, B. fluorescens, B. proteus vulgaris, B. pyocyaneus and B. subtilis. These bacteria were chosen because they possess the property of liquefying gelatine, this property in turn being dependent upon the intracellular proteolytic enzymes. Twenty-five cubic centimeters of liquid containing great numbers of B. liquefaciens were exposed in an open vessel to the radiation from a quartz-mercury vapor burner, operating at 140 volts, 3.3 amperes, at a distance of 10 cm., until they were dead as was indicated by negative results on plating. By means of a centrifugalizing machine the dead bacteria were thrown down and subsequently ground up in a mortar with sand and 30 per cent alcohol. In this way the intracellular enzymes were extracted from the dead bacteria. All of the bacteria named above were treated after this manner. Ten cubic centimeters of the alcoholic extract of the different kinds of dead bacteria were introduced into separate test-tubes containing gelatine. Ten cubic centimeters of liquid containing the different kinds of living bacteria were also introduced into tubes containing gelatine. These tubes were permitted to stand at room temperature

for ninety-six hours. At the end of this time the extent to which the gelatine had been liquefied in the different tubes was measured. The measurements are given in table 1.

TABLE 1

Under the different kinds of bacteria are shown the extent of liquefaction of gelatine by the living bacteria and by extracts of the dead bacteria

BACTERIA	LIQUEFACIENS	PRODIGIOSUS	FLUORESCENS	PROTEUS VULGARIS	PYOCYANEUS	SUBTILIS
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Extent of liquefaction by living bacteria.....	12	8	6	6	5	4
Extent of liquefaction by extract of dead bacteria...	10	7	6	5	4	4

It may be seen that the gelatine in the tube containing living B. liquefaciens was liquefied 12 mm.; that containing living B. prodigiosus, 8 mm.; B. fluorescens, 6 mm.; B. proteus vulgaris, 6 mm.; B. pyocyaneus, 5 mm.; and B. subtilis, 4 mm. It may also be seen that the extract of dead B. liquefaciens had liquefied 10 mm. of gelatine; B. prodigiosus, 7 mm.; B. fluorescens, 6 mm.; B. proteus vulgaris, 5 mm.; B. pyocyaneus, 4 mm.; and B. subtilis, 4 mm. If the amount of gelatine liquefied by the living bacteria be compared with that liquefied by the extract of the corresponding dead bacteria, it will be found that there is very little difference in the extent of liquefaction. This is taken to mean that, while the ultra-violet rays had killed the bacteria, it had affected very little their intracellular enzymes. These experiments would seem to render untenable the theory that ultra-violet rays kill living cells by destroying their intracellular enzymes.

The following experiments were carried out to show that ultra-violet radiation kills living cells by coagulating their protoplasm. Several drops of water containing great numbers of paramecia were introduced into a shallow glass vessel and covered with a quartz plate. The glass vessel was then placed on a block of ice under a quartz mercury vapor burner operating at 140 volts, 3.3 amperes, at a distance of 5 cm., and in this position the organisms were exposed for twenty minutes. A drop of the liquid containing the dead paramecia was mixed with a drop containing living ones on a glass plate and covered with a cover glass. Having located under a microscope a dead organism and a living one lying close together a micro-photograph was

made of them. Similarly micro-photographs were made of paramecia killed by heating to 45°C. and 90°C. respectively. These photographs are shown in figure 1. The upper organisms under *A*, *B* and *C* are living transparent animals; the lower one under *A* was killed by heating to 90°C., the lower one under *B* by heating to 45°C. and the lower one under *C* by exposure to ultra-violet radiation. By comparing the lower organisms under *B* and *C* it may be seen that there is no difference in the appearance of these two organisms, both being slightly more opaque than the living organisms. The lower organism under *B* was killed by the coagulation of its protoplasm by heat and since there is no difference in the appearance between this one and the lower

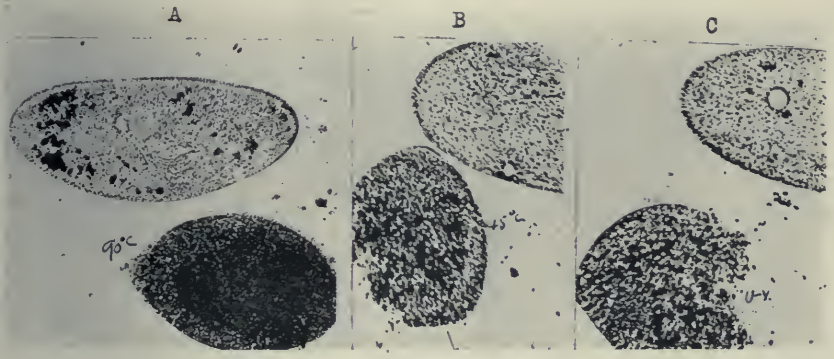

Fig. 1. Microphotographs of paramecia. The upper ones under *A*, *B* and *C* are the normal transparent living animals; the lower one under *A* was killed by heating to 90°C.; the lower one under *B* by heating to 45°C; the lower one under *C* by exposure to ultra-violet radiation.

organism under *C*, which was killed by exposure to ultra-violet radiation, it would seem to be fair to assume that the latter was killed by the coagulation of its protoplasm by the radiation. By comparing the lower organism under *A* with that under *B* it may be seen that the lower one under *A* is very much more opaque than the lower one under *B*. This greater opacity is explained by the fact that proteins are more firmly coagulated at a temperature of 90°C. than at a temperture of 45°C. It will be noticed also that the lower organisms under *B* and *C* which were killed by heating to 45°C. and by exposure to ultra-violet radiation respectively had begun to disintegrate while the lower one under *A* had not begun to do so because of the firmer coagulation of the protein of this organism heated to the higher temperature.

Henri (1), Hertel (2) and others observed that when protozoa were exposed to ultra-violet radiation the body became swollen, water drops appeared on the surface and the organisms finally disintegrated, but they did not observe any coagulation produced by the radiation. The failure of these observers to obtain conspicuous coagulation in the organisms was due to the fact that the radiation to which they exposed the organisms was not of sufficient intensity to coagulate firmly the protoplasm. If paramecia are heated to 40°C. they are killed after a time, but very little indication of coagulation is produced as is indicated by the fact that there is very little decrease in the transparency of the organisms thus killed. By increasing the temperature, however, at which the organisms are killed, the protoplasm becomes firmer and the animals more opaque. Similarly by killing the organisms by exposure to ultra-violet radiation of low intensity, a very inconspicuous amount of coagulation is produced, and hence there is very little change in the transparency of the organisms. If the intensity of the radiation is increased, however, the coagulation of the protoplasm, as well as the opacity of the animals, becomes more marked.

CONCLUSION

Exposure of living cells to ultra-violet radiation of sufficient intensity to kill the cells does not decrease to any appreciable extent the activity of the intracellular enzymes.

Evidence is presented in this paper to show that ultra-violet radiation kills living cells by coagulating their protoplasm.

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THE EFFECT OF THYROID FEEDING ON THE CATALASE CONTENT OF THE TISSUES

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It has been observed that when animals are fed thyroid or an extract of the gland they lose weight and strength. The increased oxygen intake and carbon dioxide output of such animals show that oxidation is augmented while the increased nitrogen elimination indicates an increased tissue destruction. Magnus-Levy (1) observed an increased carbon dioxide output in a man fed upon thyroid extract and an increased oxygen intake in cases of exophthalmic goiter. Fritz Voit (2) found that thyroid feeding increased protein metabolism in dogs. Anderson (3) observed a decrease in metabolism in cases of myxedema as was indicated by a decreased oxygen intake and carbon dioxide output, and that the metabolism was increased to normal by thyroid feeding. As a result of these and similar observations it is generally accepted that the effect of thyroid feeding or of hypersecretion of the glands as in exophthalmic goiter is to increase oxidation and tissue destruction, while in myxedema there is a decrease in metabolism. It has been observed that when oxidation is increased or decreased in a tissue the catalase content is correspondingly increased or decreased (4). Since thyroid feeding causes an increase in oxidation a corresponding increase in catalase should be found if the relationship between oxidation and catalase is to hold. It has also been observed that when the catalase content of a tissue is decreased the tendency of that tissue to undergo autolysis is correspondingly increased (5). Since thyroid feeding increases autolysis in muscular tissue, for example, as is indicated by a loss in weight and strength of the muscles, there should be a corresponding decrease in catalase in the muscles and in all other tissues in which autolysis is increased. It is known that the autolyzing enzymes in common with all the ordinary enzymes are easily oxidized and destroyed.

The object of this investigation was to determine if thyroid feeding increases the catalase content of certain tissues, which would account for the increased oxidation in animals fed thyroid, while in other tissues, such as the muscles and fat, it causes a decrease in oxidation which would account for the increased autolysis in these tissues. The animals used in these experiments were cats. They were placed in separate cages and fed twice a day 5 grams of desiccated thyroid mixed with 60 grams of ground meat. It was found necessary to vary the diet frequently by mixing the thyroid with different kinds of meat in order to induce the cats to eat. Fresh white fish, canned salmon, beef sausage and liver were among the meats used. The control or normal cats were fed the same kinds and amounts of food as the thyroid cats except that there was no thyroid added to their food. Even with every inducement it was found that certain cats refused to eat after the first day or two. The data given in this paper were obtained from cats that had eaten thyroid for at least five days. After this period of feeding the cats were used as soon as they refused to eat or when they showed great emaciation. None of the animals were fed thyroid longer than two weeks.

After etherizing the cats approximately 25 cc. of blood were collected from each cat and allowed to clot. The blood vessels of the animals were then washed free of blood by the use of large quantities of 0.9 per cent sodium chloride at 38°C., as was indicated by the fact that the wash water gave no test for catalase. The heart, the back (latissimus dorsi, trapezius) and leg (biceps, semi-membraneous) muscles were removed and ground up in a hashing machine. The clotted blood was pressed through several thicknesses of cheese cloth and ground up in a mortar. The catalase content of the muscles was determined by adding 1 gram of the hashed muscle to 45 cc. of hydrogen peroxide in a bottle and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted burette previously filled with water. The volume of gas was read off directly from the burette where it had displaced the water. After reducing this volume to standard atmospheric pressure the resulting volume was taken as a measure of the catalase content of the gram of material. In determining the catalase content of the blood, 10 drops of blood were added to 500 cc. of hydrogen peroxide in a large bottle and as the oxygen gas was liberated it was conducted through a rubber tube to a large inverted graduated cylinder previously filled with water, After reducing the volume of gas which was read off directly from the cylinder to stand-

ard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 10 drops of blood. The hydrogen peroxide used in all these determinations was prepared by diluting commercial hydrogen peroxide with an equal volume of distilled water. It was found very necessary to use the same make of hydrogen peroxide in all the determinations since the different makes gave different results. For this work a stock supply of about 200 liters of hydrogen peroxide was purchased and kept in a container in a dark and cool place. The results of the determinations for the blood and heart of the normal and the thyroid-fed cats are given in table 1.

TABLE 1

After blood and heart are given the number of cubic centimeters of oxygen liberated in ten minutes from hydrogen peroxide by 10 drops of blood and 1 gram of hashed heart respectively

	CAT										AVERAGE AMOUNT OF OXYGEN
	1	2	3	4	5	6	7	8	9	10	
											cc.
Blood											
Normal cat	560	430	970	1040	640	720	630	630	750	890	726
Cat fed thyroid	1280	2285	1850	1520	1940	2220	2560	2200	1570	1460	1888
Heart											
Normal cat	210	219	228	248	228	220	228	212	204	213	221
Cat fed thyroid	154	126	162	198	198	184	190	160	115	120	161

It may be seen in the table that the average amount of oxygen liberated from 500 cc. of hydrogen peroxide in ten minutes by 10 drops of blood of the normal cats was 726 cc.; that of the cats fed thyroid, 1888 cc. of oxygen. The average amount of oxygen liberated by 1 gram of the hashed heart of the normal cats was 221 cc. of oxygen, while the average for the hearts of the cats fed thyroid was 161 cc. From these results it is evident that thyroid feeding increased the catalase content of the blood by approximately 160 per cent as is indicated by the increase from 726 cc. to 1888 cc. of oxygen, while it decreased the catalase content of the heart by approximately 30 per cent as is indicated by the decrease from 221 cc. to 161 cc. of oxygen. The catalase content of the leg and back muscles and in some cases of the fat was determined, but the results were not very uniform and for that reason they were not included in the table. On the whole, however, it might be said that the catalase content of these tissues and hence oxidation was probably decreased by thyroid feeding.

It has been shown that the catalase content is an index to the amount of oxidation in a tissue, being greatest where the amount of oxidation is greatest and least where oxidation is least. From this it follows that thyroid feeding increases oxidation in the blood and decreases it in the heart and probably in the fat and skeletal muscles. Furthermore, it has been observed that when oxidation is decreased in a tissue the tendency of that tissue to undergo autolysis is increased. This was shown in starvation, for example, where oxidation is decreased in the skeletal muscles and fat, with a corresponding increase in the rate of autolysis resulting in the carrying into solution of these less vital tissues, while oxidation in a more vital organ, such as the heart, remains normal, with a corresponding high resistance to autolysis. The mechanism by which thyroid feeding produces a loss in skeletal muscles and fat would seem to be the same as that which causes the loss in starvation, namely increased autolysis made possible by decreased oxidation in these tissues. The effect of starvation on the heart, however, is different from that of thyroid feeding in that starvation does not decrease oxidation in this organ while thyroid feeding does, with the resulting increase in autolysis. This decreased oxidation with resulting increase in the rate of autolysis of the heart may explain the harmful effect on the heart encountered in thyroid feeding for obesity. It may also account for the characteristic heart disturbances in exophthalmic goiter where there is a hypersecretion of the thyroid glands. The increased oxidation in animals to which thyroid is fed may be accounted for by the great increase in catalase and hence in oxidation in the blood.

Many more cats were used in this investigation than the numbers indicate in the table. Most of these were the animals that refused to eat the thyroid after a day or two of feeding. Some of these animals were used as soon as they refused to eat, while others were kept and starved until they began eating again. The results from these cats indicated that thyroid feeding began to decrease the catalase in the heart after about two days, while it required four or five days' feeding to increase the catalase content of the blood.

CONCLUSIONS

1. Thyroid feeding increases the catalase of the blood and decreases it in the heart and probably in the fat and skeletal muscles.

2. The increased catalase of the blood may account for the increased oxidation in animals to which thyroid is fed, while the decreased cata-

lase in the heart, skeletal muscles and fat may account for the increased autolysis in these tissues, the idea being that when oxidation is decreased in these tissues a smaller amount of the autolyzing enzymes is oxidized and destroyed, resulting in an increase in the rate of autolysis.

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(5) Burge: This Journal, 1917, xliii, 58.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH NO. 67

RHEOTROPIC RESPONSES OF EPINEPHELUS STRIATUS BLOCH

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CONTENTS

I. INTRODUCTION

A. The problem

The tropical fish known as hamlet or grouper (*Epinephelus striatus* Bloch) is very favorable for biological experimentation. It is easily obtained and is a hardy animal; in response it is deliberate, but definite. When considerable numbers of hamlets are held in confinement they often manifest a tendency to crowd together closely, though without any regular arrangement, their heads pointing in all directions. This characteristic may have helped to give them the common name of "grouper."

While engaged in a study of the reactions of this fish, in connection with certain work on its central nervous system, I made use of an apparatus of the following nature: In a large spawning trough (fig. 1, *A*), such as is used in fish hatcheries, I suspended by two supports (*B*, *B*) a cage (*D*), about 36 inches long by 18 inches wide and 10 or 12 inches deep, made of galvanized "chicken wire." A current of fresh sea water was introduced at one end of the cage, at an angle of about 30° with the horizon, by a 1-inch hose pipe, for the purpose of affording a

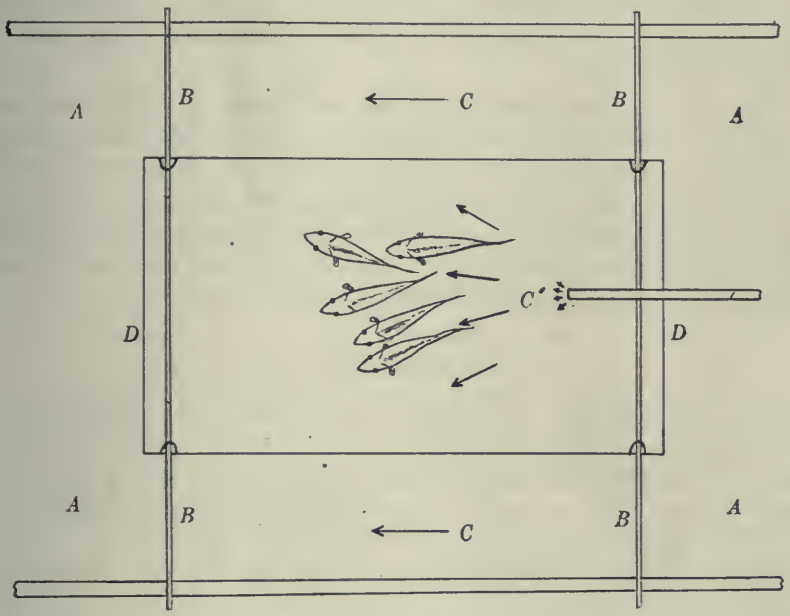

Fig. 1. Diagram of cage as seen from above. *A*, Large spawning trough; *B*, rods supporting cage; *C*, current of spawning trough; *C'*, additional currents from hose pipe; *D*, wire cage suspended from *B*.

better supply of water than was furnished by the sluggish current of the trough, flowing in the same direction.

When several normal fishes had been put in the cage for temporary storage, it was noticed that their orientation was no longer promiscuous, as in quiet water, but that an unusual position was assumed by nearly all of them (fig. 1). The cause of this was not at once apparent; but a little experimentation showed that the new orientation was in response to the stimulus of the seawater delivered through the hose pipe. Contrary to the usual rheotropic response of fishes, they had

their heads directed *away* from the hose pipe, most of the time with the body axis in line with the current, so that a group of fishes showed a fanlike arrangement corresponding to the spreading currents of water *C*, as shown by the arrows in figure 1.

In order to determine the exact nature of this reaction, experiments were undertaken both on groups of fishes and on individuals. The study of groups showed that this peculiar orientation was not altogether constant, but that some of the fishes assumed from time to time positions more or less oblique to the current. The precise significance of this was manifest only when the actions of individual fishes were observed continuously.

In an effort to ascertain the precise mechanism by which these reactions were brought about, and if possible to determine the nature of the stimulus inducing them, a small but strong localized current of water was directed in succession against different areas of the body. This revealed varying sensitivity on different areas; furthermore the effect of a narcotic on these areas indicated both the position and the probable nature of the end organs involved in these responses.

B. Review of literature

Up to about fifteen years ago it was generally held that the orientation and locomotion of organisms in a current of water—whereby the anterior end is directed against the current and a swimming motion causes either an advance or the maintenance of a comparatively stationary position against the flow—was due to the direct mechanical action of the current and was in the nature of a reaction to pressure. Stahl in 1884 described such a phenomenon in Myxomycetes and Verworn (1) in his Allgemeine Physiologie (p. 428) interprets it as a positive response to pressure stimulation. Lyon (2) (p. 157) says that reactions of blinded fishes [Fundulus?] to currents flowing through troughs may *perhaps* be caused "by higher pressure on one part than on the other, through differences in the velocity of the water striking the two parts." He here introduces the theory of unequal pressures on various parts of the fish's body in contrast with "the gross mechanical one of Radl."

The one early exception to the theory of pressure stimulation by currents seems to have been the idea advocated by Schulze (3), that the lateral-line organs were stimulated by the movement of water against them. This view has, however, been adequately disproved by Parker (4).

In the same year Tullberg (5) (p. 20) carried out experiments in which he eliminated the ear of certain fishes and found the operated animals to be insensitive to water currents. He therefore concluded that the ear is the chief receptor of this stimulus, which in his opinion affects principally the cristae acusticae of the ampullae. Parker (6) (pp. 202–203) contended, on the other hand, that the failure of fishes to orient in a normal fashion to the current when their ears had been destroyed is caused by an interference with the ear, "though the primary stimulus for this form of response might be received by the skin."[1]

In confirmation of this view—i.e., primary cutaneous sensitivity to currents—he (4) (p. 61) showed that specimens of Fundulus heteroclitus in which the lateral-line nerves had been severed responded normally,—i.e., swam against the current in a glass tube,—and he (6) (p. 202) also succeeded, after cutting of the lateral-line nerves and the spinal cord (in an anterior region), in inducing normal responses to a current of water directed against the sides of the body posterior to the cuts. Moreover, he excluded the possibility of stimulation through the ear or lateral-line senses by severing the appropriate nerves and draws this conclusion (4) (p. 63): "Surface waves and current action" . . . . "must stimulate the general cutaneous nerves (touch)."

However, his attempts to inhibit the action of the cutaneous nerves by the application of cocaine, and thus to show directly what the indirect method (the elimination of ear and lateral-line organs) had rendered probable, were unsuccessful.

In 1904 Lyon, basing his conclusions upon the results of ingenious experiments with Fundulus, scup, stickleback and butterfish surrounded by movable environments, put forward another and totally new explanation of rheotropism. He contended that "the primary cause of orientation [and locomotion of fishes] in streams of some uniformity of motion is an optical reflex." "The essential element of stimulation is the environment [apparently moving, but in reality stationary], not the currrent;" the latter "does not directly stimulate." He, however, adds that cutaneous sensations [touch?]—contact of the body with stationary solid objects and even "the sliding contact between fish and [rushing] water"—may sometimes be the cause of such orientation and locomotion. But even these responses, like those of a strictly optical reflex nature, are to be regarded, he thinks, as

[1] i.e., stimulation of the skin (tactile corpuscles) directly by small currents. This, it should be noted, is different from the indirect (optical reflex or thigmotropic—by solid object) stimulation, which is described below.

compensating motions; "the current playing only the passive part of sweeping the fish against objects on the bottom."

In view of Lyon's observations and general conclusion that the current itself does not stimulate the skin of Fundulus directly, except as it acts like a solid object of considerable size, it seems desirable to determine whether, in other fishes, the integument is sensitive to water-currents, and also whether the eyes have any essential part in the rheotropic reactions.

II. DESCRIPTION OF EXPERIMENTS

A. Posterior[2] *and lateral orientation*

1. In groups of fishes. The positions assumed by a group of fishes under the conditions already described gave, in general, the fanlike appearance shown in figure 1; but it was also to be seen that, from time to time, one or more individuals assumed a different position, the body swinging around so that its long axis was almost or quite perpendicular to the current; and sometimes, though rarely, an individual would be headed more or less directly into the current in the manner of the hitherto described reactions of fishes generally. In order to test roughly the quantitative relations at any given instant between these various positions, several series of observations were made, both during the day and at night, on a group of seven fishes which had become habituated to their surroundings. One such series is recorded in table 1; all the others are nearly identical with it.

From table 1 it will be seen (1) that, although the majority of fishes (67.1 per cent) tailed into the current, many individuals (31.9 per cent) so placed themselves that the current hit the side of the body; and (2) that less than 1 per cent headed into the current. These records were taken at about two-minute intervals. While they show that the hamlet in its normal responses to a current may assume one or the other of two positions, they do not afford sufficient evidence of

[2] In describing the various positions of orientation which a fish may assume, I shall use *posterior* orientation to denote positions in which the tail is directed toward the oncoming current or to points not more than 45 degrees from it on either side; *lateral* orientation to denote positions in which the long axis of the body is perpendicular to the direction of the current or makes an angle with the perpendicular on either side not greater than 45 degrees; and *anterior* orientation to positions whereby the head is directed straight into the current or to points not more than 45 degrees from it on either side.

TABLE 1

ccessive positions of orientation of a group of seven hamlets observed at about two minute interva

	NUMBER OF INDIVIDUALS IN EACH OF THE THREE POSITIONS AT THIRTY SUCCESSIVE COUNTS																												TOTAL	AVERAGE	PER
umber of the observation...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	210	7	100
rientation																																	
1. Posterior...	6	6	4	3	5	6	5	5	4	4	5	5	5	5	6	5	6	5	4	6	7	5	2	3	5	2	3	5	4	5	141	4.7	67
2. Lateral.....	1	1	2	4	2	1	2	2	3	3	2	2	2	2	1	2	1	2	3	1	0	2	4	4	2	5	4	2	3	2	67	2.2	31
3. Anterior....			1																				1								2	0.06	0

the exact nature of this reaction—whether the two positions are due to differences between individual fishes or are phases of one reaction. Single groupers, however, when studied continuously showed that both positions are assumed in the course of one reaction.

2. In individual fishes. Individual hamlets were tested in a spacious oblong aquarium (fig. 2) provided with plane glass front and back to allow uninterrupted observation from the sides as well as from above. A glass tube 1 cm. in diameter and so directed as to make about equal angles with two adjacent sides of the aquarium, delivered a strong current (*C*) diagonally across the tank. The volume of this current was approximately 0.1 liter per second. It is the only one which is significant for the purposes of this investigation. Those peripheral to this were not of sufficient strength or regularity to influence the fish in any consistent manner. These currents were studied by the use of floating and suspended objects, and the results plotted show their main features.

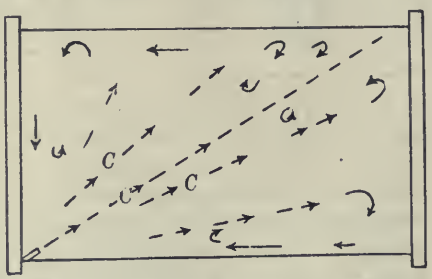

Fig. 2. Diagram of aquarium (20 by 30 inches) and currents as seen from above. *C*, Main diagonal current.

The fish under investigation (fig. 3) seemed to prefer the region of the strongest current; that is, it remained near the source of the current, shifting from one position of quiet to another, settling to the bottom, or remaining suspended at a fixed place in the current for a few moments, and then again changing position slightly. All the while, however, the fish assumed either posterior or lateral orientation to the

main diagonal current (*C*). It held this position in the tank indefinitely. When left in the current for two or three hours no change of general position was noted. When the current was shut off, the fish under investigation would swim to the bottom or to one of the corners of the tank, where it would remain relatively quiet. In figure 3, one out of several records is reproduced to show a typical reaction. The diagonal line (*C*) shows the direction of the main current. The elapsed time in minutes, from the beginning of observations, is given in table 2 for each of twelve successive positions. These positions are indicated in figure 3 by straight lines, the anterior end of the fish being denoted by a short cross line, and the successive positions by consecutive numerals placed near the end representing the tail.

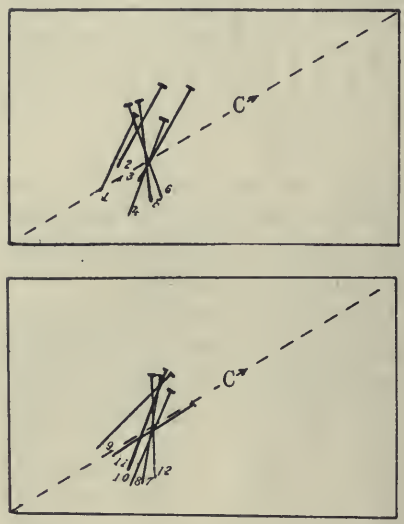

Fig. 3. Twelve successive positions occupied by a normal fish with reference to the main diagonal current, *C*. To avoid confusion only six positions are indicated on each of the two diagrams. Position 11 was retained longer than any other; it is therefore regarded as the most significant orientation. One complete reaction is regarded as occupying the interval of time between two successive assumptions of such a position.

It should be noted, however, that in this particular experiment, contrary to the most of them, the percentage of posterior and lateral orientations is nearly equal. That posterior orientation is the purpose of shifting the position, is suggested by the fact that the fish remained stationary for a considerable time only when it was almost directly tail into the current, position 11. After this period (about two minutes) it again begins a series of changes in position, like those shown in figure 3, which lasts for about four minutes, whereupon another period of rest ensues. What I have called one complete reaction, then, requires about seven minutes. It is most important to note that the fish did not at any time head into the current.

The two different experiments, one with groups and the other with individuals, are consistent in showing that posterior and lateral orientation to a current is the normal reaction of *Epinephelus striatus*.

TABLE 2

Time elapsed in assuming the positions, 1 to 12, shown in figure 3

	NUMBER OF THE POSITION											
	1	2	3	4	5	6	7	8	9	10	11	12
Elapsed time in minutes	0	½	¾	1	1¼	1½	2	3	3½	3¾	4¾	7
Time intervals between positions in minutes		½	¼	¼	¼	¼	½	1	½	¼	1	2¼

B. Experiments on regional sensitivity

A study of the relative sensitivity of various parts of the body was undertaken with the hope of finding some evidence as to the nature of

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AUTHOR'S CORRECTION

(Insert opposite page 444, Volume xliii, 1917)

The sentence (page 444, paragraph 2) beginning: "It should be noted, however," should be interchanged with the first part of a sentence on page 450 (paragraph 1), viz., with "It should be noted that . . . . the positions were posterior."

Sept

reaction, a swimming or backing away from the current. It is possible, however, to divide the body into five regions based on their relative susceptibility to stimulation by such a current. In the order of promptness of reaction these are as follows: lip region (seven seconds); caudal fin (sixteen seconds); dorsal fin, posterior part (twenty-two seconds);[3] cheek and operculum (twenty-five seconds); sides of body(about thirty seconds). The belly was not tested because of its inaccessibility. Thus it appears that the lip region is by far the most sensitive part of the integument tested. If stimulation of the lips is prolonged, the hamlet becomes very vigorous in its attempts to escape.

[3] That the fins are not essential in rheotropism is indicated by the fact that when either dorsal or caudal fins are removed, the normal reaction is unaltered. It was also noticed that fishes whose fins had become badly frayed by long captivity were normal in their responses to currents shown in figure 3.

When "cornered" by the current it literally stands on its head, a termination of the negative reaction which is extremely unusual among fishes.[4] This high sensitivity suggests at once an explanation of posterior and lateral rheotropism, for it may be that stimulation of the lips by the current in the aquarium or cage was so strong as to produce decided irritation, and thus to cause the fish to place that portion of its skin in a less exposed position.

C. Summary of normal rheotropism

The foregoing experiments show:

1. That to a moderate artificial water-current a normal orientation of *Epinephelus striatus*, in groups or individually, is posterior or lateral, as phases of one complete reaction, but almost never anterior.
2. That the lips are the most sensitive integumentary region, other regions being less sensitive in the following order; tail>, dorsal fin>, side of head>, middle of body.
3. That the peculiar posterior and lateral reaction to a current is perhaps an attempt to protect from the current the highly sensitive lips.

D. The end organs concerned in rheotropism

1. Method of determination. In searching for the end organs concerned in rheotropism, it was, of course, necessary to consider all possible sensory cells. My conclusions relative to the significance of equilibration (semi-circular canals), muscle sense, and pressure sense in these reactions are, for the most part, based upon observations only. The lateral-line organs, eyes and cutaneous receptors, on the other hand, were experimentally eliminated, and each operated fish was carefully studied to detect any resulting variation of the response from that of the normal individual. It was established by these experiments that the end organs concerned in rheotropism in the hamlet are located in the integument and are probably the tactile corpuscles.

2. Observations and experiments: a. Observations. When confined in large volumes of still water, groupers are seen usually to lie inactive on the bottom of the tank. In captivity they swim about very little, seem-

[4] It is important to note that the same reaction can be induced by the use of a fine glass rod (tactile stimulation), and also that the variation in regional sensitivity to such stimulation corresponds exactly to that described for stimulation by the current.

ing to prefer muscular repose to exertion, the fin movements being few and slow; pectoral fins are vibrated about twenty-three times per minute. The application of a localized current of little force was sufficient to start the fishes from a position of complete rest, but when they were beyond the range of the current, they again settled to the bottom. This behavior indicates that the agreeableness of muscular effort is not sufficient to cause any prolonged swimming. On the other hand, the exertion and possible fatigue involved in maintaining a relatively constant position in a current which is broad enough to cover the whole fish might be expected eventually to produce a negative reaction. Instead of this, however, the fishes remained for an hour or two in the strongest part of the current (fig. 3), seeming to prefer it to the quiet water. This leads one to the conclusion that the muscular effort necessitated in these reactions is not in itself a deterrent factor. Many times, too, when a localized current was directed against the side of the fish, lying at the bottom near the wall of the aquarium, it was observed that one of the pectoral fins—extended horizontally to the wall—served to keep the body of the fish from contact with the side of the tank. In these cases the effort involved in maintaining this position did not cause the reaction time to vary.

With a view to ascertaining what importance, if any, attaches to the pressure sense, I made use of a current of water directed through the glass tube (1 cm. in diameter) already referred to in other experiments. If the pressure sense is a factor in rheotropic response, it is to be expected that the response to a very strong current would be more prompt than to a weaker one. Accordingly I repeatedly subjected the same fish successively to a weak current (about 1/28 liter per second) and to a stronger one (about 1/8 liter per second). Though the latter was of sufficient force to produce an appreciable indentation of the skin and musculature of the body, the reaction time was not shorter than in the case of the weaker current. I may also add that fishes can be pressed by the hand against the side of the aquarium with considerable force without causing any definite response. In his study of the pressure sense as a possible cause of rheotropism, Lyon (2) (p. 154) enclosed fishes (silver sided minnows) in long stoppered bottles which were floating down the stream. He found that under these conditions, with all pressure stimulation thus eliminated, the fishes responded normally, by swimming in a direction opposite to the drift of the bottle, in an attempt to keep the visual environment constant. My experiments, though not of such fundamental importance as Lyon's,

tend to substantiate his conclusion that [considerable] pressures do not cause or influence the rheotropic reactions.

Observations were also made upon the equilibration of hamlets. They were studied in still water and when subjected to a current sufficiently strong to cause a change in the direction of the dorso-ventral axis. Any differences between the rheotropic response of fishes whose dorso-ventral axis is normal and those in which this axis has been displaced would suggest that the organs of equilibrium may be involved. An individual fish when in quiet water usually lies in such a position that its dorso-ventral axis makes an angle of 10° to 15° with the normal (fig. 4). When this angle was doubled by a current from a glass tube directed against the side of the fish there was no variation in the time required to produce a negative reaction. Moreover the motion which restores the fish to an approximately vertical position usually follows, and never precedes, this rheotropic response; whereas, if the fish is carefully put in the same oblique position by a slow displacement with the hand, instead of by the current, the righting movement takes place much more promptly. It seems, then, that any slight disturbance in the hamlet's equilibrium which the current might cause would neither produce nor in any way affect the observed behavior. This conclusion is in accord with Parker's conclusion (6) (p. 203) to which reference has already been made.

b. Experiments. Lateral-line organs can be excluded from the list of possible rheotropic receptors for two reasons: First, when the current is directed immediately against the lateral-line canal upon a limited mid-body area, the slow response (thirty seconds) characteristic of regions both dorsal and ventral to the lateral-line, but excluding it, is neither quickened nor retarded. Secondly, a hamlet in which all of the lateral-line nerves had been severed responded normally to the current. This experiment confirms Parker's results (4) (6) from a similar test made upon Fundulus.

In order to determine whether the visual organs are essential to these reactions, experiments were also performed successively on several individuals after enucleation of both eyes. Fishes thus blinded were subjected to conditions of stimulation identical with those in the tests which were made upon unoperated hamlets (fig. 2) and the successive positions which they assumed were recorded. One of these records is reproduced (fig. 5, table 3) for comparison with figure 3, which shows the consecutive orientations of a normal fish in a like environment.

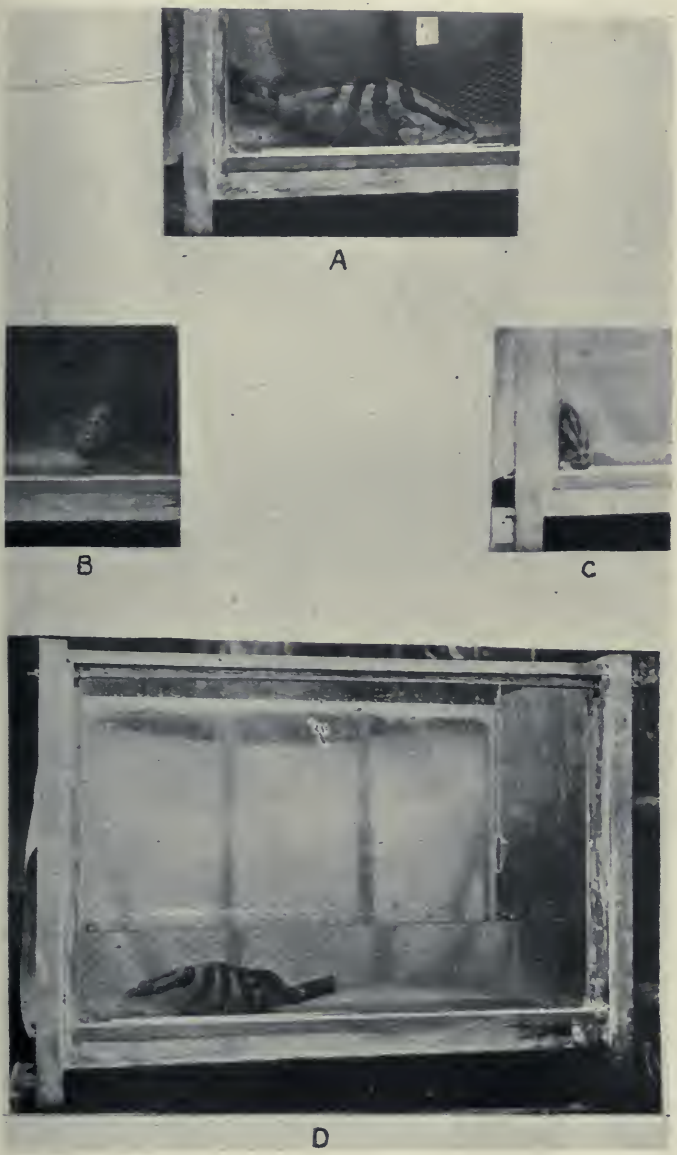

Fig. 4. Photographs of resting fishes tipped at a characteristic angle from the vertical. *A*, Side view; *B* and *C*, front views; *D*, side view of fish, showing the whole aquarium.

The two records, while showing slight variations, are remarkably alike in the time of response and the number of different positions assumed in changing from an almost lateral orientation to an approximately posterior one. It should be noted that in this series of orientations 75 per cent of the positions were posterior, and that, as in figure 3, no anterior positions were assumed. It seems, then, that the eyes of the hamlet are not the essential rheotropic end organs.

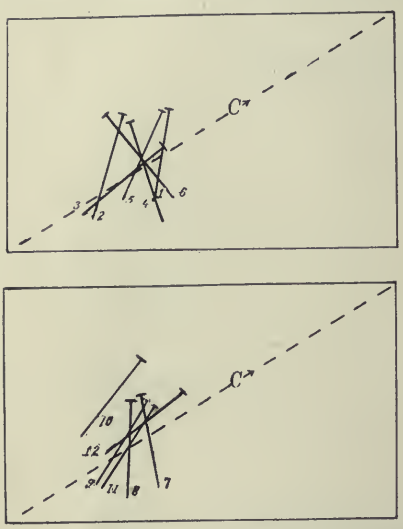

Fig. 5. Twelve successive positions assumed by a blinded fish in response to the current *C*. As in figure 3, only six positions are shown in each diagram.

In an effort to locate the cells which are stimulated by the current, the skin was stripped from one of the more sensitive body areas. It was impossible to obtain any response from a current which was directed against the subcutaneous structures (muscles, etc.) thus exposed. In a few cases indefinite reactions, which were much slower than normal, were observed, but they were not characteristically rheotropic in nature. These results lead one to the conclusion that the end organs concerned in rheotropism are located in the integument.

TABLE 3

Time elapsed in assuming positions 1–12 (fig. 5)

	NUMBER OF THE POSITION												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Time elapsed in minutes.....	0	½	¾	1	1¼	1½	2	2½	3	3½	4	4½	7
Time intervals between positions in minutes.....		½	¼	¼	¼	¼	½	½	½	½	½	½	2½

The problem of determining the particular type of sense organ which is sensitive to these currents is resolved, then, into a physiological

study of the hamlet's cutaneous end organs. Of these only the tactile corpuscles are significant, because the receptors for chemical, photic and thermal stimuli plainly can not be involved in these rheotropic responses.

It has been stated previously that the areas of greatest cutaneous sensitivity in the case of both touch and current stimulation have the same distribution. Cocaine is known to inhibit the functioning of the end organs of touch. It was, therefore, used to eliminate, functionally, the tactile corpuscles, in order that their relation to rheotropism might be determined. Of all available areas the lips were chosen for the application of this reagent because the experiments had shown that their stimulation gave the most marked and peculiar responses. Whether the lips are stimulated by a fine glass rod or by a moderate water current, the fish performs the very curious reaction of either backing away violently or of standing on its head. The method of treatment with the cocaine was as follows: the fish was removed from the experiment tank and the lips were immediately immersed in a 0.1 per cent solution of sulphate of cocaine for about ten seconds; this was supplemented by bathing the lips with the same solution applied by means of a soft cloth at about ten-second intervals for fifty seconds. After this treatment the fish was returned to the tank and its responses to stimulation by a glass rod and by a weak current (about 1/28 liter per second) were noted. By a repetition of the treatment it became evident that there was a slowing down in time of response with increased exposure to the solution and that this was precisely the same for both types of stimulation. Two repetitions of the first treatment—in all about three minutes—were usually sufficient to inhibit completely all responses to either type of stimulation; neither the glass rod nor the localized current then produced any reaction. In subsequent trials the lips were treated continuously—without periodic subjection to stimulation—for a period of about three minutes. The effect was the same as in the preliminary treatment just described. After such administration of cocaine the fish swam about in a slightly abnormal manner, manifesting an irritation due, doubtless, to the drug. This insensitivity of the lips lasted about a minute, sometimes a few seconds longer. Then a gradual functional recovery occurred until, at the end of three to four minutes, normal responses could be obtained by the use of either stimulus. It is most significant that the time of disappearance of normal sensitivity, as well as that of its reappearance, was absolutely the same for both kinds of stimulation.

This fact indicates that those sensory cells which are stimulated by touch and defunctionized by cocaine are also the cells which are the primary end-organs of the rheotropic response.

3. Summary of end-organ determination. 1. The end organs of the hamlet essentially concerned in rheotropism are located within the integument.

2. The regional distribution of sensitivity to a water-current and to touch is the same.

3. Cocaine applied to the lips for about three minutes renders those organs insensitive both to touch and to currents.

4. These facts indicate that the end organs of touch serve also as the essential end organs of current stimulation in the hamlet.

5. Other sensory cells may be more or less affected by currents in some fishes, but they appear to be only accessory end-organs of rheotropism, and in the responses described in this paper they evidently play no part at all.

III. DISCUSSION

There is much evidence to show that the rheotropic responses of the hamlet, as suggested for Fundulus by Parker (4) (6), are effected chiefly by the tactile corpuscles. His attempt to prove this by immersing the entire fish in a solution of cocaine did not succeed because the general action of the drug entirely destroyed all sensitivity and movements of the fish; but the great sensitivity of the lips of the hamlet has afforded an excellent opportunity to study changes in the fish's behavior resulting from the local application of this narcotic. In this case regional anaesthesia, producing insensitivity to touch, is as satisfactory as general narcotization would be, because it causes a most unique rheotropic response totally to disappear.

Some of Lyon's experiments (2) on rheotropism, from which he concluded that the reaction of Fundulus to currents is chiefly to compensate the transporting effect of the current, seem to furnish evidence that the integumentary cells (tactile corpuscles) were directly concerned in the rheotropism which he observed. Among these is experiment 9 (p. 157), in which a blinded fish, without touching any solid object—often required for orientation by fishes without eyes—headed into the rushing current. This certainly may be interpreted as a response to direct tactile stimulation of the integument by the water, and Lyon himself admits that this may be called a *true* rheotropism. In his opinion, however, it is due to the "sliding contact" (stereotropism)

between fish and water, although he admits the possibility of another interpretation involving the idea of unequal pressures on different parts of the body of the fish. Whether the rheotropism induced by this "sliding contact" is the equivalent of stereotropism, with which Lyon believes it is closely related, is a question needing further investigation.[5]

The comparative importance, in the behavior of Fundulus, of these two types of impressions—optic and cutaneous—is, I think, suggested by some of Lyon's interesting experiments. An example of this is his experiment 3 (p. 153). Here a normal fish, surrounded by a rapidly moving artificial environment, is immersed in a current of water flowing in the same direction as the environment, but less rapidly. The fish swims in the direction of, but faster than, the current flow, following the moving environment in rate as well as direction. This is unquestionably a case of optical response. When the environment stops moving, the current still flows on in the same direction, but with a gradual decrease of speed due to friction; but the fish, having been carried passively by the current, turns, without a reference point, and faces [swims against?] the current. This may be due, as Lyon says, to an apparent reversal of the visual field; but, since *blinded* fishes (experiment 9), *without* touching a reference point, also orient against the current, it seems equally logical to interpret the turning of fishes with eyes (experiment 3) as the result of the normal rheotropic response to direct tactile stimulation of the integument, which had, during the movement of the optical field, been subordinated to the sight-reflex.

If this be a proper interpretation of the results with Fundulus, we have in the hamlet a reversal of the relative importance of the two kinds of stimuli. Here, under the experimental conditions described, all optic stimuli were, apparently, subordinated to tactile impressions; the direct effect of the current being predominant and able to cause entirely normal reactions in the absence of eyes. It is, however, not quite satisfactory to compare the two sets of experiments (those by Lyon and by myself) from this point of view, because the relative amounts of cutaneous and optic stimuli in each are indefinite and variable. It is certain that, in my experiments, there was relatively little stimulation of the eyes, because the fish remained in an approximately

[5] It seems probable that the currents of a narrow trough would not be sufficiently strong nor distinct from one another to simulate solid objects, but that there would be an almost insensible gradation between them.

constant position, and that, in Lyon's movable-environment experiments, their total stimulation was much greater. It can not be said whether the tactile corpuscles were subjected to a proportionate stimulus or not. It may be that the hamlet, too, would orient to a movable environment regardless of contemporaneous tactile stimulations[6] by the current; but the facts remain that the tactile-corpuscles do, of themselves, effect orientation, and that this orientation under the above circumstances is unaltered by the presence of eyes. This orientation, it seems to me, is caused by a direct stimulation of the integument by the water currents as such, and to it we should apply the term rheotropism. The response so well described by Lyon as due to optic reflex might then be called a rheoscopic response in view of the fact that it is due to the optical effect of a flowing or moving environment.

How the current stimulates these tactile end organs is still a matter of speculation. It may be that differences of velocity in different portions of a current provide slight local variations of force sufficient to cause a definite response on the part of the fish. How the stimulation results in orientation is a further question, for the mechanism and sensation may or may not be the same for rheotropism that they are for stereotropism.

The author wishes to express his appreciation to Dr. E. L. Mark for the privilege of working at the Bermuda Biological Station, and to Dr. Mark and Dr. W. J. Crozier for valuable assistance and advice in the work.

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[6] It would be interesting to determine the relative importance of the eyes and cutaneous elements (tactile corpuscles) as sense organs in the orientation and rheotropic motions of many other fishes.

FURTHER EVIDENCE REGARDING THE RÔLE OF THE VAGUS NERVES IN PNEUMONIA

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A former communication[1] recorded the discovery "that section of the vagus nerves protects the respiratory cells and prevents their exhaustion in pneumonia. In pneumonic dogs in which both vagus nerves have been cut, the rate of respiration remains normal throughout the disease. In such animals there is no dyspnoea."

It was necessary in that research to cut the right vagus nerve within the chest and to obtain complete recovery from that operation before cutting the left vagus nerve in the neck. Some days after this second operation the dogs were inoculated with pneumonia. The disease ran its usual course, except for the extraordinary fact that the typical dyspnoea was entirely absent. Obviously, the section of the vagus nerve within the chest and the recovery of the animal without infection are troublesome and time-consuming procedures. This method is essential to the demonstration that the exhaustion[2] of the respiratory mechanism characteristic of pneumonia does not take place when the path of afferent impulses from the lungs to the bulbar respiratory cells is severed by the section of the vagi. It is not, however, essential to the demonstration that the dyspnoea in pneumonia depends on impulses passing from the lungs through the vagus nerves. The dependence of the dyspnoea upon vagal impulses may be shown by cocainizing the vagi while the pneumonia is at its height. The following protocol is evidence of this.

Experiment June 15, 1916. At 4 p.m., 24 cc. of a broth culture of Friedländer's bacillus were injected into the right bronchus of an anaesthetized dog weighing 8 kilos.

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[1] Porter and Newburgh: This Journal, 1916, xlii, 175.

[2] Discovered by Newburgh, Means, Porter: Journ. Exper. Med., 1916, xxiv, 583.

June 16, 9 a.m. Rectal temperature, 40°C. Respiration labored; 80 per minute. The dog was placed on the operating table and lightly but sufficiently etherized. The vagus nerves were exposed in the neck and were surrounded by a layer of absorbent cotton.

The absorbent cotton was wet with a 1 per cent solution of cocain.

9.30 a.m. Respiration, quiet, "easy," 20 per minute.
10.00 a.m. Respiration, 16.
12.00 m. Respiration, 16; temperature, 40°.
1.00 p.m. Respiration, 15.
2.00 p.m. Respiration, 30.
3.00 p.m. Respiration, 60; temperature, 40°.
3.15 p.m. A few drops of cocain solution were dropped on the cotton surrounding the vagus nerves.
3.30 p.m. Respiration, 16.
5.00 p.m. Respiration, 14; temperature, 39° (the beginning of the fatal fall).
6.00 p.m. Respiration, 14; temperature, 37.5°. The dog lies upon his side; he cannot stand.
6.30 p.m. Respiration, 14; temperature, 37°. Dog semi-conscious.
7.00 p.m. Respiration, 14; temperature, 37°. Dog in coma.
7.10 p.m. Death.

Autopsy. Red hepatization of the right middle and both lower lobes of the lungs.

CONCLUSION

Cocainizing the vagus nerves changes the violent dyspnoea of pneumonia into quiet, normal breathing.

OROKINASE AND SALIVARY DIGESTION STUDIES IN THE HORSE[1]

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INTRODUCTION

The work reported in this paper may be divided into four main groups or parts, as follows:

I. Orokinase—an enzyme produced by the glands in the mouth, which activates the saliva.

II. Bacteria of the mouth as activating agents.

III. Amylolytic action of mixed saliva obtained from the mouth and esophagus.

IV. The amount of complete starch digestion in the mouth.

The work was planned and outlined, the tissue extracts prepared and the operations were performed upon the experimental horses by Palmer. Anderson carried out the bacteriological studies and assisted in the operations and preparation of the tissue extracts. Malcomson studied the amounts of starch conversion in the mouth. Peterson studied the amylolytic action of mixed saliva. The entire group participated in the studies with the activating substances.

I. OROKINASE

Orokinase is the name proposed by Palmer for the enzyme produced in the mouth and found in the saliva, which makes active the inert saliva emptied into the mouth from the salivary glands. We believe, and our experiments prove, that this enzyme, orokinase, is produced by the buccal glands, and possibly by the lingual glands.

[1] Published with the approval of the Director as Paper No. 65 of the Journal Series of the Minnesota Agricultural Experiment Station.

That we should find such an activating substance was suspected before we began our studies. The idea was presented through a study of the literature. Ellenberger and Hofmeister (1) state that mixed saliva of the horse has a very powerful diastatic action, whereas saliva collected from the parotid ducts is inactive. Mathews (2) in reviewing this fact suggests the possibility of a co-ferment or kinase produced by the mucous membrane of the mouth. He also adds that "the late Dr. Cook told the writer that if the human mouth is carefully washed out with a sterile solution of water or dilute antiseptic, the saliva collected from the ducts may be inactive, whereas the saliva which has been in contact with the mucous membrane of the mouth is very active."

Our first work, then, consisted in confirming Ellenberger and Hofmeister's work, and especially since some workers do not accept this view but are of the opinion that horse saliva is inactive under all conditions. Why these investigators have failed to demonstrate the amylolytic activity of mixed horse saliva, will be discussed under part III.

We have been able to confirm Ellenberger and Hofmeister's statement that saliva is inactive before it reaches the mouth, by studying the amylolytic activity of parotid fistula saliva of three horses, and the glycerine or water extracts of the parotid, submaxillary and sublingual glands of eight horses. The same methods of study were employed as those used by Palmer (3) in his studies on ox saliva.

Basing our conclusions on approximately one hundred examinations of the substances named, we conclude parotid fistula saliva when stimulated under natural conditions (feeding oats) is without trace of amylolytic activity upon cooked or raw starch at least within a period of several hours incubation. Glycerine and water extracts of the three salivary glands are also without action as indicated by clearing of the starch solution, loss of blue color with iodine, or the presence of reducing sugars.

We also agree with Ellenberger and Hofmeister that mixed saliva is very powerful. This conclusion is based upon the study of seventeen positive cases. In eleven horses active mixed saliva was collected from the mouth, and in six it was obtained from the esophagus. The details of this work are discussed in part III.

Another method of demonstrating that mixed saliva is very powerful, is by feeding raw corn and oats; these substances contain no reducing sugars before feeding, but show heavy reduction when caught from an esophageal fistula. This work is discussed in part IV.

From this work it is evident that in the mouth the previously inactive saliva becomes active, and our work was now directed towards locating this activating substance. Ellenberger and Hofmeister suggested bacteria of the mouth as the activating agents, but we were unable to confirm this, as shown in our bacteriological studies. Our efforts to locate the activating substance were first rewarded on December 7, 1916, when on this date a 50 per cent glycerine and water extract of the mucous membrane of the buccal region gave excellent results. Only a few drops of this extract were required to activate parotid fistula saliva and gland extracts from the three salivary glands. If we used a large enough quantity of this extract, it would not only activate the fistula saliva and gland extracts, but it would digest starch itself. We could, however, so reduce the amount until we had a quantity sufficient to activate the fistula saliva or gland extracts, but which would not alone digest starch within a period of two hours incubation.

Further studies with this activator "I" as we called it, revealed the following facts: Its ability to activate the saliva was destroyed by boiling. When a few drops of this activating substance were added to 50 cc. of fistula saliva, and incubated for several hours, we could not demonstrate that a small amount could activate an indefinite amount of fistula saliva if given time enough.

Our next step was to demonstrate this activating substance in a number of animals. The next three buccal mucous membrane extracts gave negative results, so we began to search anew for the activating substance. In carefully dissecting the mouth, the small buccal glands are found under the mucous membrane of the cheeks and lips, and many of the lobes are quite adherent to the mucous membrane. In fact it is difficult to dissect the mucous membrane away from its underlying parts without removing some of these glands with it. These glands form quite a large mass, just anterior to the commissures in the lower lip, and another large group is found in the buccal region just posterior to the commissures. In addition to these locations, the glands are numerous under the mucous membrane of the buccal region, and here two rows of small ducts present themselves. In some subjects there is a small deposit of dark pigment around the opening of each duct, and the ducts number about one hundred or more. The glands are also quite numerous under the mucous membrane of the lower lip and the openings of their ducts can be easily made out. In a fresh specimen a small quantity of the secretion of these glands can be squeezed out through the ducts and onto the mucous membrane where

it can be collected. In two specimens this buccal juice would activate fistula saliva, would not digest starch itself, and was destroyed by heat.

We have now demonstrated in ten horses that extracts of the buccal glands contain the activating substance. In several of these cases the mucous membrane from the buccal region and the lips was carefully removed, but they were negative in all cases. Our results in the first case (activator I) can probably be accounted for by the fact that some of these glands were removed with the mucous membrane. The activators obtained from these ten cases have not all behaved alike. Some of them would digest starch as well as activate fistula saliva; some would activate the saliva, so that the digestion was carried to the maltose stage, while others would only carry the digestion to the soluble starch or dextrin stage. They were all destroyed by heat.

Our best extracts have been obtained from horses freshly killed, but even some of these were unstable and lost their activating power after thirty-six to forty-eight hours. For example, the most powerful activator (No. III) was obtained from a horse a few hours after death; this activator was very powerful and table 1 shows in detail one experiment using this activator, but after standing twenty-four hours this extract was inactive. This activator would not digest starch when used alone and was destroyed by heat.

We did not succeed in obtaining a potent extract in every case examined. We invariably failed in cases where the horse had been dead for a few days or when the head had been frozen, and in our positive cases the activators seemed to vary in strength, even though we tried to use the same relative amount of extracting material in each case. This may be accounted for by the fact that the activator is unstable, or our methods of extracting are not ideal, or that after death substances are present in the buccal glands which destroy this activating substance.

These buccal glands and the openings of their ducts are located and pour out their secretion in places which are well adapted to activate saliva coming into the mouth. The parotid duct in the horse opens opposite the third upper cheek tooth, and as this parotid saliva flows down over the buccal mucous membrane, it directly comes in contact and mixes with the secretion of the buccal glands. The ducts of the submaxillary gland opens opposite the canine tooth, and here in this region just anterior to the commissure of the lips we find another group of glands very similar in gross structure to the buccal and very likely belonging to the same class. Similar glands are also found under the

TABLE 1

Detail of one experiment demonstrating the action of orokinase on fistula saliva and salivary gland extracts

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Nearly clear	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	Fair	More	Heavy
B_s	Nearly clear	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
C_s	Considerable	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
D_s	Considerable	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
E_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
F_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
G_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
H_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
I					No clearing					Blue					No reduction
J					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
B_s contains: Parotid gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
C_s contains: Submaxillary gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
D_s contains: Sublingual gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
E_s contains: Fistula saliva, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
F_s contains: Parotid gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
G_s contains: Submaxillary gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
H_s Contains: Sublingual gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
I contains: Buccal extract, 0.5 cc.; distilled H_2O, 1.0 cc.; starch, 5 cc.
J contains: Distilled H_2O, 1.5 cc.; starch, 5 cc.

mucous membrane of the lower lip, and the secretion of these glands mixes with the sublingual saliva.

The lingual glands on the base of the tongue may also produce orokinase, since mixed saliva coming from the esophagus is more powerful than any mixed saliva which we have obtained from the anterior part of the mouth. Extracts of the lingual glands (located in the base of the tongue) have been prepared from five horses, and in all cases the extract alone would digest starch either to the soluble starch, dextrin or maltose stage, but they did not activate fistula saliva.

We have succeeded in procuring from the mouth of one animal what we believe to be almost pure buccal juice. A grey mare which normally was a willing animal to salivate, had a parotid fistula on the right side. We would stimulate secretion by teasing with oats and corn and from the right cheek obtain a secretion much more viscid than the fistula saliva and which resembled buccal juice.[2] We know this was not parotid saliva because it did not appear like parotid secretion and because no parotid saliva was being poured out onto this cheek, and unless saliva crossed the mouth from the left side, we had pure buccal juice. In a few trials, this secretion gave good results, a small amount would activate fistula saliva. A few drops would activate 50 cc. of fistula saliva after several hours incubation and if a large enough quantity was used, it would digest starch.

Orokinase can also be demonstrated in the mixed saliva of man and horse. To demonstrate this action with mixed horse saliva, the saliva obtained from an esophageal fistula should be diluted 1 to 10 or 15, with distilled water and two drops of such a mixture used. Two drops of this mixture will not digest starch, at least within a period of two hours incubation, but when added to 1 cc. of fistula saliva, good digestion results. Table 2 shows in detail one experiment demonstrating the activating properties of two drops of a mixture of mixed horse saliva, 1 to 10 in distilled water.

To demonstrate orokinase in human saliva, the human saliva must be diluted 1 to 50 in distilled water. Two drops of this mixture will be inactive or very slightly active (varying with individuals) but when added to 1 cc. of horse fistula saliva the resulting mixture gives good digestion. Table 3 shows in detail one experiment demonstrating the activating properties of two drops of a mixture of human saliva, 1 to 50 in distilled water.

[2] Fistula saliva is clear but not viscid, and flows like water; buccal juice is clear and very viscid; mixed saliva is clear and viscid but not as viscid as buccal secretion.

TABLE 2

Details of one experiment demonstrating orokinase in mixed horse saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Slight	Clearer	Clearer	Clear	Clear	Blue	Blue	Blue	Blue	Blue	No reduction	Very slight	Slight	Fair	Good
B_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; mixed horse saliva (1–10), 2 drops; starch, 5 cc.
B_s contains: Distilled water, 1 cc.; mixed horse saliva (1-10), 2 drops; starch, 5 cc.
C_s contains: Fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

TABLE 3

Details of one experiment demonstrating orokinase in mixed human saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Quite clear	Clearer	Clearer	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Good	Heavy	Heavy	Heavy
B_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; human saliva (1–50), 2 drops; starch, 5 cc.
B_s contains: Distilled water, 1 cc.; human saliva (1–50), 2 drops; starch, 5 cc.
C_s contains: Fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

When a few drops of mixed horse saliva are added to 50 cc. of fistula saliva, and the mixture incubated for several hours, there seems to be greater digestion than in a fresh mixture of the same amounts. In the few tests we have made with human saliva, we have failed to demonstrate this property.

All attempts to artificially activate horse fistula saliva or the gland extracts have failed. Magnesium salts failed in our experience. Calcium chloride probably does not, or if it does, its action is very slight. Various degrees of acidity and alkalinity, using various acids and bases have also failed. Letting saliva evaporate at room temperature, and using a water solution of the precipitate, failed. Injecting fistula saliva into the mouth failed to activate the fistula saliva. We could not demonstrate orokinase in human saliva, which had been slowly heated in a water bath until the ptyalin had been destroyed. The heating probably killed the orokinase as well as the ptyalin.

Inactive horse saliva very likely becomes self-active with age. Glycerine extracts (50 per cent) of the salivary glands were kept in the ice box and tests made every few days until putrefactive changes set in. We were unable to demonstrate activity except in the case of sublingual extract. This became active at forty days. There was no evidence of putrefaction at this time. Fistula saliva covered with toluol was kept at incubator temperature and no activation had occurred at sixty days.

There are a number of points concerning orokinase which remain to be worked out, and our studies are being continued. A few experiments with the pig strongly point to a similar phenomena.

II. BACTERIA OF THE MOUTH AS ACTIVATING AGENTS

Ellenberger and Hofmeister (4) apparently did not associate the glands of the mouth with the process of saliva activation, but suggested the bacterial flora of the mouth as being the activating agents. In five experiments using cultures from five different horses, we failed to demonstrate activation.

The bacteria were obtained from the oral cavity by means of a sterile swab and grown on plain agar for thirty-six hours at 37.5°C. The growth was washed off with sterile water into a sterile bottle. No attempt was made to differentiate between the various bacteria, and the entire growth was used. Various amounts of the bacterial emulsion were added to 1 cc. of parotid fistula saliva and salivary gland ex-

tracts. To this was added 5 cc. of a 1 per cent starch solution, and the mixture incubated at 40°C. Digestion was studied by noting changes in the viscosity, the color with iodine and the presence of a reducing sugar.

The results obtained were negative in the five experiments and we can conclude from this work that bacteria of the mouth do not possess the property of activating the inactive horse saliva which is emptied into the mouth. If positive results had been obtained in these studies, it was our intention to isolate the various organisms found in the mouth, and attempt to determine the relative activity of the different bacteria.

III. AMYLOLYTIC ACTION OF MIXED SALIVA

Investigators differ in their opinion regarding the presence of amylolytic enzymes in the saliva of the horse. Ellenberger (5) is of the opinion that mixed saliva has strong amylolytic properties. Mills (6) states that horse saliva has a very feeble action upon starches. R. Meade Smith (7) found that horse saliva would convert crushed raw starch to sugar in fifteen minutes, and that mixed saliva had a more marked amylolytic power than individual secretions. Fred Smith (8) states that according to his observations on the horse, saliva has no chemical action on the raw starch of its food.

We have demonstrated in seventeen horses that mixed saliva possesses amylolytic properties, and that when a good sample can be obtained this amylolytic action is about as powerful as human saliva.

Methods of collecting saliva. As a rule it is difficult to stimulate salivary secretion in the horse. A few horses are found, which are habitual slobberers and from which a considerable quantity of saliva can be obtained without much difficulty. We have been unable to find a satisfactory chemical stimulant. Pilocarpine will stimulate a profuse flow of saliva, but the drug itself will digest starch and enough of it will be excreted in the saliva to demonstrate this action. This fact was shown to be the case by Palmer (9) in his studies on ox saliva, and in repeating this work with pilocarpine, we have been able to confirm his statements. In one horse, possessing a parotid duct fistula, 0.5 grain of pilocarpine hydrochloride was administered subcutaneously. A pronounced flow of saliva occurred in about ten minutes and this saliva showed marked amylolytic properties. Fistula saliva from this horse had been previously tested many times when secretion had been stimulated by feeding oats, and was always found to be negative.

In some cases we were able to stimulate a fair amount of secretion by irrigating the mouth with dilute acetic acid, and giving inhalations of strong acetic acid. Teasing with grains or forage, or placing a bit in the mouth also assisted in some cases. In two horses saliva was being secreted, at the time of collecting the sample, and these cases gave good results.

When secretion was stimulated, it was necessary to collect the saliva by means of a spoon, as the animal would swallow as soon as a small quantity collected in the mouth. In the majority of cases only 5 to 10 cc. of saliva could be obtained, and in some animals it was very difficult to collect. In the cases in which it was very difficult to collect the saliva, the small amount of material collected seemed to be a mixture of buccal secretion and mucus, and these samples invariably gave poor results. There was a direct relation between the ease with which the saliva was collected and the amount of digestion. When it was difficult to stimulate and only a small quantity of mouth secretion was obtained, the results were either negative or very poor. This fact in our opinion very largely explains or accounts for the negative or poor results reported by some workers. We obtained negative results in a number of horses, but if we could procure these same horses at a time when the flow of saliva was easily stimulated, we invariably obtained good digestion.

The collection of the mixed secretions from the esophageal fistula was, of course, attained without any difficulties. Every five to ten minutes the horse would swallow from 5 to 20 cc. of mixed mouth secretions.

Methods of study. The usual methods of noting changes in viscosity, color with iodine and the presence of a reducing sugar were employed. The cooked starch used in our studies consisted of a 1 per cent solution of Dakomin corn starch, and the uncooked starch solution was prepared by grinding whole corn or oats in a mill, adding water, straining through cheese cloth, and using the liquid portion. The tubes containing the mixtures under observation were invariably incubated at 40°C.

Results. In eleven horses a potent secretion has been obtained from the mouth, but in several horses we have failed to demonstrate amylolytic activity in the mouth secretions. We not only failed to obtain positive results in some horses, but even the eleven positive cases gave varying results. The degree of activity was directly proportional to the ease with which we obtained our sample. The following table gives a summary of the relative activity in the eleven positive cases.

TABLE 4

Summary of eleven positive cases showing when changes became pronounced

	CLEARING IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120
Number of positive cases.....	3	4	2	1	1	2	—	4	2	3

Table 5 shows in detail one experiment demonstrating the amylolytic activity of mixed horse saliva, obtained from the mouth. The saliva in this case was obtained from an aged grey mare. The secretion was obtained without much difficulty, and was stimulated by teasing with oats. Digestion was not as strong as that obtained in some horses, neither was it as weak, and the results in this case are suggestive of what can normally be expected. It will be noticed that clearing was not recorded until fifteen minutes, and that at this time slight reduction had occurred. Digestion was not complete in this case at two hours, as indicated by the blue color with iodine. The amount of digestion in this case was similar to that reported by Meade Smith.

With the exception of one horse, the mixed secretions obtained from esophagus were very powerful. The particular animal which gave the digestion below par was a large grey mare, with a parotid fistula on the right side. The secretion swallowed by this mare differed from that in any of the other horses, and was of a consistency similar to that of egg white. This secretion possessed weak amylolytic action, but had strong activating properties when mixed with the inactive fistula saliva. This secretion was evidently largely composed of buccal and lingual secretions. Masticated corn or oats obtained from the esophageal fistula in this mare, however, contained about as much sugar as in the other cases studied.

In five horses the secretions collected from the esophageal fistulae were of a uniform potency, and were somewhat more viscid than the parotid fistula saliva. Even in the horses with parotid duct fistulae, (on one side) the secretions collected from the esophagus were not like those found in the above grey mare, but were similar to those obtained from the horses possessing only esophageal fistulae. In these five horses the mixed secretions possessed very powerful amylolytic action. The starch solution became clear in one to five minutes, the blue color with iodine disappeared within this time, and at these intervals the reduction was very heavy. Table 6 shows in detail one experiment demonstrating the powerful amylolytic activity of mixed saliva, collected from an esophageal fistula.

TABLE 5

Details of one experiment comparing amylolytic action of mixed horse saliva obtained from the mouth, with that of human saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Slight	Slight	Clearer	Clearer	Clear	Blue	Blue	Blue	Blue	Blue	Very slight	Slight	Fair	Good	Much
B_s	Clear	Clear	Clear	Clear	Clear	No color	No color	No color	No color	No color	Heavy	Heavy	Heavy	Heavy	Heavy
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Mixed horse saliva, 1 cc.; starch, 5 cc.
B_s contains: Mixed human saliva, 1 cc.; starch, 5 cc.
C_s contains: Horse parotid fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

TABLE 6

Details of one experiment comparing amylolytic action of mixed horse saliva obtained from esophageal fistula with that of human saliva

NO. OF TUBE	CLEARING IN MINUTES				COLOR WITH IODINE IN MINUTES				REDUCTION IN MINUTES			
	3	5	15	30	3	5	15	30	3	5	15	30
A_s	Nearly clear	Clear	Clear	Clear	Blue	Faintly blue	No color	No color	Heavy	Heavy	Heavy	Heavy
B_s	Clear	Clear	Clear	Clear	faintly blue	No color	No color	No color	Heavy	Heavy	Heavy	Heavy
C				No clearing				Blue				No reduction

A_s contains: Horse saliva from esophageal fistula, 1 cc.; starch, 5 cc.
B_s contains: Human saliva, 1 cc.; starch, 5 cc.
C contains: Distilled water, 1 cc.; starch, 5 cc.

In a number of experiments we have studied the amylolytic activity of mixed saliva, obtained from esophageal fistulae, compared to that of human saliva on cooked and uncooked starch. When acting upon cooked starch, the two salivas are of about equal activity. Upon raw corn or oats starch prepared by grinding these grains, adding warm water and straining through cheese cloth, the horse saliva is more powerful than the human. In fact horse saliva seems to attack the raw grains as readily as the cooked. Human saliva would also digest these raw grains to a very marked degree.

IV. AMOUNT OF COMPLETE STARCH DIGESTION IN THE MOUTH

Ellenberger (10) has demonstrated that horse saliva can digest raw starch, by showing the presence of a reducing sugar in the food contents escaping from an esophageal fistula, when the diet is composed of sugar-free starches. He reports that on a diet of corn, the food caught from an esophageal fistula one to three minutes after feeding shows the presence of much reducing sugar.

R. Meade Smith (11) on the other hand states that examination of the substances escaping from an esophageal fistula in the horse fed on starchy food, shows that practically no conversion of starch into sugar occurs in the mouth. Smith, however, does believe that horse saliva possesses amylolytic properties, but that it requires at least fifteen

minutes action before digestion can be demonstrated. For this reason, he argues that it is not to be expected that food coming from an esophageal fistula would contain a reducing sugar. Smith is also of the opinion that the digestion started in the mouth is continued in the stomach, and that this digestion is of considerable importance.

In experiments upon six horses we have been able to confirm Ellenberger's work, and have been able to show that on a diet composed of raw corn or oats, the food escaping from the esophageal fistula shows heavy reduction with Fehling's solution, whereas the grain itself or the horse saliva possesses no reducing properties. The first horse, an aged bay gelding, was fed a handful of corn, which was thoroughly masticated and swallowed within three minutes. The corn was caught from the esophagus into a clean beaker, distilled water added, the mixture strained through cheese cloth, and the liquid portion tested at once with Fehling's solution; following which heavy reduction appeared. A handful of corn from the same ear was ground in a hand mill, water added, the mixture strained through cheese cloth, and the liquid portion tested with Fehling's solution; following which no reduction occurred. Mixed mouth secretions escaping from the fistula were also tested, and they gave negative results. Similar tests were made with oats and wheat. The oats showed heavy reduction, but the wheat very little. The wheat also caused the animal to become choked, causing considerable inconvenience, and we have not repeated this experiment.

In five horses we have attempted to make a quantitative determination of the amount of sugar present in the swallowed food, and thus determine the amount of complete starch digestion in the mouth. The starch in the grains was converted into sugar by the official government method (12), and the percentage of sugar determined by Benedict's method. This gave us the total possible amount of sugar. One hundred grams of whole raw corn or oats was then fed, and caught from the fistula into a suitable vessel. Dilute HCl was added and thoroughly mixed with the food to stop further enzyme action. The mixture was then strained through cheese cloth or run through a force filter and the percentage of sugar determined. A determination was made later, and if the results checked with the first, we knew the enzyme had been destroyed.

The amount of complete starch conversion which had taken place in the mouth was not as great as we had anticipated, and our methods were subject to considerable error. We are inclined to think, however,

that our percentages are too low rather than too high. A point worthy of mention is the fact that our horses were all old animals, and they did not masticate the grain as well as young horses and considerable grain was retained in the mouth, which was difficult to remove or estimate the amount. Old horses with poor teeth usually allow some food to remain in the cheeks, and to guard against this, the animals were watered before and after each feed, but this would not remove all of the grain.

Experiments upon these five horses show that approximately 0.5 to 1 per cent of starch in the corn, and 1 to 2 per cent of the oats starch was completely digested in the mouth. Table 7 shows the results of this work.

TABLE 7

Amount of complete starch digestion in the mouth

ANIMAL	TOTAL POSSIBLE PERCENT		PERCENTAGE OF COMPLETE DIGESTION IN MOUTH			REMARKS
	Corn	Oats	Corn	Oats	Wheat	
Bay Gelding..	59.89	49.26	Not estimated, heavy reduction	Not estimated, heavy reduction	Very slight reduction	Good mastication
Black Gelding	59.89	49.26	0.41	1.2	Not estimated	Poor mastication
Bronco Pony	59.89	49.26	0.56	2.7	Not estimated	Poor mastication
Grey Mare....	59.89	49.26	0.58	1.1	Not estimated	Poor mastication
Roan Gelding	59.89	49.26	1.04	1.3	Not estimated	Fair mastication
Sorrel Mare...	59.89	49.26	0.47	0.65	Not estimated	Very poor mastication

In three horses a number of additional tests were made whereby we did not add the acid to the food escaping from the fistula, but allowed the digestion to continue for several hours at incubator temperature. When tested, it was found that on an average 9.7, 13.4 and 6.38 per cent of the corn starch, and 8.4, 12 and 4 per cent of the oats starch had been digested in the three horses respectively. (The third animal had very poor teeth and mastication was very imperfect.)

It is known that corn contains 55 to 60 per cent and oats about 50 per cent of digestible carbohydrates, and our estimations checked well

with these, and of this amount the above amounts were digested after several hours. But here again these figures may not be truly representative of the amounts digested, because it is a well established fact that the products of enzyme activity will stop the action of the enzyme, and this was certainly the case in our experiments. It is also possible that salivary amylase will attack only certain of the carbohydrates in the grains.

SUMMARY

1. Saliva obtained from the parotid ducts or extracts of the salivary glands will not digest starch.

2. It is difficult to stimulate secretion and collect mixed saliva from the mouth of the horse.

3. Saliva collected from the mouth is seldom, if ever, as powerful as that obtained from an esophageal fistula.

4. Mixed mouth secretions obtained from an esophageal fistula have a very powerful amylolytic action.

5. The amylolytic action of mixed horse saliva is equal to that of human saliva on cooked starches, and greater than that of human saliva when acting on raw starches.

6. Mixed horse saliva attacks raw starch as readily as cooked starch.

7. The inactive saliva secreted by the salivary glands is activated in the mouth by the secretions of the glands in the mouth.

8. The name orokinase has been proposed for the activating enzyme found in the mouth secretions.

9. Orokinase can be demonstrated in the mixed mouth secretions of the man and horse.

10. Attempts to artificially activate fistula saliva or gland extracts have failed, but the gland extracts become self-active with age.

11. On a diet of raw corn and oats, food caught from an esophageal fistula a few minutes after feeding, shows the presence of considerable reducing sugar.

12. Salivary digestion started in the mouth is very likely continued in the stomach, and this digestion is more important in the horse than most investigators have been lead to believe.

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AN APPLICATION OF BOYLE'S LAW TO PULSE WAVES IN CLINICAL MEASUREMENT OF BLOOD PRESSURE

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In a recent paper by Erlanger (1) dealing with some conditions occurring in the estimation of blood pressure by the indirect or armlet method, some conclusions are reached which seem incorrect in that they are reached apparently without due regard for the physical laws which are involved. An experimental analysis of the statements made by him has led me to quite opposite conclusions which are presented in the present paper.

The conditions underlying the first deduction which Erlanger made and stated are: An inextensible artery which is inelastic but is easily collapsible and is subjected to diastolic and systolic pressure within, and also subject to pressure without, the artery being surrounded by and placed in a so-called "compression chamber" which chamber is filled with an incompressible fluid which is connected with a manometer whose indicator is moved by a minimum translocation of fluid in the chamber. The pressure outside the artery and in the chamber can be applied to the artery at any desired phase or level; for example, when the inside pressure is at diastolic, or at systolic, or at any level between these two (2).

It is noted here that Erlanger first postulates a chamber with an incompressible fluid and at the same time connected with a manometer which permits movement of the fluid. These two conditions are not in agreement.

Erlanger's deductions are: If a pressure now equal to the diastolic be applied during the diastolic phase in the artery, no oscillations will be produced in the manometer during the pulsations of inside arterial pressure. For, he argues, if the inside pressure rises above the diastolic, the vessel is already completely filled, and being inextensible, cannot expand further and therefore cannot transmit the increase of inside or arterial pressure when it rises above the diastolic level. But

I wish here to point out that if the pressure in the chamber is at the diastolic level and the pressure within the artery is also just at the diastolic level, then it does not at all follow that the artery must necessarily be filled with fluid. Since the artery is readily collapsible (though not elastic) it may be only partly filled, or it may be entirely flat and empty. It may be in any degree of fullness or emptiness. But one must know the amount of fluid within the artery before he can tell whether a rise in arterial pressure will be transmitted to the chamber. As a matter of fact, not unless the artery is completely filled with fluid at the diastolic pressure and the chamber pressure just equal to it is applied without allowing the artery to collapse the slightest amount, can the result obtained by Erlanger be possible.

I have devised an improved apparatus giving more nearly the postulated conditions and also embodying the features outlined by Erlanger. The apparatus is shown in figure 1. My modification eliminates the tambour used by Erlanger, which is a desirable change since the use of the tambour introduces an elastic membrane into the system which is contrary to the desired theoretical condition. In figure 1, *A* is an inelastic artery; *B*, a rigid chamber surrounding the artery filled with water; *C* is a minute capillary tubular air space to show pressure conditions in the chamber. (It is true that theoretically, as with Erlanger's tambour, I have introduced the objectionable elastic substance into the system; but the advantage of the capillary tube of air is that its volume is so minute that the translocation of fluid is practically negligible.) *D* is a valve through which the desired amount of air can be forced into the small capillary tube.

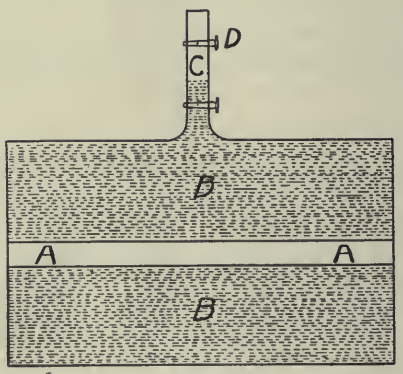

Figure 1

Another statement made by Erlanger in this same paper (3) deals with the same inelastic but collapsible artery within the same compression chamber but filled with an elastic medium, namely air. His conclusion is, in effect, that with the conditions just stated and with a given pulsatory volume change of the artery, the transmitted oscillations are proportional to the initial chamber pressure. For example he gives in figure 2, (4) a diagram which would indicate that if a pres-

sure of say 100 mm. were in the air-filled chamber, and then the artery was expanded say 1 cc. of volume, the pulsatory wave transmitted to the tambour (or other manometer) would show an oscillation of say X mm. Now repeat the observation with the chamber pressure doubled (or raised to 200 mm.) but with the same volume of expansion of the artery which is 1 cc. According to Erlanger there would now be double the size of oscillations recorded by the chamber tambour or 2 X mm.

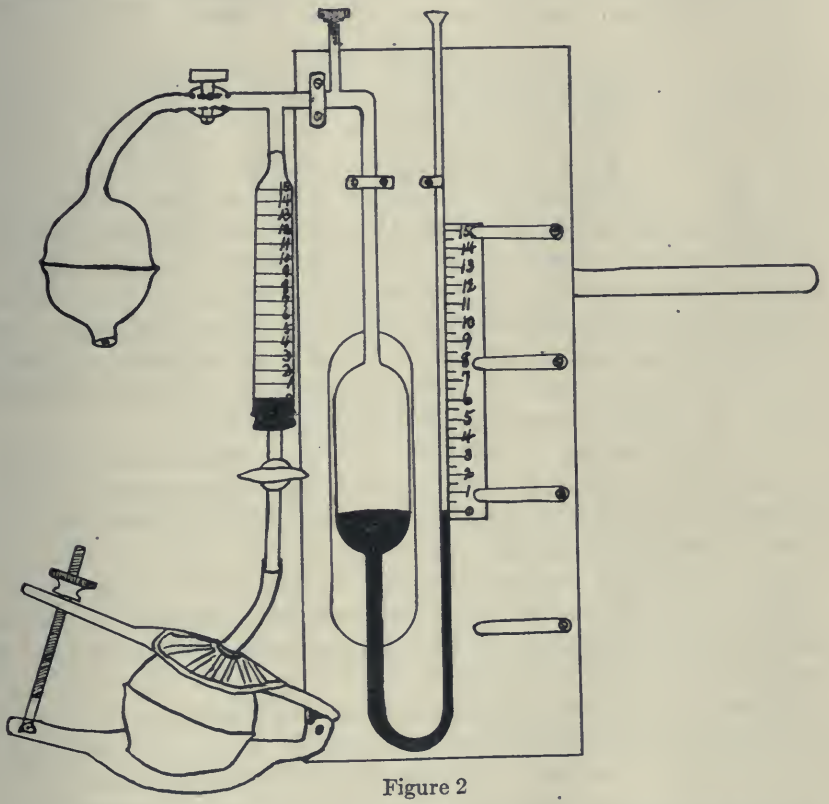

Figure 2

So Erlanger in his diagram shows the transmitted oscillations growing in size as the chamber pressure is increased; beginning at slightly above the diastolic they increase until they are almost doubled when the chamber pressure has reached a point just below systolic level.

But as a matter of fact they do not behave in this way at all. *The fallacy in Erlanger's hypothesis lies in the fact that he regarded only the manometric pressure of the chamber, whereas he should have regarded the*

absolute pressure. The absolute pressure is of course the manometric pressure plus the barometric pressure. In other words the oscillations obtained by varying pressures are in the ratio of $\frac{P}{P'}$, where P is the absolute pressure (or the sum of the manometric and the barometric pressures) and P' is the absolute pressure caused by the compression. $P' - P =$ the oscillations. Boyle's law is $PV = K$, where P is pressure, V the volume, and K a constant, $\frac{K}{V'} = P'$ where V' is the volume produced by adding an incompressible fluid to the air chamber, and P' is the new pressure produced by this addition. A concrete case may be taken. With the air chamber set at 100 cc. capacity, 1 cc. of fluid was forced in by compressing the air to 99 cc. volume. Beginning with a barometric pressure of 747 mm. and zero manometric pressure, the theoretical rise caused by the above experiment is 7.54 mm. of mercury. Beginning with the manometric pressure in the chamber of 50 mm. which is a total or absolute pressure of 797 mm., the oscillation expected as shown by theoretical calculation is 8.05 mm. Beginning with a chamber pressure of 100 mm. the oscillation expected theoretically is 8.55 mm., etc. The theoretical ratio of the oscillation here to the absolute pressure at the beginning is 0.0101 : 1.

The ratio of the size of oscillation at 50 mm. beginning pressure, as compared with the size of oscillations at a beginning pressure of twice that amount, or 100 mm., is 8.05 : 8.55 or 1 : 1.06 plus (instead of 1 : 2 as per Erlanger hypothesis).

The ratio of the size of the oscillation at 0 mm. beginning pressure, as compared with the size of oscillations at a beginning pressure of 100 mm. was 7.54 : 8.55 or 1 : 1.13, instead of 1 : *infinity* which is absurd! (as demanded by Erlanger's hypothesis).

The apparatus shown in figure 2 was used to make these experiments. It consists of a cylinder holding 100 cc. connected on one side with a manometer and provided on the other side with a bulb for forcing in the desired amount of fluid (1 cc. or more). A compression bulb gave the desired initial pressures when these were above the atmospheric or barometric pressure.

The table gives the data; first as obtained from theoretical calculation, second from actual readings as obtained from one of a large series of experiments.

The accord of the two is as close as we expected. Volume of air used, 100 cc. Volume of displacement by compression, 1 cc. Barometric pressure on the day of the experiment, 747 mm.

BAROMETRIC PRESSURE		BEGINNING MANOMETRIC PRESSURE		ABSOLUTE PRESSURE	THEORETICAL OSCILLATIONS CALCULATED	EXPERIMENTAL OSCILLATIONS FOUND	RATIO	
							Theoretical	Experimental
mm.		*mm.*		*mm.*	*mm.*	*mm.*		
747	+	0	=	747	7.54	7.5	1.01	1.004
747	+	50	=	797	8.05	8.0	1.01	1.004
747	+	100	=	847	8.55	9.0	1.01	1.062
747	+	150	=	897	9.06	9.0	1.01	1.062

$$PV = K \quad P'V' = K$$

$$\frac{K}{V'} = P' \quad P' - P = \text{size of oscillation.}$$

$$\frac{\text{Absolute pressure P}}{\text{Absolute pressure P}'} = \text{Ratio of oscillation}$$

It is generally known that the volume of the compression chamber determines the relation between the magnitude of oscillations and arterial pressure. It may be remembered here that this relation is a simple numerical one, viz., a chamber of twice the volume will give an oscillation of one-half the magnitude with a given pulsatory arterial volume change.

CONCLUSIONS

A simple apparatus is described for helping to show the workings of certain physical laws regarding the indirect transmission of arterial pulse waves such as are used in clinical measurement of blood pressure.

Erlanger states that with an inelastic but collapsible arterial segment surrounded with a rigid chamber and the chamber filled with incompressible fluid (water) when the arterial (inside) pressure is at diastolic, and the chamber (outside) pressure is closed at exactly the diastolic level, no pressure waves can be transmitted to the chamber from the artery. I find that this is not true except where the artery at the closing of the chamber is fully distended with fluid, for when the arterial segment is only partially filled, the pressure waves of the artery are wholly transmitted to the chamber (excepting only when the volume occupied by the translocation of fluid in the filling of the artery is less than the compression volume of the tambour, manometer or capillary tube caused by the pulsatory change of arterial pressure).

Erlanger also states that the oscillations of pressure in the air filled arm band or air filled compression chamber, with a given volume pulse change would be proportional to the manometric pressure of the arm

band. This does not hold. On the contrary I find, upon testing this hypothesis with the help of the physical apparatus described above, that the oscillations of volume occupied by a given mass of gas produces inversely proportional oscillations of *absolute pressure.* Or in other words, *the absolute pressure of a given mass of gas is inversely proportional to its volume.* In short, it is another way of stating Boyle's law that the absolute pressure of a gas is proportional to the concentration of the gas.

Therefore the results of the present work are in harmony with Boyle's law, but are contrary to Erlanger's hypothesis.

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THE INFLUENCE OF THYROID FEEDING UPON CARBOHYDRATE METABOLISM

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The relation of the thyroid gland to carbohydrate metabolism has been discussed from various view points. In Basedow's disease spontaneous glycosuria or alimentary glycosuria has been frequently recorded. Flesch (1) found alimentary hyperglycemia in 61 per cent of his cases of Basedow's disease and also was able to cause alimentary hyperglycemia by thyroid feeding or transplantation of the thyroid gland. Schulze (2) reported that alimentary glycosuria is not so frequent in Basdeow's disease (25 per cent), but a very small amount of epinephrine, which was insufficient to cause glycosuria in the normal condition, called forth marked glycosuria in 80 per cent of the cases of Basedow's disease examined. The tolerance for carbohydrate is considered to be increased in myxoedema. In cases of myxoedema and obesity, glycosuria was found after the use of thyroid preparations.

Hirsch (3) and Underhill and Saiki (4) demonstrated a decrease in the utilization of dextrose given by mouth or subcutaneously introduced in dogs after thyreoparathyroidectomy. Hirsch (5) showed later that although thyreoparathyroidectomy leads to a lowering of the tolerance for carbohydrate, thyroidectomy with undisturbed parathyroids produces no such effect. Demonstrating that thyroidectomy with preservation of the parathyroids increases the tolerance for sugar and inhibits epinephrine glycosuria, Eppinger, Falta and Rudinger (6), (7) came to the conclusion that the influence of parathyroidectomy upon carbohydrate metabolism is just contrary to that of thyroidectomy. As to the influence of thyroidectomy with preservation of the parathyroid upon epinephrine glycosuria, Underhill and Hilditch (8) could not confirm the results of Eppinger and his coworkers. Performing the same operation, Pick and Pineless (9) proved its inhibitory influence upon epinephrine glycosuria in young goats, but this was not the case in rabbits. McCurdy (10) observed an increased tolerance for dextrose injected intravenously in dogs from which the thyroid together with two parathyroids had been removed. In thyreoparathyroidectomized cats, Miura (11) demonstrated that this operation does not show any influence upon alimentary galactosuria, but it decreases epinephrine glycosuria. From the studies of

Eppinger, Falta and many others, it was shown that thyroid, chromaffin tissue and hypophysis act in an accelerative manner upon metabolism whereas pancreas and parathyroid behave in the opposite way. The relation of various ductless glands must, therefore, be considered in discussing the influence of the thyroid gland upon carbohydrate metabolism.

When thyroid preparations are introduced into animals many pathological symptoms are called forth. Glycosuria is sometimes seen. Carlson and his coworkers (12) found that desiccated thyroid given by mouth is toxic for many animals and different genera exhibit great variation in their susceptibility, dogs, cats, foxes and ducks being the most resistant of all animals fed, rodents and primates the least. The most constant symptoms were emaciation and diarrhoea with death in convulsions or depression. French (13) also confirmed the toxicity of thyroid for rabbits, rats and guinea pigs, control experiments with other organs or tissues showing no toxic symptoms. When fresh thyroid was given by mouth 20 to 50 grams per day, the rabbits died in two to ten days. Giving fresh thyroid to rats and cats by mouth, Cramer and Krause (14) found that the glycogen content of the liver decreases to a minimum. In dog experiments they showed also that thyroid feeding causes a slight but distinct lowering of the tolerance for glucose. They concluded that thyroid, when administered to normal animals, acts specifically on only one aspect of carbohydrate metabolism; it inhibits the formation and storage of glycogen in the liver. Finding a change of the curve of the respiratory quotient in experimental hyperthyroidism, Cramer and McCall (15) concluded that thyroid hormone discharges and oxidizes the liver glycogen and this explains the increased oxidation of carbohydrate, protein and fat, produced by thyroid feeding.

Mobilization of liver glycogen can be induced by many factors, namely excessive muscle work, fasting, epinephrine injection, pancreas extirpation, piqûre, irritation of splanchnic nerves, intoxication with narcotics or phosphorus, etc. Though it is a well known fact that epinephrine injection discharges liver glycogen, Schwarz (16) made an interesting contribution which shows that extirpation of both adrenal glands also induces the total loss of liver glycogen in rats. On the other hand, Pollak (17) proved that rabbits, made glycogen-free by fasting and strychnine, show an increased glycogen content of the liver, in a quantity only equaled by carbohydrate-fed animals, after injections of small increasing doses of epinephrine. Kahn and Starkenstein (18) confirmed Schwarz's results in rat experiments, but in rabbit experiments they could not demonstrate any such influence. Investigating the relation between the thyroid and adrenal glands, Cramer (19) considers that the thyroid hormone stimulates the secretion of epinephrine and this produces a discharge of glycogen from the liver.

From the brief references mentioned above it will be seen that the problem of the influence of the thyroid gland upon carbohydrate metabolism is very complicated. At the suggestion of Prof. Frank P. Underhill, I have investigated a few points concerning this problem, namely the influence of thyroid feeding upon the glycogen content of the liver, blood sugar content, assimilation limit for dextrose and also upon epinephrine hyperglycemia and glycosuria.

METHODS

Rat experiments. Full-grown white rats were fed on paste, prepared according to Osborne and Mendel with 70 per cent of rat biscuits and 30 per cent of lard. Fresh thyroid glands of pigs obtained from the slaughter house or desiccated thyroid (Parke, Davis and Company) was added to the diet. In case of *fresh* thyroid glands, the amount for each animal per day was measured at the beginning of the experiment and kept in a freezing room. For control animals lamb meat was prepared in the same manner. Though the influence of the parathyroidectomy upon carbohydrate metabolism has become clear, the result of feeding this organ is yet unknown. The thyroid preparations used in these experiments may have contained a very small amount of parathyroid. The rat eats thyroid very willingly. When *desiccated* thyroid was used, it was mixed with the paste in the proportion of 1:9. For control experiments comminuted, desiccated, coagulated egg was mixed with the paste.

Before the real experimental period, all the animals were fed on paste regularly for at least three days. The urine was collected, following the method suggested by Osborne and Mendel (20). The rat does not eat much in the day time, and it is very difficult to compel it to eat a certain amount of food in a desired time. In my present work, thyroid or meat was given at 6 p.m. After the animal ate this food, the paste was given and left during the night. The next morning, the rest of the paste was taken away and no food was given in the day time. Water was given freely. On the last day, thyroid or meat was allowed as usual, but the paste was put in the cage at 10 p.m. At 9 the next morning the animal was killed. When thyroid was given the appetite of the animal became poor. The amount of food for each control animal was regulated so that it was about equal to that of the thyroid-fed animal.

Glycogen was determined by Pflüger's method (21), (22). The glycogen was hydrolyzed and the reducing sugar obtained was estimated by Allihn's gravimetric method.

When the animal was decapitated, the blood was collected in a glass containing potassium oxalate. The sugar content of the blood was determined by the Lewis-Benedict method (23).

The epinephrine content of the adrenal gland was determined by a color method with mercuric acetate. Comparing various color methods,

Borberg (24) proved that they can be used for the approximate determination of the epinephrine content of the adrenal gland itself. Underhill and Fine (25) and Comessatti (26) obtained a satisfactory result by the color produced by mercuric chloride. The procedure used in the present work was as follows: Both adrenals were ground in a mortar with 0.2 gram of sand and 5 cc. of physiological saline solution. The mixture was kept in a stoppered tube in a cold room for three hours. After centrifuging, 2 cc. of the upper clear part were removed to a small test tube. One cubic centimeter of saturated mercuric acetate solution was added. Protein was precipitated immediately and a pink color developed in the upper clear portion. For comparing the depth of the color, adrenalin chloride (1: 1000, Parke, Davis and Company) was diluted in the proportion of 1: 25, 1: 30 and 1: 35 with distilled water, and mixed with mercuric acetate solution in the same manner. The color obtained from the adrenal glands of normal rats was nearly as deep as that of the 1: 30 dilution.

Rabbit experiments. Full-grown rabbits were fed on oats and carrots. Water was given freely. Before the experimental period, all the animals took 75 grams of carrots and 50 grams of oats per day for at least two days. For producing hyperthyroidism, fresh thyroids of pigs were used, calf's liver being used for control experiments. The two latter foods were cut into small pieces and fed to the animals by hand.

The urine was collected by compression of the bladder through the abdominal wall. The blood sample was taken from an ear vein and its sugar content was determined by the Lewis-Benedict method. The blood sugar content in the morning of the first experimental day shows the normal value, the blood sample having been taken before the first thyroid feeding. The sugar in the urine was estimated polarimetrically after removing the coloring matters and levorotatory substances with saturated mercuric acetate solution. Total nitrogen of the urine was determined by Kjeldahl's method.

THE INFLUENCE OF THYROID FEEDING UPON THE GLYCOGEN CONTENT OF THE LIVER

In Cramer and Krause's experiments (14), a glycogen content of the liver of the thyroid-fed rats was barely discernible, the amount in the control animals being on the average about 2 per cent. The amount of thyroid (sheep's) fed was described as one lobe, one-half lobe, etc.,

per day. The duration of feeding was two to eight days. Even a single administration of one lobe of thyroid seemed to be enough to diminish liver glycogen. In my experiments the amount of thyroid and other food was determined exactly. The animals seemed a little weaker after two or three days of thyroid feeding, the appetite usually being affected. Diarrhoea, which Carlson (12) and Gudernatch (27) described as a remarkable symptom after thyroid feeding, was not observed with my experimental conditions. Glycosuria was never found. In autopsy, no marked macroscopical changes were seen, excepting in one case only (Rat III in table 1) where the alimentary tract showed a slight hyperemia. In all cases the stomach and small intestine contained chyle, showing that they were in a stage of active digestion. Judging from the color reaction, the epinephrine content of the adrenal gland of the thyroid-fed rats did not differ from that of the control animals. Three rats, not recorded in the tables, took no food at the end of the thyroid period and they died in one or two days, although thyroid was omitted from the diet. The results are detailed in tables 1 and 2.

From tables 1 and 2 it will be seen that feeding fresh thyroid decreased the glycogen content of the liver markedly. This confirms the results of Cramer and Krause. The thyroid-fed animals always lost in body weight. Although in some control experiments (Rats III, IV and V in table 2) the amount of food was regulated so that it was not enough to maintain their body weight, the glycogen content of the liver remained normal. The sugar content of the blood was normal at the end of the period.

Desiccated thyroid was also fed. Neither glycosuria nor diarrhoea was present. The results are detailed in table 3.

In these cases, the regulation of the amount of food was not so strictly observed. But a decrease of glycogen content of the liver caused by thyroid feeding can be clearly seen. Autopsy showed no noteworthy macroscopical changes. Chyle was found both in the stomach and small intestine in all cases.

In a discussion of the glycogen content of the liver the extent of digestion and absorption of the food also must be taken into consideration. The decrease of the glycogen of the liver of the thyroid-fed rats can hardly be explained by the insufficient digestion and absorption of the food, because (1) no noteworthy change in the appearance of the alimentary tract could be observed; (2) the feces were

TABLE 1

The influence of fresh thyroid feeding upon the glycogen content of the liver and the sugar content of the blood

RAT	I		II		III		IV		V		VI	
Number												
Body weight, grams	154.6		196.6		220.1		154.7		198.0		184.6	
Food	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day	5.0	8.0	5.0	8.0	5.0	8.0	5.0	8.0	3.0	9.0	3.0	9.0
Second day	5.0	8.0	5.0	8.0	5.0	8.0	5.0	8.8	3.0	6.0	3.0	5.1
Third day	5.0	5.2	5.0	9.0	5.0	9.0	5.0	9.0	3.0	6.0	3.0	4.1
Fourth day	5.0	9.0	5.0	9.0	5.0	9.0	5.0	8.2				
Fifth day	5.0	6.6	5.0	8.2	5.0	9.0						
Loss of body weight at end of period, gram	−3.2		−8.6		−11.3		−7.2		−10.8		−13.3	
Blood sugar content, per cent	0.11		0.12		0.11		0.11		0.12		0.13	
Weight of liver, gram	6.1		8.3		7.6		5.3		5.3		6.8	
Glycogen content of liver: Milligram	7.4		13.6		22.9		trace		31.6		64.5	
Glycogen content of liver: Per cent	0.12		0.16		0.29				0.60		0.95	

TABLE 2

Control experiments. The glycogen content of the liver and the sugar content of the blood after meat feeding

RAT	I		II		III		IV		V	
Number										
Body weight, gram	153.7		213.0		182.2		194.2		145.1	
Food	Meat	Paste	Meat	Paste	Meat	Paste	Meat	Paste	Meat	Paste
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day	5.0	8.0	5.0	8.0	3.0	7.0	3.0	7.0	0	7.0
Second day	5.0	8.0	5.0	8.0	3.0	7.0	3.0	7.0	0	5.0
Third day	5.0	8.0	5.0	8.0	3.0	3.0	3.0	5.0	0	4.0
Fourth day	5.0	8.0	5.0	8.0						
Fifth day	5.0	8.0	5.0	8.0						
Change of body weight at end of period, gram	+15.5		+0.3		−11.1		−10.5		−3.7	
Blood sugar content, per cent	0.13				0.10		0.09		0.11	
Weight of liver, gram	6.5		8.6		6.0		6.5		6.3	
Glycogen content of liver: Milligram	322.7		344.3		165.8		247.9		218.5	
Glycogen content of liver: Per cent	4.97		4.00		2.76		3.81		3.47	

TABLE 3

The influence of desiccated thyroid feeding upon the glycogen content of the liver

RAT		I	II	III	IV	V*	VI*
	Number						
	Body Weight, gm.	222.3	247.6	310.1	187.3	237.5	211.1
Food		Thyroid + Paste	Thyroid + Paste	Thyroid + Paste	Thyroid + Paste	Egg + Paste	Egg + Paste
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
First day		7.1	11.3	12.6	11.5	14.7	9.3
Second day		9.8	17.6	7.8	11.6	11.8	13.5
Third day		5.3	13.5	6.6	4.7	9.0	12.7
Fourth day		6.6	6.4	9.9	6.4	9.0	9.0
Change of body weight at end of period, gram		−17.5	−24.0	−22.6	−19.8	+0.8	+14.4
Weight of liver, gram		7.8	8.3	7.7	8.9	7.4	7.5
Glycogen content of liver	Milligram	40.7	trace	trace	trace	148.7	290.2
	Per cent	0.52				2.01	3.07

* Control experiments.

normal both in amount and character; (3) there was also poor glycogen formation in the liver when dextrose was parenterally administered. The latter point will be discussed in a later section of this paper.

THE GLYCOGEN CONTENT OF THE LIVER OF THYROID-FED RATS AFTER OMITTING THYROID FROM THE DIET

Kojima (28) found that after thyroid feeding pronounced histological changes are produced in the pancreas of rats. The question, whether the decrease of glycogen content of the liver bears some relation to the changes in the pancreas or is due to hypersecretion of the adrenal glands or to other factors, is yet to be decided. But it is interesting to know whether the changes, wherever they may be, resulting from thyroid feeding and causing the decrease of glycogen content of the liver, can or cannot be repaired very easily by omitting thyroid from the diet.

Four rats were fed first on fresh thyroid and the paste for a few days, and then on thyroid-free diet. Two, three or thirteen days after omitting thyroid from the diet, the glycogen content of the liver was determined. The results are detailed in table 4.

TABLE 4

The glycogen content of the liver of thyroid-fed rats after omitting thyroid from the diet

RAT		I			II			III			IV		
	Number	I			II			III			IV		
	Body weight, grams	251.5			141.3			149.1			155.4		
Food			Thyroid	Paste		Thyroid	Paste		Thyroid	Paste		Thyroid	Paste
		day	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*
First period with thyroid		1	3.0	9.0	1	3.0	9.0	1	3.0	6.0	1	5.0	9.0
		2	3.0	8.2	2	3.0	5.2	2	3.0	6.1	2	5.0	6.6
		3	3.0	9.0	3	3.0	4.5	3	3.0	6.5	3	5.0	3.7
											4	5.0	5.0
											For 13 days biscuit was given freely		
			Meat	Paste		Meat	Paste		Meat	Paste		Meat	Paste
		day	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*	*day*	*gm.*	*gm.*
Second period without thyroid		1	3.0	8.0	1	3.0	5.0	1	0	6.0	14	3.0	7.0
		2	3.0	8.0	2	3.0	5.0	2	3.0	4.8	15	0.7	6.0
								3	3.0	5.4			
Change of body weight at end of second period, gram		−17.7			−8.0			−12.5			+1.3		
Weight of liver, gram		7.7			5.8			5.0			6.5		
Glycogen content of liver	Milligram	180.9			209.1			187.4			302.3		
	Per cent	2.35			3.61			3.75			4.65		

From table 4, it will be seen that glycogen is very easily stored in the liver after omitting thyroid from the diet, notwithstanding that the loss of body weight still remains. It is scarcely probable, therefore, that the changes causing the loss of liver glycogen are of a serious morphological nature.

THE FORMATION OF LIVER GLYCOGEN BY PARENTERAL ADMINISTRATION OF DEXTROSE IN THYROID-FED RATS

Grube (29) and many other investigators found that perfusion of the liver with blood or physiological saline solution containing excessive dextrose (1 per cent or more) can increase the glycogen content of the

liver in a few hours. Gumprecht (30) demonstrated that subcutaneous injection of dextrose into fasted rabbits can cause glycogen storage in the liver as high as 3.9 per cent. He injected 20 grams of dextrose in eight to nine hours. Before going on with the experiments on thyroid-fed rats, Gumprecht's experiments were repeated with fasted rats. After a certain period of fasting a 10 per cent dextrose solution, sterilized by boiling, was injected subcutaneously and intraperitoneally into the animals. The subcutaneous injection (7.5 to 15.0 cc.) was performed three hours before and the intraperitoneal injection (5.0 to 7.5 cc.) two hours before decapitation. The glycogen content of the liver was increased distinctly by this procedure. On the average, the liver of the fasted rats into which dextrose was injected contained 1.28 per cent glycogen, the liver of the control animals showing only 0.31 per cent glycogen.

Fresh pig thyroid was given to four rats for three days and then dextrose was injected as described for fasted rats. The average of glycogen content of the liver was 0.7 per cent. The same experimental conditions of pure thyroid feeding was performed in Rats V and VI in table 1. The average of glycogen content of the liver of these last mentioned rats was 0.77 per cent. Storage of liver glycogen seems, therefore, to be affected by thyroid feeding. The details can be seen in tables 5 and 6.

TABLE 5

The formation of liver glycogen by parenteral administration of dextrose in fasted rats

RAT		I	II	III	IV	V	VI
	No.						
	Body weight, grams	152.2	166.8	196.4	100.8	149.3	175.7
Duration of fasting, hours		48	57	72	48	57	72
Loss of body weight, gram		−19.2	−17.7	−19.0	−9.5	−14.4	−27.9
Dextrose injection		none	none	none	0.75 gm. subcutan. 0.5 gm. intraperit.	1.5 gm. subcutan. 0.75 gm. intraperit.	1.5 gm. subcutan. 0.75 gm. intraperit.
Weight of liver, gram		3.8	4.2	5.1	3.1	4.6	6.0
Glycogen content of liver	Milligram	13.6	trace	29.3	35.8	63.2	78.7
	Per cent	0.36		0.58	1.16	1.38	1.31

TABLE 6

The influence of parenteral administration of dextrose upon the glycogen content of the liver of thyroid-fed rats

Dextrose injected { 1.5 gm. subcutaneously 3 hours / 0.75 gm. intraperitoneally 2 hours } before decapitation

RAT	I		II		III		IV	
No.								
Body weight	195.7		187.0		262.2		166.1	
Food	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste	Thyroid	Paste
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
First day	3.0	4.7	3.0	9.0	3.0	9.0	3.0	8.1
Second day	3.0	4.7	3.0	2.6	3.0	9.0	3.0	4.0
Third day	3.0	4.5	3.0	3.8	3.0	9.0	3.0	5.5
Loss of body weight at end of period, gram	−12.0		−11.8		−14.2		−9.0	
Weight of liver, gram	5.8		5.1		6.3		5.0	
Glycogen content of liver: Milligram	54.3		5.6		60.0		40.5	
Glycogen content of liver: Per cent	0.95		0.11		0.95		0.81	

As thyroid feeding increases oxidative process in the organism, it may be that the sugar injected was utilized immediately and had no chance to form glycogen. Another explanation may be that the thyroid-fed animal eliminates the excessive sugar much quicker than the normal animal, and thus cannot get any chance to store the sugar as glycogen. But the rabbit experiments for the carbohydrate tolerance after thyroid feeding, which will be described shortly, seem to make these explanations improbable. Cramer and Krause (14) described that after thyroid feeding, besides the loss of glycogen in the liver, glycogen content of other parts of the body sometimes decreased. The loss of liver glycogen and the inability of the liver to store dextrose as glycogen may be caused either by a disturbance of the glycogenetic function of the liver or by an increased glycogenolytic function of the liver. Starkenstein (31) and Wohlgemuth (32) could not demonstrate any change of diastatic action of the liver and blood after epinephrine glycosuria and piqûre. Macleod and Pearce (33) reported that stimulation of the splanchnic nerve, which caused a marked increase in the reducing power of the blood of the vena cava opposite the liver, did not cause any increase in the glycogenolytic power of liver extracts. Though pancreas diabetes shows a low glycogen content of the liver.

Nishi (34) proved by his perfusion experiments that the glycogenetic function of the liver is not affected in this disease. The complicated relation of the liver, thyroid, pancreas, adrenal, sympathetic nervous system and sugar center has been examined by numerous investigators (35) (36). But many points are not yet decided. Eppinger and his coworkers showed that thyroid preparations stimulate the sympathetic nervous system. The hypothesis, suggested by Cramer (19), that the influence of thyroid feeding upon liver glycogen is caused by the mediation of the adrenal glands, may be very probable. But for solving such a problem further investigations are necessary. An intimate relation between the adrenal glands and the glycogenolysis in the liver was demonstrated by Macleod and Pearce (37). On the other hand, though Fränkel (38) and Bröking and Trendelenburg (39) reported that they found an increased epinephrine content of the blood in Basedow's disease, Gottlieb (40) tried to explain the results of those investigators without assuming any excessive epinephrine in the blood. Neubauer and Porges (41) found that phosphorus, which causes the loss of liver glycogen, makes also the adrenal glands lose their chromaffin property, and that frequent adrenal injections are sometimes able to prevent the loss of liver glycogen resulting from phosphorus poisoning. On the other hand, Underhill and Fine (25) found no remarkable change of epinephrine content of the adrenal glands after hydrazine poisoning, which usually causes the loss of liver glycogen as well as phosphorus poisoning.

THE INFLUENCE OF THYROID FEEDING UPON THE SUGAR CONTENT OF THE BLOOD AND THE UTILIZATION OF DEXTROSE PARENTERALLY ADMINISTERED

Though it seems to be believed by many investigators that the tolerance for carbohydrate is increased in hypothyroidism and is lowered in hyperthyroidism (42) there are found some contradictory reports on this problem. The complicated and probably antagonistic relation between the thyroid and parathyroid glands, and varying susceptibility of different kinds of animals must be kept in mind. Observations in Basedow's disease cannot be compared unconditionally with those of experimental hyperthyroidism. Cramer and Krause (14) could not find any glycosuria in rats after thyroid feeding. In their dog experiment only a slight lowering of the carbohydrate tolerance

TABLE 7

The influence of thyroid feeding upon the sugar content of the blood and the utilization of dextrose parenterally administered

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT		URINE		DEXTROSE	
			Thyroid	Carrots	Oats	9 a. m.	4 p. m.	Volume	Total nitrogen	Injected intraperitoneally	Recovered in urine
		kgm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*cc.*	*gm.*	*gm.*	*gm.*
I*	1	2.14	10	75	50	0.10					
	2		15	75	31	.	0.11	110	0.823		
	3		15	75	5	0.11	0.10	105	0.914		
	4		15	33	5	0.11	0.11	74	0.979		
	5	1.75	15	16	0	0.08				12.33	
II	1	2.74	15	75	50	0.10	0.10	62	0.893		
	2		15	75	50	0.11	0.09	125	1.088		
	3		15	75	7	0.11	0.11	50	0.870		
	4	2.46	15	40	0	0.12		111	1.426	12.33	2.19
III	1	2.40	15	75	50	0.11	0.10	107	0.891		
	2		15	75	41	0.12	0.12	127	0.836		
	3	2.27	15	75	0	0.11		144	1.015	12.33	2.60

* The animal was found dead in the morning of the 6th day. The urine obtained measured 32 cc. and contained 0.144 gm. nitrogen and a trace of reducing sugar.

TABLE 8

Control experiments. The sugar content of the blood and the utilization of dextrose parenterally administered in calf's liver fed rabbits.

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT		URINE		DEXTROSE	
			LIVER	CARROTS	OATS	9 a. m.	4 p. m.	Volume	Total nitrogen.	Injected intraperitoneally	Recovered in urine
		kgm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*cc.*	*gm.*	*gm.*	*gm.*
I	1	2.34	15	75	50	0.09	0.10	141	0.745		
	2		15	75	50	0.10	0.09	167	0.880		
	3		15	75	50	0.11	0.10	71	0.697		
	4	2.32	15	75	31	0.11		65	0.614	12.33	2.25
II	1	2.68	15	75	50	0.09	0.09	143	1.294		
	2		15	75	50	0.11	0.09	107	0.882		
	3		15	75	50	0.08	0.10	111	0.761		
	4	2.54	15	75	0	0.10		100	0.970	12.33	2.45

was noticed. Böe (43) could not demonstrate any remarkable hyperglycemia in rabbits after injection or feeding of thyroid preparations. In my experiments with fresh thyroid-fed rabbits, the sugar content of the blood and the utilization limit of dextrose intraperitoneally administered were examined. A 10 per cent dextrose solution, sterilized by heat, was injected in the peritoneal cavity without anesthesia. In no case was glycosuria observed until dextrose injection. In tables 7 and 8 the details are shown.

From these tables it will be seen that the sugar content of the blood and the utilization limit of dextrose parenterally administered are not affected by thyroid feeding.

THE INFLUENCE OF THYROID FEEDING UPON THE EPINEPHRINE HYPERGLYCEMIA AND GLYCOSURIA

As regards the influence of thyroidectomy upon epinephrine glycosuria, contrary results have been reported. (Eppinger, Falta and Rudinger (6) ,(7), Underhill and Hilditch (8), Underhill (44), Pick and Pineles (9), Grey and de Sautelle (45)). Böe could not demonstrate any influence of hyper- or hypothyroidism upon epinephrine hyperglycemia. My experiments in this direction are detailed in tables 9 and 10.

Epinephrine (adrenalin chloride 1: 1000, Parke, Davis and Company) was injected subcutaneously in the proportion of 1 milligram per kilo of body weight. In no cases was glycosuria demonstrated on the first and second experimental day. As the thyroid-fed animals became very weak, the urine of the last experimental day was collected six and one-half hours after epinephrine injection. The animals were alive two hours later, but found dead the next morning.

From tables 9 and 10, it will be seen that feeding of fresh thyroid does not affect epinephrine hyperglycemia or glycosuria markedly. These results are in harmony with those of Böe's experiments.

SUMMARY

Fresh thyroid gland of pigs or desiccated thyroid (Parke, Davis and Company) administered by mouth in doses of 3 to 5 grams (fresh) or 0.5 to 1.7 gram (desiccated) per day, decreased the glycogen content of the liver of white rats distinctly in three to five days. Control animals, fed on the same diet with the addition of muscle tissue or

TABLE 9

The influence of thyroid feeding upon the epinephrine hyperglycemia and glycosuria

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT (PERCENTAGE)				URINE		
						Before epinephrine injection	Hours after epinephrine injection					
			Thyroid	Carrots	Oats		2	4	6	Volume	Total nitrogen	Sugar
		kgm.	*gm.*	*gm.*	*gm.*					*cc.*	*gm.*	*gm.*
I	1	2.60	15	75	50					67	1.076	0
	2		15	75	33					118	1.500	0
	3	2.38	15	75	0	0.11	0.46	0.40	0.26	104*	0.605	4.44
II	1	2.44	15	75	50					116	0.953	0
	2		15	75	50					164	0.926	0
	3	2.26	15	75	0	0.10	0.40	0.32	0.24	92*	0.648	3.19

* The urine was collected from 9 a.m. to 6.30 p.m. Epinephrine was injected at 12 m.

TABLE 10

Control experiments. The epinephrine hyperglycemia and glycosuria in calf's liver fed rabbits

RABBIT	EXPERIMENTAL DAY	BODY WEIGHT	FOOD			BLOOD SUGAR CONTENT (PERCENTAGE)				URINE		
						Before epinephrine injection	Hours after epinephrine injection					
			Liver	Carrots	Oats		2	4	6	Volume	Total nitrogen	Sugar
		kgm.	*gm.*	*gm.*	*gm.*					*cc.*	*gm.*	*gm.*
I	1	2.54	15	75	50					110	0.976	0
	2		15	75	50					97	0.870	0
	3	2.48	15	75	0	0.12	0.31	0.33	0.18	153*	0.712	4.72
II	1	2.80	15	75	50					33	0.942	0
	2		15	75	50					60	1.150	0
	3	2.88	15	75	0	0.10	0.37	0.30	0.17	144*	0.879	4.53

* The urine was collected from 9 a.m. to 6.30 p.m. Epinephrine was injected at 12 m.

egg, do not show any such change, even when the food amount is regulated so that they lose as much in body weight as the thyroid-fed animals.

The influence of thyroid feeding upon liver glycogen can be very easily removed by omitting thyroid from the diet. The liver shows its normal glycogen content two or three days after the cessation of thyroid administration, even when the loss of body weight has not been regained. This phenomenon seems to show that the changes resulting from thyroid feeding and causing the loss of liver glycogen, are not of a serious morphological nature.

When dextrose is introduced parenterally to fasted rats which show a very low glycogen content of the liver the amount of liver glycogen increases markedly in a few hours. This does not seem to be the case in the thyroid-fed rats.

Experimental hyperthyroidism does not change the sugar content of the blood in either rats or rabbits.

Spontaneous glycosuria does not result from thyroid feeding in either rats or rabbits.

The tolerance of thyroid-fed rabbits for dextrose, parenterally administered, does not differ from that of normal animals.

Nearly the same degree of hyperglycemia and glycosuria can be induced by epinephrine injection in thyroid-fed as in control rabbits.

The adrenal gland of thyroid-fed rats contains approximately the same amount of epinephrine as that of normal rats.

I desire to express my thanks to Prof. Frank P. Underhill, to whom I am greatly indebted for his suggestions, help and criticism; also to Prof. Lafayette B. Mendel for his kind advice.

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VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

III. Flexion Reflex Variations, Compared with Those of the Nerve-Muscle Preparation

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The object of this research was to compare the variations in threshold for faradic stimulation of the flexion reflex in the spinal cat, with similar variations in the nerve-muscle preparation, using the same muscle in each case, and keeping other conditions as uniform as possible, while the two sets of determinations were being made. The results were intended primarily to serve as a control on further quantitative studies on reflexes, in which it was important to know to what minimal values the variations in reflex thresholds would be reduced under the most favorable conditions of stimulation; that is, in animals unaffected by drugs or other experimental procedures except those incident to spinal transection. In other words, an answer was sought to the question: How smooth will the curve plotted for flexion reflex thresholds appear compared with the curve for nerve-muscle thresholds, when these curves are obtained from the same muscle, and from an animal whose tissues are in as nearly normal condition as possible?

The spinal cat was prepared by a modification of Sherrington's method, described in detail in a previous paper,[1] in brief, the carotids were tied, brain pithed, artificial respiration supplied and the animal kept at approximately normal body temperature by an electric heating pad. To avoid interference from the scratch reflex, the cord was cut in the neighborhood of the tenth dorsal vertebra. The femur and foot were held in clamps, immobilizing the leg at knee and ankle; the tendon of the tibialis anticus muscle was cut at its distal end, and a thread carried from it in the direction of its normal pull to a myograph lever whose effective tension on the muscle was about 35 grams. The peroneus longus muscle was excised to a point above the entrance of

[1] Porter: This Journal, 1912, xxxi, 141.

its motor nerve, and the femoral and the hamstring nerves were sectioned, thus paralyzing most of the muscles concerned in the flexion reflex aside from the tibialis anticus. An electrode of a form later to be described, was placed on the posterior tibial nerve and the break induction shock necessary to cause the least detectible movement of the writing point of the myograph lever on the moving smoked drum was determined; the lever magnified the movement of the muscle twice. Readings were made approximately once a minute for from one-half to one hour; the peroneus nerve in the thigh was then sectioned and the electrode applied to its distal end, giving a nerve-muscle preparation with the same muscle as before, and a second series of threshold determinations made.

Several attempts were made to secure simultaneous observations on reflex and nerve-muscle, stimulating the peroneal nerve in the hip without cutting it, using a modification of the electrode mentioned above, but conductivity was always diminished in the neighborhood of the electrode, so the attempts were abandoned. The plan of obtaining simultaneous records from homologous muscles on the two legs was not tried. The inductorium was a large one with secondary 13 cm. long and 6 cm. in diameter; it was calibrated by comparison with a coil already calibrated by the Martin method;[2] a part of the calibration curve is shown in figure 1. Secondary coil positions are measured from complete superposition over the primary, which is position 0. It is evident that secondary coil positions from about 25 outward, will give the greatest accuracy in quantitative work, since a relatively large movement of the secondary yields only a small increment in the value $\frac{M}{L}$; hence the secondary has been used within these limits in the present research. The Z units referred to in this paper are obtained

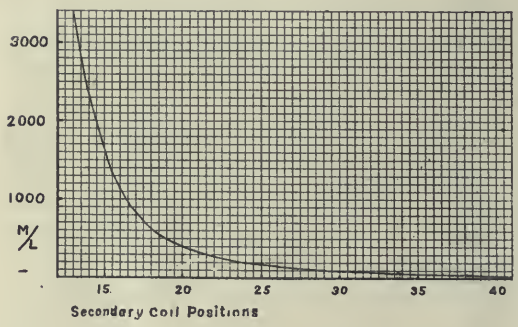


Fig. 1. Calibration curve for the inductorium used. (Martin calibration.)

[2] Martin: The measurement of induction shocks, New York, 1912.

by multiplying the value $\frac{M}{L}$ for any given secondary position, by the current in amperes through the primary coil; the latter value has been 0.1 ampere in most experiments.

The threshold of a reflex determined by the method I have described may be affected by a number of possible variables: (1) The nerve has abnormal conditions of irritability near the point of ligation or section. Gotch[3] found alterations in the electrical response 8 mm. from the point of section; Forbes[4] reports similar results. (2) The electrodes at their point of application may introduce variables. I had much difficulty in a previous research[5] in using the quantitative method, because of the short-circuiting of a varying fraction of the stimulating shock by the minute drop of liquid which collected between the platinum wires of the Sherrington shielded electrode;[6] the removal of this drop gave an apparent lowering of threshold of from 0.7 to 1.5 Z units. In justice to the Sherrington form it should be said that, if it be carefully applied so that the nerve is not compressed, and unless the minutest variations in threshold are to be followed, it can be used entirely successfully; for example, I have determined the threshold with it at the beginning of an experiment and found it 2.4 Z units; six and a half hours later the threshold was 3.7 Z units, so that no serious local impairment could have been caused by its application. (3) The central nervous connections are held by Sherrington[7] to be responsible for the greater variability in threshold of the reflex arc as compared with the nerve trunk. (4) The nerve trunk and (5) the myo-neural junction may vary in conductivity.[8] (6) The muscle fibers themselves may conceivably vary in excitability. It was my purpose to eliminate variations at the point of stimulation as far as possible, and then to compare the variations in threshold of the group of structures comprising the reflex arc with similar variations in the nerve-muscle group, intending thereby to demonstrate the variability introduced by the central connections between neurone and neurone. In such a study it would be desirable to utilize an arc with the fewest possible central connections, and

[3] Gotch: Journ. Physiol., 1902, xxviii, 32.

[4] Forbes and Gregg: This Journal, 1915, xxxix, 172.

[5] Porter: This Journal, 1915, xxxvi, 171.

[6] Sherrington: Journ. Physiol., 1909, xxxviii, 375.

[7] Sherrington: The integrative action of the nervous system, New Haven, 1906, 14.

[8] Cf. Burridge: Journ. Physiol., 1911, xli, 285.

those of as low a threshold as possible, that the results might represent minimal variations in reflex arc conduction; there are observations which indicate that the flexion reflex, and in particular the contraction of the tibialis anticus muscle, approximates these requirements. First, the flexion reflex is one of the few to respond to weak single shocks;—crossed extension can be readily elicited by single shocks but in my experience commonly has a higher threshold than flexion.[9] Second, among the muscles concerned in the flexion reflex Sherrington[10] found some with higher thresholds than others, but none with a lower than tibialis anticus; I have found the same result. In an experiment to test the point the tendons of insertion were cut, the muscles isolated as much as possible from each other and contraction observed directly while the tendon was held in the hand; the thresholds were as follows in Z units: tibialis anticus 4.7; peroneus longus 5.1; extensor longus digitorum 11.8; biceps (posterior portion) 4.9; tensor fasciae latae 8.1; semitendinosus 4.7; sartorius (portion inserted on patella) 5.5; gracilis 5.2. These figures are averages of from two to seven determinations on each muscle; the stimulating electrode was on the posterior tibial nerve and single break shocks were used. Finally, if speed of transmission be a criterion of the number of central connections concerned, then the flexion reflex has few, for Sherrington[11] found with strong shocks little difference between the rate through the reflex arc and through a corresponding length of nerve trunk.

For the purpose of avoiding the difficulties mentioned above incident to the use of the Sherrington electrodes a form of liquid electrode suggested by those used by Lucas[12] has been employed; it is shown in longitudinal section, once and a half natural size, in figure 2. A glass tube *b* with a constricted neck *i* has a short piece of rubber tube *c* drawn over its end tightly for a short distance; closing the lumen of the rubber tube is a glass ball *e* which has been melted on to a platinum wire *f* ending in a loop; a copper wire *g* is soldered to the platinum wire. The nerve *à* has a ligature *dd'* attached to its end; the space *h* is filled with a paste of kaolin in Ringer's solution, mixed to the consistency of cream. Water distilled from glass and chemically pure salts are used

[9] Porter: Loc. cit.

[10] Sherrington: Journ. Physiol., 1910, xl, 28.

[11] Sherrington: Integrative action of the nervous system, New Haven, 1906, 19.

[12] Lucas: Journ. Physiol., 1913, xlvi, Proc. p. xxxii; 1913, xlvi, 480; 1908, xxxvii, 114; 1906, xxxiv, 375.

in making this up; the kaolin is Baker's, washed twice in distilled water and twice in Ringer's solution. Ringer's solution alone was not used in the tube *b*, because of the ease with which it permitted bubbles to enter. The same paste surrounds the nerve at its emergence from the tube at *i*. The wire *g* is connected to one terminal of the induction coil; the other terminal is connected to a platinum needle thrust deeply into the shoulder muscles (later experiments) or to the tracheal cannula (earlier experiments); *f* is made the cathode. The current in passing from one electrode to the other reaches its greatest density at *i* and stimulates the nerve at that point, as it does at the corresponding points in the Lucas models.

The electrode is applied as follows: the nerve—posterior tibial, for example,—is carefully separated from surrounding tissues for a distance of 4 or 5 cm., is ligated, and cut at the distal end; a glass tube is selected from among several of different sizes kept on hand, with an opening at *i* as nearly as possible of the diameter of the nerve; the ligature is

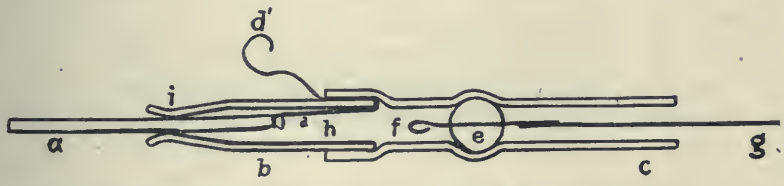


Fig. 2. Liquid electrode, once and a half natural size; description in text.

passed through the tube and the nerve drawn in after it to a distance of about 1.5 cm. With a fine-pointed pipette the glass tube is now filled with the kaolin-Ringer mixture; the lumen of the rubber tube surrounding the platinum loop has previously been filled with the mixture, and the rubber tube is now forced over the glass one, thus holding the ligature tightly, preventing the nerve from slipping out. If bubbles are by accident included in the glass tube, the glass ball *e* is pinched forward with the fingers until they are forced out at *i*. A thread is passed through a small portion of the tibialis posticus, or neighboring muscles, and tied about the tube at *i*, care being taken that the nerve is neither pulled, nor forced to make an angle at *i*, which would compress it at that point, since a relatively slight pressure there raises the threshold and necessitates rearrangement of the electrode. The nerve is surrounded with the kaolin-Ringer paste at the point of its emergence from the tube; the rubber tube is made long

enough to pass out of the wound, the edges of which are closed over the electrode with spring clips. In applying the electrode to the peroneal nerve in the thigh, the same procedure is followed, except that in some cases it has been found unnecessary to attach the electrode to the underlying muscles. The peroneal nerve is a part of the sciatic trunk at this point and must be dissected away from it; when thus separated its size is nearly the same as that of the posterior tibial, and usually the same electrode has been used for both. A nerve held in an electrode of the type described has had its blood supply seriously interfered with, but otherwise it is in practically a normal environment; it is bathed in Ringer's solution, and at the point of stimulation the liquid,

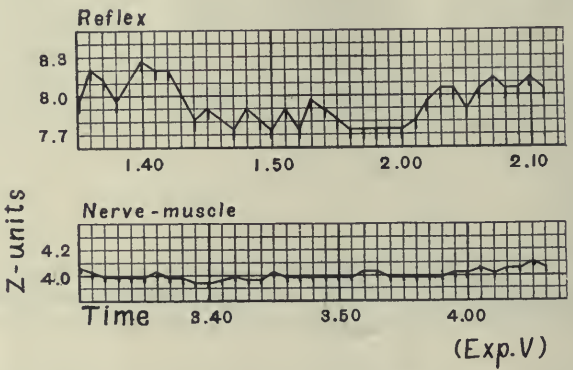


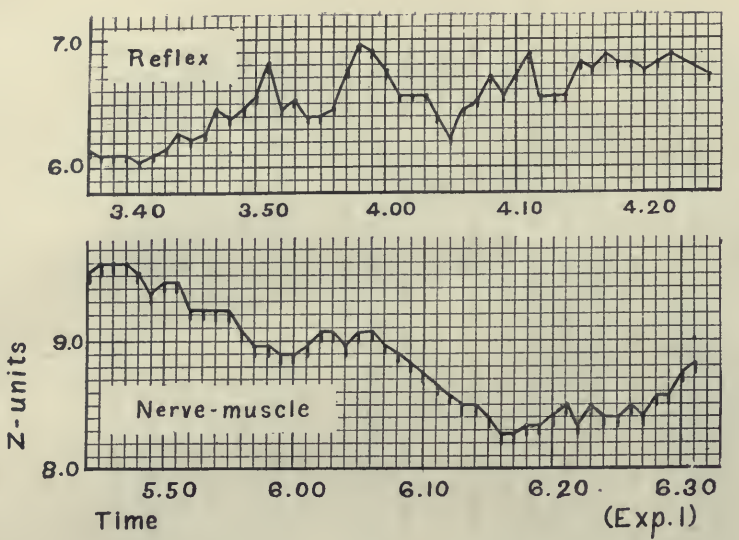
Fig. 3. Variations in threshold of reflex and nerve-muscle preparations in the spinal cat, using the same muscle, tibialis anticus, in each case. Alterations in threshold between successive readings greater for reflex than for nerve-muscle. Changes in direction more frequent in the reflex curve than in the nerve-muscle curve. Abscissae; time; ordinates, Z units (Experiment V).

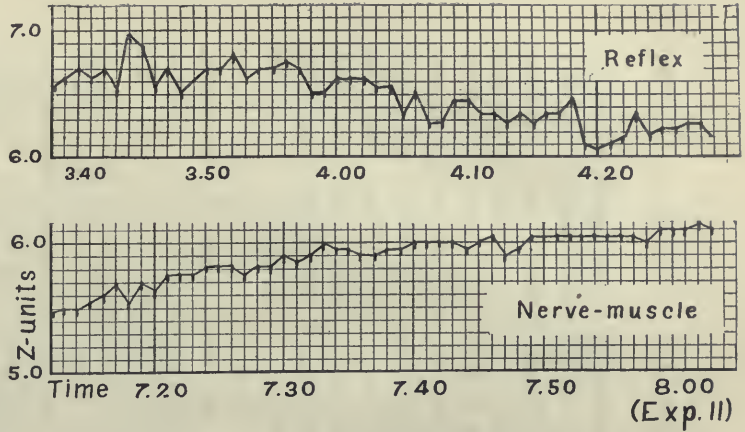
and therefore the resistance, remains constant except for possible changes in the resistance of the nerve itself; the ligated end is presumably so far away as not to be able to influence irritability at the point of stimulation.

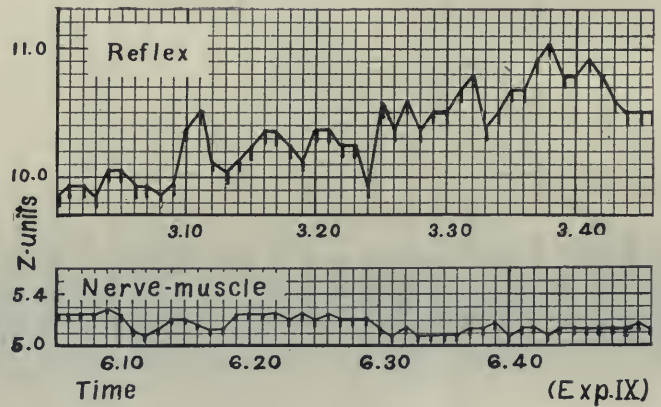
In determining the threshold the secondary coil was approximated to the primary by 1 mm. increments, while the myograph lever wrote on a moving drum, and the point at which the least detectible movement of the lever occurred was taken as the threshold, when this was confirmed by a second shock three to five seconds later. I have not found that an interval as short as this between shocks affected the threshold. Determina-

tions were made once a minute, in most cases, and continued for periods of twenty minutes to an hour, the series of nerve-muscle readings following those for the reflex as promptly as possible; data from an experiment are shown plotted in figure 3. The accuracy of the determinations in this and other figures is indicated by the length of the short vertical lines; in figure 3, for example, the threshold for the nerve-muscle was determined with an accuracy of 0.04 Z unit, corresponding to a movement of the secondary coil of 1 mm. with the secondary out 39 cm. from complete superposition over the primary, and 0.1 Amp. through the primary. From figure 3 it is evident that the alterations in threshold between successive readings is greater for reflex than for nerve-muscle, and the direction of change towards a higher or lower threshold alters oftener for reflex than for nerve-muscle, making the curve for the latter the more irregular of the two. Curves on a smaller scale are given in figure 4 for other experiments. No reason could be assigned for the gradual but pronounced lowering of nerve-muscle thresholds in experiments I and III, amounting to 2 Z units in experiment III, or more than ten times the maximum change in any minute; in contrast, the nerve-muscle of experiment V (fig. 3) altered a total of only 0.16 Z units during thirty-six minutes (highest 4.10, lowest 3.94 Z units) or about three times the maximum change in any minute (0.05 Z unit). The relatively high nerve-muscle threshold in both these experiments may have been due to poorly fitting electrodes, which allowed part of the current to be short circuited. In experiment IX the movement elicited was flexion at knee, instead of contraction of the isolated tibialis anticus muscle. Table 1 gives a summary of two important features for all the successful experiments; the difference in threshold between successive readings one minute apart (columns 2 and 3) is from 1.6 to 7.6 times as great for reflex as for nerve-muscle preparations; and the number of "peaks" in the reflex curve is greater in each experiment than the number in the nerve-muscle curve (columns 4 and 5), which is the quantitative indication that the nerve-muscle curve is the smoother of the two.

At times, for stretches of several minutes, the nerve-muscle has shown no alteration in threshold within the limits of error of the determination (fig. 3 and fig. 4, experiments II, III and IX). The reflex has never remained as steady as this; the longest period of unaltered threshold I have found for reflex has been four minutes and for nerve-muscle eight minutes. The frog nerve-muscle preparation removed


Reflex
7.0
6.0
3.40
3.50
4.00
4.10
4.20
Nerve-muscle
Z-units
9.0
8.0
5.50
6.00
6.10
6.20
6.30
Time
(Exp. I)


Reflex
7.0
6.0
3.40
3.50
4.00
4.10
4.20
Nerve-muscle
Z-units
6.0
5.0
Time
7.20
7.30
7.40
7.50
8.00
(Exp. II)


Reflex
11.0
10.0
3.10
3.20
3.30
3.40
Z-units
Nerve-muscle
5.4
5.0
6.10
6.20
6.30
6.40
Time
(Exp. IX)

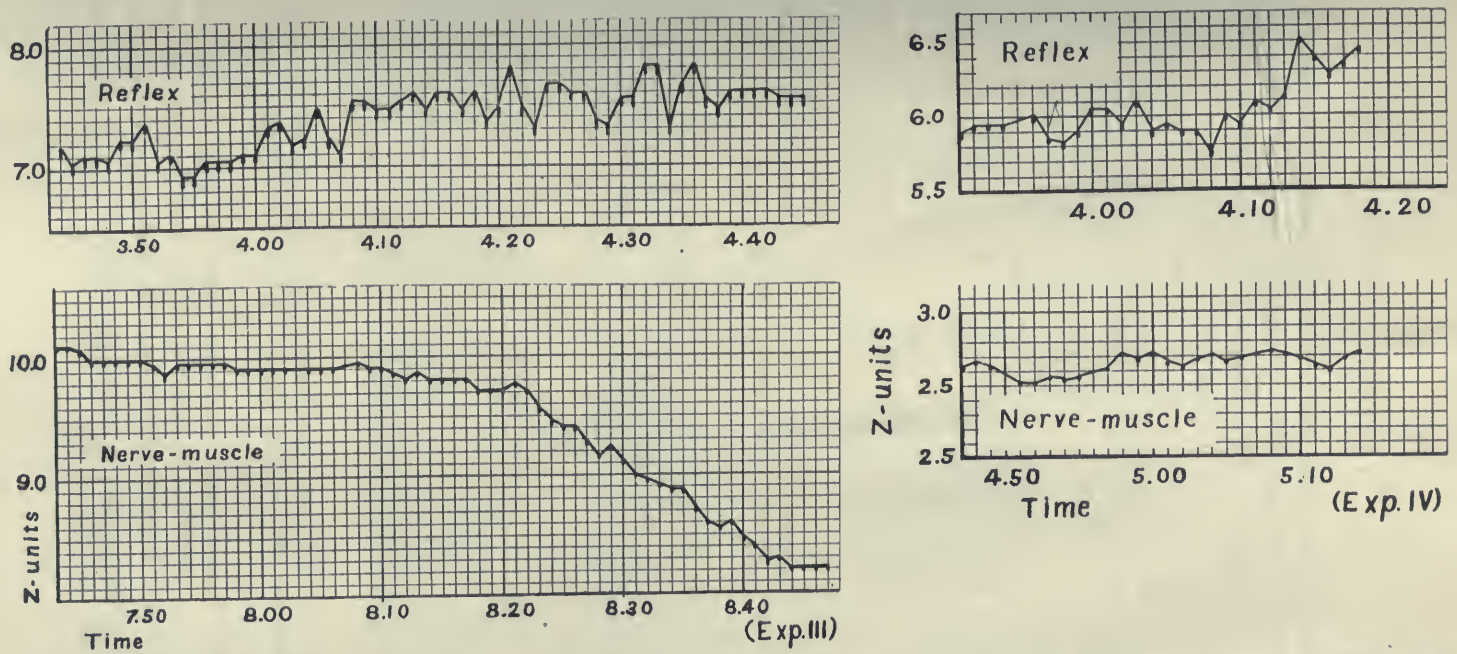


Fig. 4. Variations in threshold of reflex and nerve-muscle preparations; five experiments. Flexion at knee in Experiment IX; in all others, contraction of tibialis anticus muscle.

from the body was reported by v. Fleischl[13] many years ago as having a steady threshold, with so little variation, in fact, that it has been used by him and others[14] in calibrating the induction coil. It could hardly

TABLE 1

EXPERIMENT NUMBER	MAXIMUM VARIATIONS IN THRESHOLD IN Z UNITS, BETWEEN SUCCESSIVE DETERMINATIONS ONE MINUTE APART.		NUMBER OF "PEAKS" IN THE CURVES FOR REFLEX AND NERVE-MUSCLE RESPECTIVELY, FOR EQUAL LENGTHS OF TIME. (THE MEASURE OF SMOOTHNESS OF THE CURVES.)	
	Reflex.	Nerve-muscle.	Reflex.	Nerve-muscle.
I	3.5	2.0	10	7
II	4.3	1.5	13	9
III	4.5	1.5	14	7
IV	4.0	1.0	7	6
V	3.8	0.5	9	6
VI	2.5	0.9*	11	6
VII	2.8			
VIII	3.0			

* Exclusive of the change shown at 4.31, figure 4.

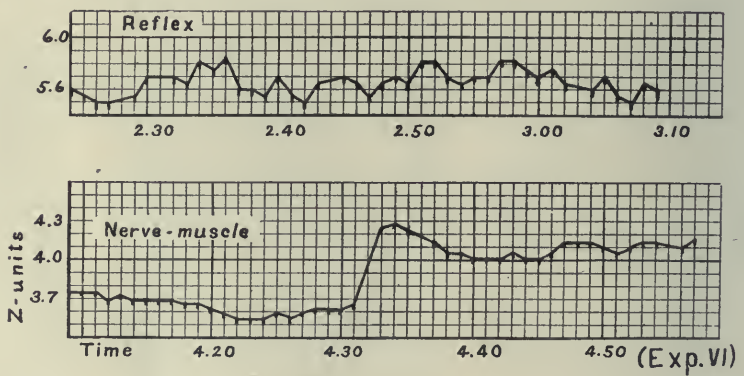


Fig. 5. Threshold of reflex and nerve-muscle preparations compared. Rise in threshold of the latter without apparent cause at 4.32 (Experiment VI).

be expected that the nerve-muscle preparation in the body of the cat would retain so constant a threshold. The frog preparation removed from the body is presumably undergoing a steady deterioration; the

[13] v. Fleischl: Sitzungsberichte der Königlichen Akademie der Wissenschaften, Wien, 1875, Bd. lxxii, Abt. III, 41.

[14] Martin: Loc. cit.

cat tissue, with intact circulation, might well show improvement as well as deterioration, dependent on alterations in blood supply or blood content. Gruber,[15] using height of contraction as an index to the condition of the nerve-muscle preparation, finds prompt improvement on increasing blood pressure. I have seen sudden changes in threshold at times without obvious reason therefor, figure 5 shows a case; just before the sudden rise in threshold at 4.31 the forelegs of the animal were strongly extended, suggesting partial asphyxia, but artificial respiration was proceeding as usual and no other experimental procedure was altered; stability of threshold in the nerve-muscle preparaton, therefore, while the rule, cannot be counted on with certainty.

The comparative smoothness of the reflex curve is brought out when it is compared with a similar curve of threshold changes of the crossed-extension reflex; figure 6 is a record of the simultaneous behavior of the two reflexes, flexion being recorded as already described, crossed extension by the contraction of the quadriceps group of muscles, the patellar tendon being attached to a myograph lever and the femur held firmly by a pin passing through its lower end and into a block of wood; flexion varies in threshold from 5.50 to 5.95 Z units, crossed extension from 8.40 Z units to 14.30 Z units. The semi-rhythmic waves of variation in crossed-extension threshold with their crests at 4.10, 4.25 and 4.36 have been noted in a similar record from another animal. The smoothest flexion reflex curve obtained, was that of experiment VI (fig. 5), where the threshold for forty-five minutes showed a total variation of only 0.4 Z unit; the greatest change in any single minute was 0.3 Z unit (at 2.36, fig. 5) so that the total alteration was approximately 1.3 of this maximal single change.

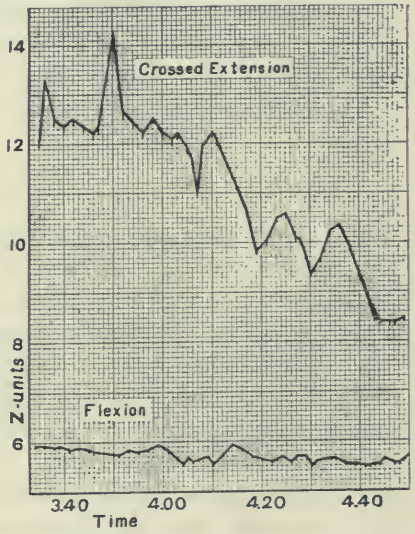


Fig. 6. Simultaneous variations of reflex threshold in flexion and crossed-extension reflexes (Experiment IX). Crossed-extension markedly less constant in threshold than flexion.

[15] Gruber: This Journal, 1913, xxxii, 221.

The central connections between the neurones of the arc, that is, the synapses, have been held responsible for the greater variations in threshold of the reflex as compared with the nerve-muscle. This does not mean that any given synapse shows changes in resistance to the passage of the nerve impulse, corresponding to the undulations of a reflex curve such as that of figure 2; such a curve is not incompatible with the supposition that each synapse is obeying the "all-or-none" law. If we postulate a sufficient number of independent arcs involved in the reflex contraction of the tibialis anticus, each with its own synaptic resistance and each following the "all-or-none" law, we may explain all the observed variations in threshold; the complete dropping out of a group of synapses with low resistance will raise the threshold for the entire muscle to a new level, and their return to function will lower it.

SUMMARY

1. A liquid electrode (modified from Lucas) is described, by which mammalian nerve in situ may be stimulated for periods of several hours, under more uniform conditions at the point of stimulation than is possible with the platinum wire form of electrode.

2. The difference in threshold between successive readings one minute apart, may be from 1.6 to 7.6 times as great for the flexion reflex, (tibialis anticus muscle), as for the nerve-muscle preparation, the same muscle being used in each case.

3. The curve of reflex threshold variations changes its direction more frequently than the nerve-muscle curve for equal lengths of time; that is, the nerve-muscle curve is the smoother.

4. The nerve-muscle threshold has been observed to alter as much as 2 Z units (roughly ten times the maximum minute-to-minute alterations) in the course of an hour. It is the rule for such a change to be gradual, but an exception is described.

5. The nerve-muscle threshold showing least alteration remained for thirty-six minutes without exceeding in total change 0.16 Z unit, or about three times the maximum change in any single minute.

6. The reflex threshold showing least alteration remained for forty-five minutes within the limits of 0.4 Z unit, or 1.3 times the maximum change in any minute.

7. The threshold for crossed-extension varies more widely than the threshold for flexion. In the experiment described, the total variation

for crossed-extension was 5.90 Z units; for flexion, 0.45 Z unit, during the same period of time.

I am indebted to my wife, Helen Nichols Porter, for much assistance in this investigation.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 68.

THE BEHAVIOR OF HOLOTHURIANS IN BALANCED ILLUMINATION

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I. The behavior of phototropic animals when so situated as to be illuminated from opposite directions gives important information regarding the nature of the mechanism of stimulation. It was held by Loeb (1) that a phototropic organism, when placed at the center of the line joining two equal sources of illumination, should move in a direction perpendicular to this line. The accuracy with which this kind of response is obtainable in a suitable organism is demonstrated by Patten's (2) experiments with the negatively phototropic larva of the blowfly. Patten further found that the angular deflection of the larva's path from the perpendicular was so related to the percentage difference in intensity between the two lights, when these intensities were caused to alter in a graded series of ratios, as to produce a smooth curve when these values were plotted on the axis of ordinates and abscissae, respectively. On the basis of this graph, and taking into account the blowfly larva's method of locomotion, Patten was able to show (3) that the responses appear to be determined by the presence of two photosensitive surfaces inclined to each other at a definite angle. The definite path of progression adopted by the larva under the influence of two opposed unequal beams may be predicted on the assumption that the animal ceases to deflect from its original locomotor path—which in these experiments is a straight line normal to that connecting the sources of light—as soon as the luminous intensity on these mutually inclined surfaces is made equal by the larva's position.

II. There may be deduced from this principle the corollary that, if the photoreceptive surfaces of an animal are so arranged as to be sensibly *parallel*, no definite position should be assumed by this organism when bilaterally illuminated. For, in such a case, if the balanced lights were *equal* in intensity, it would be impossible for the animal to place itself in a position which would result in unequal illumination of the two sides; while, if the lights were *unequal*, the amount of light received by

either side could not in any position be made equal to that incident upon the other. It is assumed, of course, that the longitudinal axis of the animal remains straight when orientation is completed.

Thus an animal of this kind might be clearly phototropic according to other criteria, and yet fail to satisfy the requirement of orientation under bilateral illumination which Loeb's contention demands. The existence of such organisms, however, would add further support to the assumption made by Patten, namely, that the exhibition of a definite path of locomotion under these conditions depends upon the final equality of illumination on each of the two photosensitive surfaces.

III. Certain pedate holothurians have been found to be different from most other echinoderms, in that they exhibit a pronounced bilaterality in their structure, coupled with a fixed direction of progression (4), (5), (6). Typical tropistic behavior is thus made possible. It has been shown that some of these holothurians (Holothuria surinamensis, H. captiva) are photo-negative, that they are sensitive to light over their whole surface, that they give well defined reactions to shading, and are non-reactive to a sudden increase in light intensity, yet orient away from the light with conspicuous precision. It is impossible to conceive, as I have already pointed out (4), that these animals are oriented by light in any way other than through the direct action of illumination upon their integument.

One of the holothurians referred to, H. captiva, is the most outstandingly bilateral of all the species which I have studied. Its lateral surfaces are so nearly straight and parallel that, to all intents and purposes, they may be regarded as strictly so; that is, longitudinal elements of its surface are parallel. I have previously described the accuracy with which it orients away from the light. Experiments were subsequently made to determine the behavior of this species when illuminated from opposite sides.

Inasmuch as the whole surface of H. captiva is sensitive to light, the higher sensitivity of the oral end should not greatly obscure the interpretation of such experiments. When horizontal light is employed, the sides of the animal may be regarded as equivalent to two photosensitive areas. This particular species of holothurian, fortunately, tends to preserve a straight position of the body.

In my present study upon this animal, the tests were made in a dark room, the animals being placed in a flat-sided aquarium with their long axes perpendicular to the line connecting the two sources of light. The lights used were two tungsten incandescent filaments, placed at

various distances from the holothurian in different experiments. In some cases the animal was induced to begin crawling in a direction perpendicular to that of the light beams before the latter were turned on. The intensity of the lights was varied by using filaments of different wattage (25 to 100 watts). Since the characteristic features of the results soon became evident in these experiments, no great refinement was necessary in the physical precision of the light adjustments.

IV. In no case did Holothuria captiva assume a path of locomotion definitely related to the intensities of the acting lights. In no case was it constrained to move, after the fashion of the blowfly larva, along a path inclined to the direction of the beams. A number of animals were found to move first toward one light, then toward the other; this was the case when the lights were of nearly equal intensity. Many individuals circled in an aimless, indeterminate way (as when illuminated from above), while others became curled into the shape of the letter S, the oral end, and frequently the cloacal end as well, being bent away from the stronger light.

When the intensity of the stronger illumination was made eight or nine times that of the weaker one, the holothurians almost invariably oriented precisely and away from the stronger light. But when they had moved somewhat toward the weaker light, their movements became quite irregular.

To meet the criticism that possibly the incandescent filaments as sources of light were too small as compared with the size of the animals, the latter being 6 to 8 cm. in length, experiments were tried with sunlight reflected from two mirrors, each measuring 15 by 20 cm. The holothurians were in a dark chamber, an opening on either side (10 by 8 cm.) allowing the sunlight reflected from the mirrors to enter the box from opposite directions. One or several lightly smoked glasses were then interposed between one mirror and the corresponding opening in the dark chamber. The behavior of the holothurians under these conditions was qualitatively identical with that in the trials with electric lights. Hence I conclude that H. captiva does not maintain a predictable path of locomotion under the influence of bilateral illumination.

V. The validity with which this result may be applied to the criticism of photic stimulation theories depends largely on the share taken by the general surface of the animal in photoreception. That this share is a considerable one, is shown by the following:

a. Excised pieces of the "dorsal" integument (including skin and muscle layers) which have been cut from the midbody surface of H.

surinamensis or H. captiva exhibit local wrinkling contractions when a spot of intense light is thrown upon them. The wrinkling movements cease when the strong light is removed. When first prepared, the pieces of the body wall are strongly contracted; but if they be allowed to lie in seawater for half an hour or so, or if they be subjected to gentle traction, they become somewhat relaxed, although the cut edges remain curled. It was on such relaxed preparations that the light test was made. Tube feet on isolated portions of the "ventral" surface also bend away from the light.

b. A phenomenon closely akin to "circus movements," and probably in principle identical with them, appears when these holothurians are exposed to general illumination from above, provided one side of the animal's surface has been rendered insensitive to light. In the narcotizing, cocaine seems to give the best results, but chloretone was also used. In the case of either narcotic, a strong solution (in seawater) was employed and the holothurian, while being held out of water, had one side painted over with the solution several times. The parts so treated soon become quite insensitive to light, while the untreated side is still sensitive, and its tube feet all remain normally functional. On being returned to seawater the holothurians soon assume a curved attitude, the narcotised side being the relaxed one (convex). If the animals are then strongly illuminated from above, they rapidly become curved in the opposite direction, the narcotised side being then the inner, contracted (concave) portion of the bend. Thus the holothurian bends toward the *unstimulated* side, whether the non-stimulation is determined by the relative absence of light, or by the enforced incapacity of its photoreceptors.

VI. These experiments afford evidence: (1) that the amount of light received by the photosensitive surface of Holothuria surinamensis and H. captiva determines their behavior in an illuminated field; (2) that the assumption of a definite path of progression, with respect to balanced illumination, depends on the non-parallel relation of photosensitive surfaces.

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(5) Crozier: Zool. Jahrb., Abt. Physiol., 1915, xxxv, 233.
(6) Crozier: Sci., N. S., 1916, xliii, 148.

THE EFFECT OF DEXTROSE GIVEN INTRAVENOUSLY ON BLOOD COMPOSITION AND URINARY SECRETION[1]

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In a previous paper (1) the writer has summarized the data at hand concerning the relations between the composition of the blood and diuresis. In addition to studies directed toward a broad and general explanation of these relations, the attempt has frequently been made to concentrate attention upon some single substance and its behavior in such a connection. It is desired to present here an addition to these studies in the form of a series of injections of different dextrose solutions, using the same animal throughout.

Only a few previous studies will be mentioned. Lamy and Meyer (2) could find no relation between sugar diuresis and systemic blood pressure, the viscosity, molecular concentration (as measured by the depression of the freezing point), or dry weight of the blood, or the volume of the kidney in chloralized dogs with oncometers in place. The diuresis according to them may begin at a time when the viscosity of the blood is unchanged or even increased. It is noted that in general the polyuria runs parallel to the concentration of sugar in the blood, but there are numerous exceptions to this rule. These exceptions usually take the form of a high blood sugar without diuresis.

Fisher and Wishart (3) working in Lusk's laboratory, find that after the administration of dextrose per os, the hemoglobin of the blood is much decreased, but has returned half way to normal before diuresis begins. The hemoglobin concentration is taken as a measure of blood volume, although its accuracy in this rôle is open to some question (4).

Ewing (5) determined the specific gravity of the blood, confirming Fisher and Wishart's observations for glucose administered intra-

[1] Read in abstract before the Society for Experimental Pathology, New York, December 29, 1916.

venously. He adds that diuresis may occur without change in the concentration (specific gravity) of the blood, or may be absent when the blood shows a well defined dilution. Diuresis is usually dependent on blood sugar level, but one experiment is a notable exception to this rule. When the blood sugar exceeds 0.4 per cent, the urinary sugar concentration appears to remain fairly constant, while as the blood sugar falls and diuresis decreases, the urinary concentration goes up. Ewing's injections were made rapidly.

Epstein (6) studied the relation of glycosuria to blood sugar and concluded that it depends on an increase in the total amount of sugar in the circulation, which must be determined by noting simultaneously the blood sugar concentration and the blood volume (as indicated by its hemoglobin content). When a glycosuria is precipitated by a hyperglycemia in this sense, the urinary sugar concentration must show a tendency to remain constant, since "diuresis in diabetes mellitus plays an important rôle in determining the total amount of sugar eliminated in the urine, but has no influence on its concentration or percentage." This relation is found to be so constant that Epstein thinks that there is a glycosuric constant, like that of Ambard for urea, which is higher in individuals with nephritic kidneys. In such cases a greater hyperglycemia must be present to produce the same glycosuria.

Arrous (7) working with intravenous injections, contrived a coefficient expressing the relations of the volume of fluid injected and the volume of urine excreted, which remained constant for each concentration of dextrose, regardless of the size of the dose.

Blumenthal (8) brought forward very clearly the necessity for including time in the conception of glucose tolerance and utilization, and Woodyatt (9) with his method of precisely timed injections made possible a more accurate study of the questions above outlined.

It was planned, therefore, to have the present work consist of two series of injections, using the same animal for all, that each injection might be better compared with the others. In the first series, the same quantity of glucose, per kilogram-hour, was to be administered each time, but dissolved in varying amounts of water, while in the second, the same amount of water per kilogram-hour was to be injected, but containing varying quantities of glucose. In each experiment the volume of the urine and the sugar of the blood and urine were to be followed.

The general outline of experimental technique was the same as that described in a previous paper (1). No anesthetic of any kind

was used. Blood was drawn from the jugular with pipet and needle. This procedure caused in the animal, which was of a very quiet and phlegmatic disposition, no perceptible disturbance, and the blood sugar curves give no evidence of fluctuations from this cause. Urine was removed by the suction catheter which has been described. The injections were made with a small motor-driven pump embodying the same principles as that of Woodyatt (10). The glucose used was purified by several recrystallizations from crude dextrose. For this product the writer is deeply indebted to Dr. George Peirce (formerly of this laboratory). The blood sugar determinations were according to the method of Lewis and Benedict. The heating was done in an autoclave. The urinary sugar was estimated by means of the polarimeter. The use of small tubes made it necessary to dilute only the very small samples. The urine was cleared with alumina cream, which gave a beautifully clear supernatant fluid. It was found however that there was a slight adsorption of sugar by the aluminium hydroxide; from 1 to 5 per cent, according to the concentration. Corrections were made for this adsorption.

TABLE 1

DEXTROSE GRAMS PER KILOGRAM—HOUR	WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
	40.0	20.0	10.0	2.5
	per cent	*per cent*	*per cent*	*per cent*
0.7		3.5		
1.0		5		
2.0	5	10	20	80
3.0		15		

Table 1 gives an outline of the injections made, with the quantities of water and sugar administered, per kilogram-hour. The percentage concentration of the injection fluid in each case is shown by the figures in the boxes, each figure representing an experiment actually performed. The duration of the injections varied slightly, but all ran four hours or more except the 0.7 gram to 20 cc. experiment, and in the comparative figures all have been calculated merely for the first four hours, except this experiment. The injection referred to lasted three hours, and is included merely for reference, since a different dog was used for it.

It is felt that presentation in the form of curves will be the most advantageous to the reader for grouping quickly the results. These

curves have all been drawn to the same scale. The abscissae represent time, the ordinates the amounts of the various substances. The solid line represents the volume of urine secreted, calculated as a rate, i.e., cubic centimeters per fifteen minutes. The dotted line shows the blood sugar in per cent. The interrupted (dash) line gives the concentration of sugar in the urine, again as per cent, while the dash and dot line combines the first and third as the rate of excretion, or grams per fifteen minutes for the sugar. The vertical solid line represents the end of the injection.

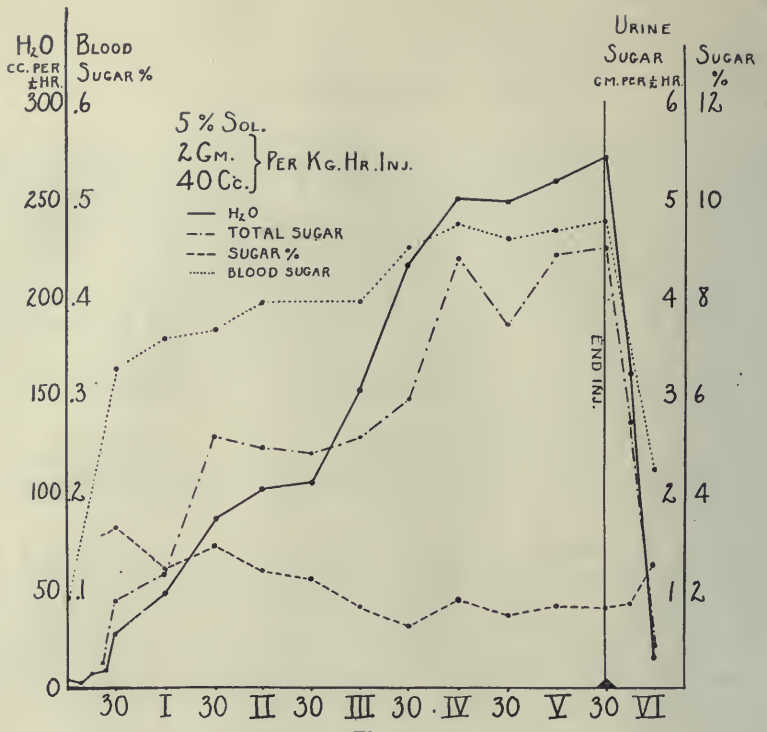
Some of the features of these injections may be best displayed by bringing together the curves from a number of experiments into composite charts.

Figure 8, the first of these charts, has four curves representing the water output rates in the experiments where the sugar injected remained constant. Each is labeled according to the concentration of the injection fluid used. As is to be expected, the more dilute solutions produce a greater diuresis, but that from the 80 per cent solution is practically as great as that from the 20 per cent solution. The diuresis begins in each case at the same time, namely about twenty to twenty-five minutes after the beginning of the injections.

Figure 9, the second composite chart, gives the rates of sugar output in the same four experiments. Here it is seen that, although the sugar intake is exactly the same per kilogram-hour in each case, the injection of 5 per cent solution, accompanied by a great diuresis, leads to a much smaller excretion of sugar than that of an 80 per cent solution, accompanied by very little diuresis. The other concentrations fit in well between. It must however be observed that this smaller excretion, in the case of the dilute injection, is principally a delayed excretion, since while with the 80 per cent injection, the sugar output shoots up most rapidly, it has, at about the fifth hour, atttained much the same level with all four concentrations of the injection.

This delay in excretion means, naturally, that there has been greater retention in the body of dextrose with the dilute than with the concentrated injections. The extent of this retention is shown numerically in table 2 in grams per kilogram-hour, for a four-hour period of injection.

This makes it seem that a copious diuresis interferes with the excretion of glucose by the kidneys, but it is probable that the true explanation is somewhat different. Zuntz (11) in metabolism experiments designed to show the protein sparing action of carbohydrate noticed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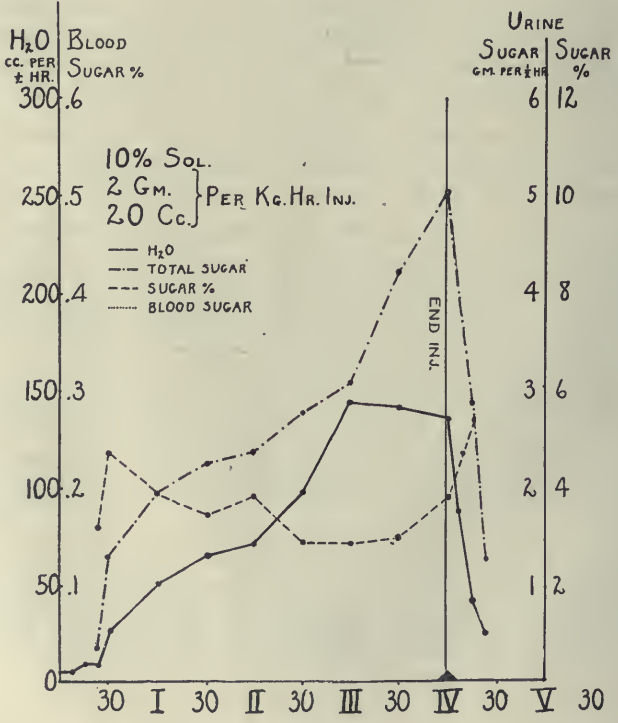
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR.
SUGAR %
5 % SOL.
2 GM. } PER KG. HR. INJ.
40 CC.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
6
5
4
3
2
1
12
10
8
6
4
2
30 I 30 II 30 III 30 IV 30 V 30 VI

Fig. 1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BLOOD SUGAR %
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SUGAR %
10% SOL.
2 GM. } PER KG. HR. INJ.
20 CC.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300
250
200
150
100
50
0
.6
.5
.4
.3
.2
.1
6
5
4
3
2
1
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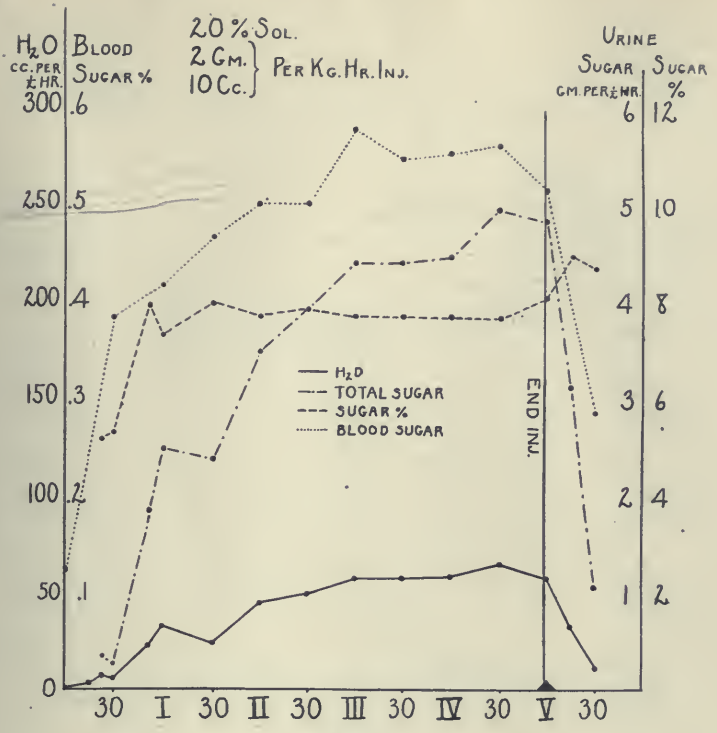
8
6
4
2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 2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BLOOD SUGAR %
20% SOL.
2 GM. } PER KG. HR. INJ.
10 CC.
URINE SUGAR GM. PER ½ HR.
SUGAR %
300 .6
250 .5
200 .4
150 .3
100 .2
50 .1
0
6 12
5 10
4 8
3 6
2 4
1 2
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
30 I 30 II 30 III 30 IV 30 V 30

Fig. 3

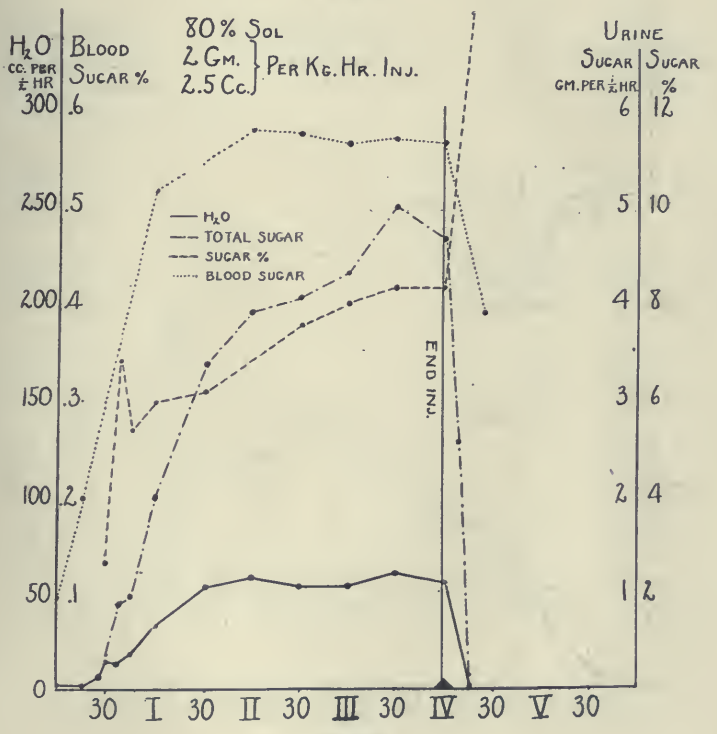
H₂O CC. PER ½ HR.
BLOOD SUGAR %
80% SOL
2 GM. } PER KG. HR. INJ.
2.5 CC.
URINE SUGAR GM. PER ½ HR.
SUGAR %
300 .6
250 .5
200 .4
150 .3
100 .2
50 .1
0
6 12
5 10
4 8
3 6
2 4
1 2
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
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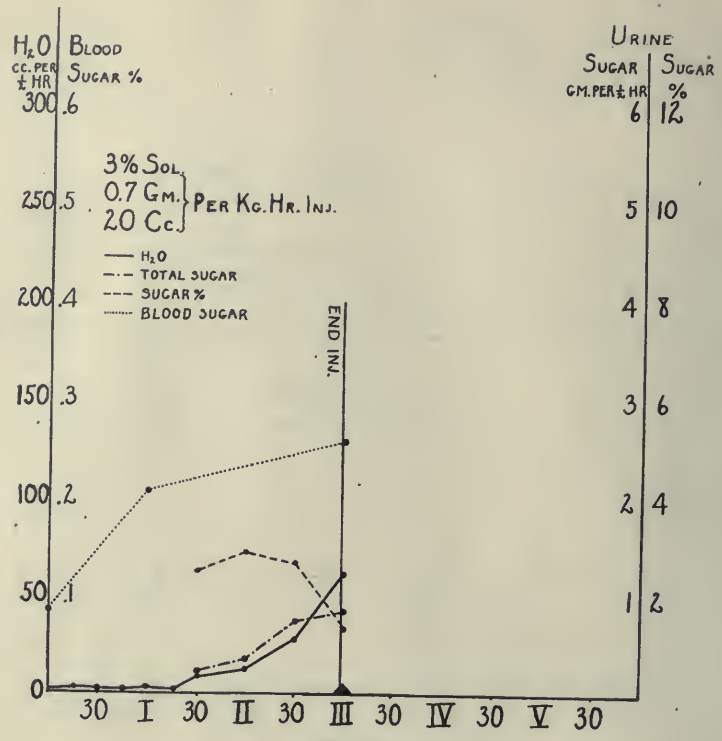
Fig. 4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BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR
URINE SUGAR %
3% SOL. 0.7 GM. 20 CC. PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300 .6 6 12
250 .5 5 10
200 .4 4 8
150 .3 3 6
100 .2 2 4
50 .1 1 2
0
30 I 30 II 30 III 30 IV 30 V 30

Fig. 5

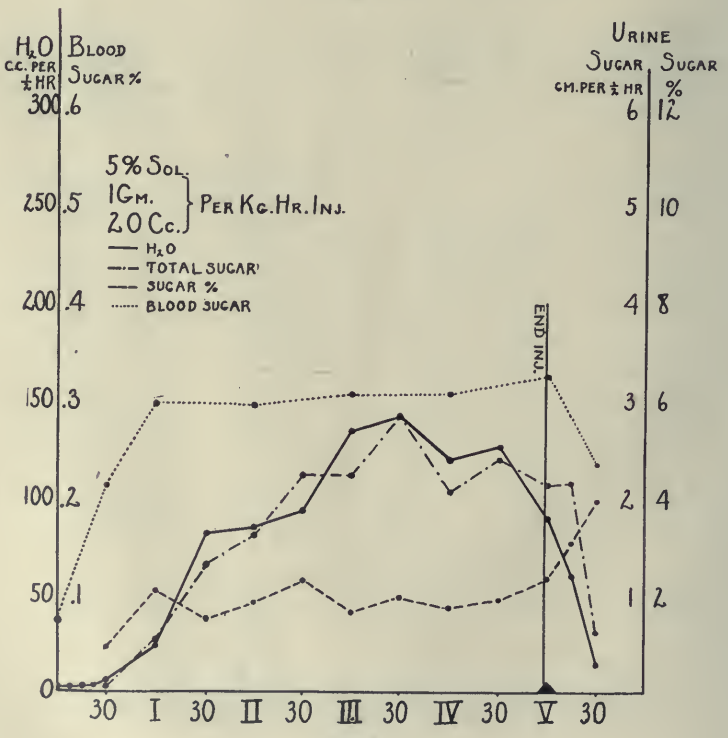
H₂O CC. PER ½ HR
BLOOD SUGAR %
URINE SUGAR GM. PER ½ HR
URINE SUGAR %
5% SOL. 1 GM. 20 CC. PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
300 .6 6 12
250 .5 5 10
200 .4 4 8
150 .3 3 6
100 .2 2 4
50 .1 1 2
0
30 I 30 II 30 III 30 IV 30 V 30

Fig. 6

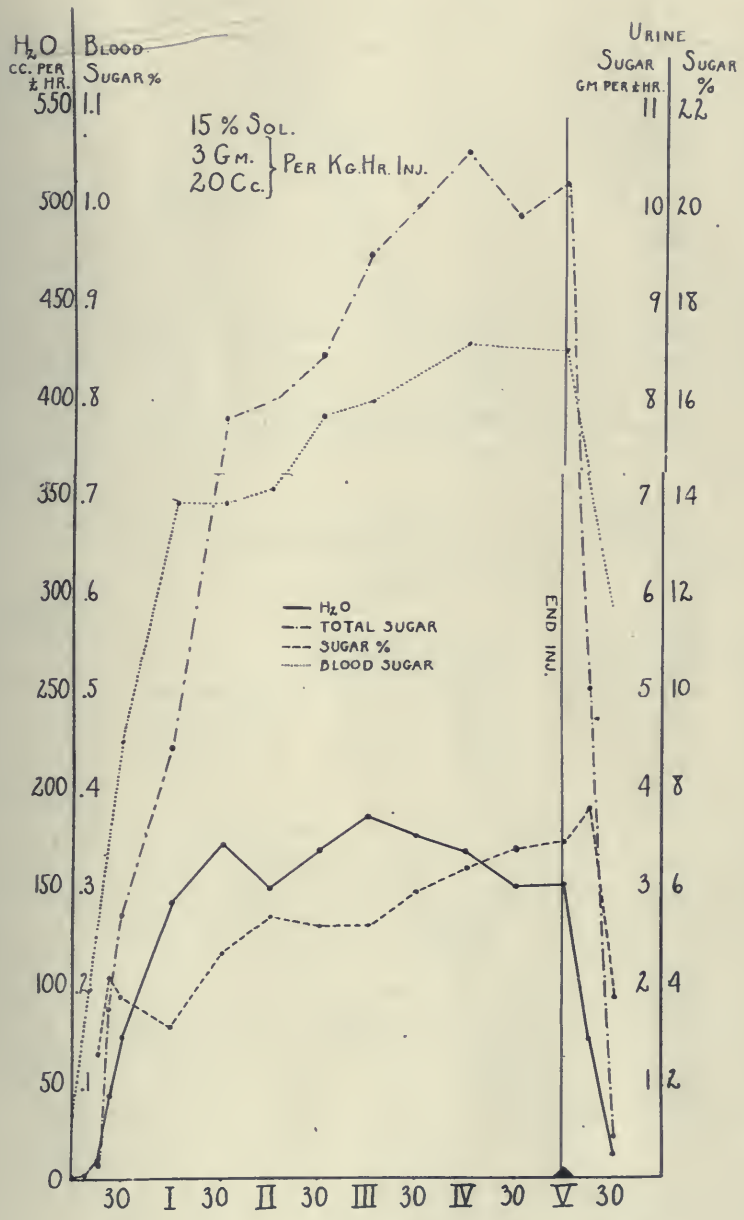
H₂O CC. PER ½ HR.
BLOOD SUGAR %
URINE SUGAR GM PER ½ HR.
SUGAR %
15 % SOL.
3 GM.
20 CC.
PER KG. HR. INJ.
H₂O
TOTAL SUGAR
SUGAR %
BLOOD SUGAR
END INJ.
550
500
450
400
350
300
250
200
150
100
50
0
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
11
10
9
8
7
6
5
4
3
2
1
22
20
18
16
14
12
10
8
6
4
2
30 I 30 II 30 III 30 IV 30 V 30

Fig. 7

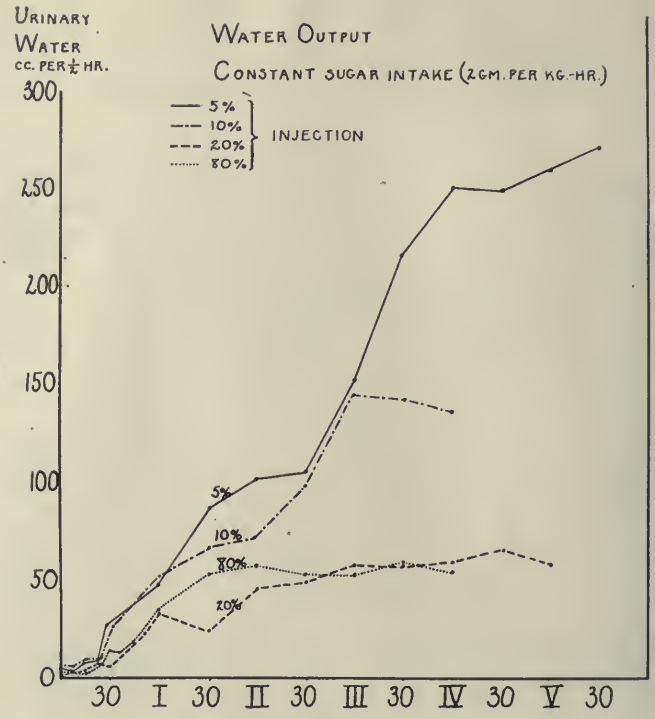
URINARY WATER CC. PER ½ HR.
WATER OUTPUT
CONSTANT SUGAR INTAKE (2 GM. PER KG.-HR.)
5%
10%
20%
30%
INJECTION
300
250
200
150
100
50
0
30 I 30 II 30 III 30 IV 30 V 30

Fig. 8

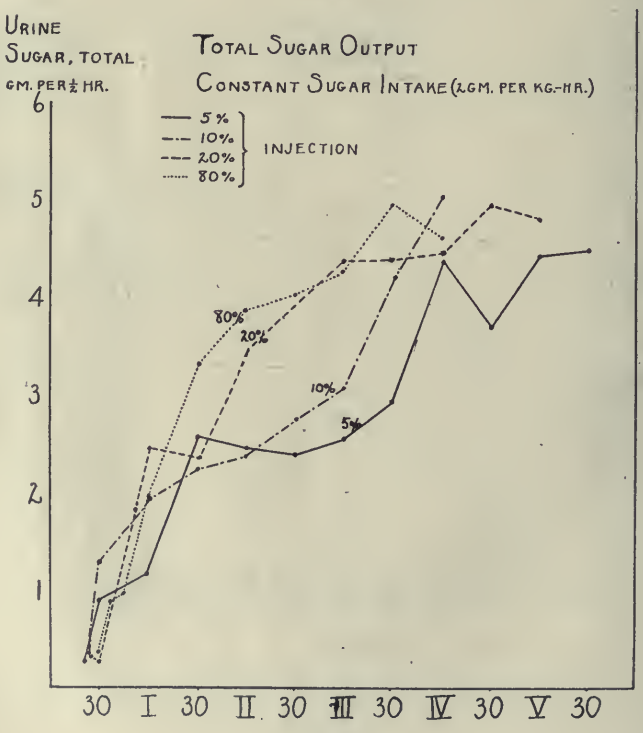
URINE SUGAR, TOTAL GM. PER ½ HR.
TOTAL SUGAR OUTPUT
CONSTANT SUGAR INTAKE (2 GM. PER KG.-HR.)
5%
10%
20%
30%
INJECTION
6
5
4
3
2
1
30 I 30 II 30 III 30 IV 30 V 30

Fig. 9

TABLE 2

DEXTROSE GRAMS PER KILOGRAM—HOUR		WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
		40	20	10	2.5
		Sugar grams per kilogram—hour	Sugar grams per kilogram—hour	Sugar grams per kilogram—hour	Sugar grams per kilogram—hour
2.0	Excreted	0.43	0.49	0.52	0.61
	Retained	1.57	1.51	1.42	1.39

that when, in his animals, carbohydrate was added to the diet, there was a sudden great increase in weight, due to a retention of water. This finding has been confirmed by Wimmer (12), Chauveau[2], Bischoff and Voit[2] and others. These experiments offer further striking confirmation by an entirely different method of investigation.

The following table (table 3) shows the fate of water injected with the sugar.

TABLE 3

SUGAR GRAM PER KILOGRAM—HOUR		WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
		40	20	10	2.5
		Water	Water	Water	Water
		cc.	cc.	cc.	cc.
2.0	Excreted	972	718.4	328	364
	Retained	788	178	94	−258

Although the diuresis was much greater with the dilute solutions, the amount of water retained was, like the sugar, also greater. The retention decreased steadily until, with the 80 per cent injection, there was an actual loss of water to the organism. Although one need not go as far as Zuntz in assigning a numerical value to the relation between the amount of water necessary for glycogen formation and the glycogen formed, yet it is clear that the retention curves of sugar and water run parallel to each other in these experiments. What has occurred, then, is an increased storage of glucose in the body, and one must seek further to make clear the effect of dilution of the injection on kidney function considered separately.

The data from the blood sugar determinations throw additional light on the mechanism by which this result is accomplished. They

[2] Quoted by Allen: Glycosuria and diabetes.

appear in figure 10. No blood sugar figures are available from the 10 per cent injection. When the strong solution (80 per cent) is injected, the blood sugar rises very sharply, nearly to 0.6 per cent, in striking parallelism with the rate of total sugar output in the urine and then remains approximately constant. With the weak solution, however, the initial rise is only in the neighborhood of one-half as great to be followed by a leisurely but steady rise through the five and one-

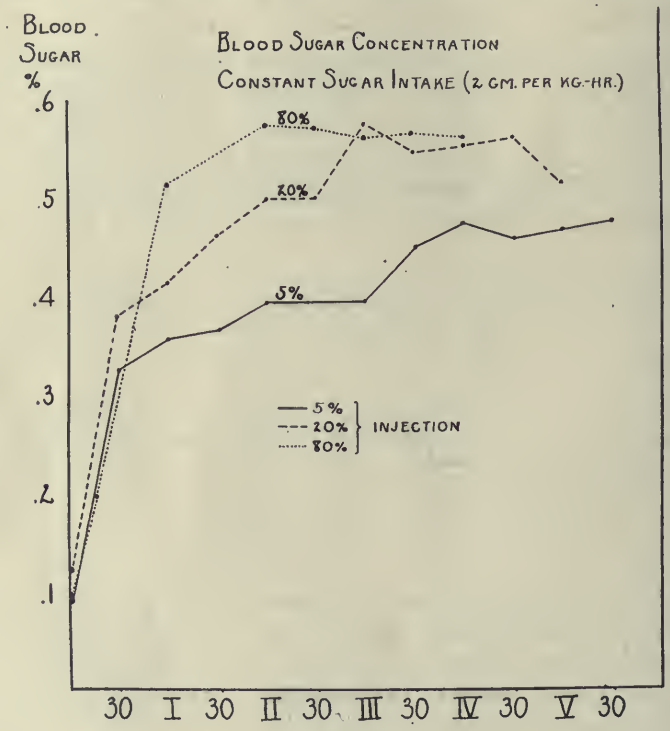

Fig. 10

half hours of the experiment. If figures for the water content of the blood were at hand, the interpretation of these findings would be assisted, but unfortunately they are not. The influence of dilution of the blood, with its attendant plethora, must be considered by indirect approach. This can be done after a glance at tables 2 and 3 and figure 10. In the 40 cc. (5 per cent) experiment, 788 cc. of water were retained, while a total of 18.7 grams sugar was excreted (see protocols). In the 2.5 cc. (80 per cent) experiment, 258 cc. of water were lost to the

body, the output being greater than the intake, and 26.6 grams sugar were excreted, the same quantity of sugar (88 grams) having been injected in each case. The animal then contained some 1046 cc. more of water and 7.9 grams *more* of sugar at the end of the first than at the end of the second experiment mentioned. The animal weighed about 11 kilograms, which would indicate a blood volume of roughly 770 cc. Now at the end of the 2.5 cc. (80 per cent) experiment, the blood sugar stood at 0.56 per cent, and even if one assumes—which is manifestly an exaggeration—that the entire 258 cc. which were lost during the experiment came from the blood and were not replaced, the circulation then contained $(770–258) \times 0.0056 = 2.87$ grams sugar, in 512 cc. of blood. Proceeding on the assumption that all of the 1046 cc. of fluid and 7.9 grams of sugar retained in excess in the 40 cc. (5 per cent) experiment remain in the plethoric blood, there would be in circulation at the end of this experiment ($1046 + 512 =$) 1558 cc. of fluid containing ($7.9 + 2.87 =$) 10.8 grams of sugar. If this were the case the concentration would be ($10.8 \div 1558 =$) 0.69 per cent, but the actual observed concentration at the end of the experiment was 0.476 per cent, so that it is evident that at least part of the 7.9 grams retained in excess has gone to the tissues along with the bulk of the retained sugar. In addition, to assume that 1046 cc. of fluid remain in the circulation is going far beyond the necessities of the situation, since it would be an increase in blood volume of 136 per cent, and it is doubtful if this can occur.

In the experiments of Fisher and Wishart, it was calculated from the hemoglobin content that the blood volume might be increased by as much as 50 per cent after the ingestion of glucose. It is unfortunate that no accurate method of determining blood volume is available. Similar calculations for the 10 cc. experiment demonstrate the same facts, even more conclusively.

One can then conclude definitely that hydremia does not account for all of the sugar which is retained in the body in the dilute injection experiments in excess over that retained in the concentrated injection experiments, and that a considerable part of this sugar leaves the circulation for the tissues. This is equivalent to saying that the presence of a greater quantity of water in the injection fluid accelerates the storage or at least the taking over of dextrose by the tissues.

These considerations now enable one to return to the question of the relative diuretic efficiency of concentrated and dilute solutions. If it is true that the presence of a plentiful excess of water assists the

flow of both sugar and water from the vessels to the tissues, what effect has it on the flow of the same substances from the blood into the urine?

Sugar in the body can scarcely affect the kidney except as it comes in contact with the renal cells while circulating in the blood. To make a fair comparison, therefore, times should be chosen during the various experiments at which equal concentrations of sugar in the blood occurred.

Since more sugar has been removed from the circulation by other means in the case of the dilute injections, the kidney, having to deal with a blood of lower glucose concentration, does not receive as great a stimulus as in the case of the concentrated injections until much later in the experiment. If, for instance, the even figures for blood sugar level of 0.1 per cent, 0.2 per cent, 0.3 per cent, etc., are arbitrarily chosen, and the rate of sugar excretion noted in each experiment at the time each of these levels was reached, a table like the following rough example (table 4) can be made.

TABLE 4

BLOOD SUGAR PER CENT	WATER CUBIC CENTIMETERS PER KILOGRAM—HOUR			
	40	20	10	2.5
	Sugar gram per 15 minutes	Sugar gram per 15 minutes	Sugar gram per 15 minutes	Sugar gram per 15 minutes
0.1	0		0	0
0.2	0		0	0
0.3				
0.4	2.75		1.2	1.3
0.5			3.0	2.25
0.6			4.5	3.75

Glycosuria begins in each experiment when the blood sugar is between 0.2 and 0.3 per cent. At 0.4 per cent the 40 cc. injection experiment is excreting 2.75 grams of sugar per fifteen minutes, while much less is being put out in the case of the more concentrated injections. From this standpoint, then, at a given level of blood sugar, the presence of an excess of water accelerates the passage of sugar from the blood into the urine, just as it does from the blood to the tissues.

Comparing figure 10 and figure 9, it will be seen that the nearest approach to parallelism exists between blood sugar and total sugar

output. This relation is maintained in all the experiments, the total sugar curve showing a tendency to hold its course more or less regardless of changes in the quantity of urine, especially the minor ones, which are compensated for in a truly remarkable way by changes in the concentration. This is represented graphically in some of the curves—the concentration changing one way, the quantity the other, and the total sugar line continuing its original course.

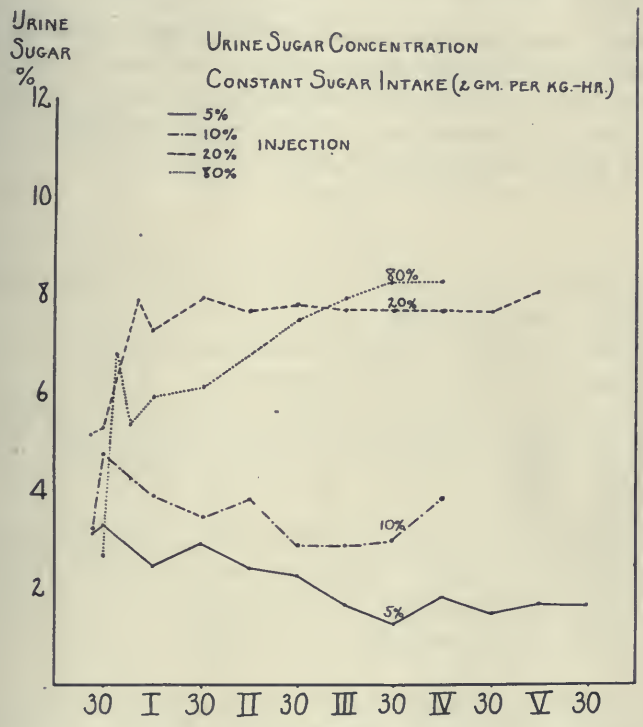

Fig. 11

Figure 11 represents the urinary sugar concentration in the first four experiments. In those cases with copious diuresis the concentration falls off as the diuresis increases. The concentration in the 80 per cent (2.5 cc.) injection is below that in the 20 per cent (10 cc.) injection at first, because the diuresis commenced earlier with the 80 per cent injection. The concentration does not seem to run parallel with the blood sugar, but varies inversely as the quantity of water available. Thus in figure 7 the steady increase in the sugar output

is obtained by a steady increase in the concentration, the urine quantity remaining constant, while in figure 3 exactly the reverse is true. Epstein's (6) statement, as noted above for diabetics, that the concentration is constant, the sugar output varying with the quantity of urine excreted, is certainly not true for a normal dog excreting sugar under these conditions. The concentration may continue to rise when the blood sugar goes above 0.4 per cent (fig. 7) contrary to the statement of Ewing (5).

The series of injections in which the amount of water given remained constant (20 cc. per kilogram-hour), figures 5, 6, 2 and 7, shows that the water output in such a case is entirely dependent on the sugar content. This is in accordance with the well known fact that distilled water given intravenously causes no diuresis.

SUMMARY

It is felt that the experimental conditions described are unusually free from disturbing influences.

Under these conditions the sugar output per unit of time shows a direct relationship with the blood sugar level.

Upon intravenous injection of a given quantity of dextrose, the amount of diuresis produced depends on the amount of water available, within certain limits. If a very concentrated solution is given, the body is robbed of water.

If large quantities of water are given with the dextrose, its percentage retention in the body is increased and water is retained along with it, so that less sugar is excreted, despite diuresis.

This retention is not confined to the blood, but occurs also in the tissues.

If more water is available, more dextrose is excreted into the urine *at a given level of blood sugar* than when less water is available.

I am much indebted to Mr. J. H. Janney of the fourth year class in the medical school for his valuable assistance in carrying out these experiments.

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FURTHER STUDIES ON THE EFFECT OF ADRENALIN UPON MUSCULAR FATIGUE

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Muscular activity in conjunction with Addison's disease is extensively discussed in the older literature. Before experimental work was begun on the adrenal glands it was well known that marked muscular weakness was characteristic of Addison's disease. In 1892, Abelous, Langlois and Charrin (1) showed that persons suffering from this malady were less capable of doing muscular work, as measured by the Mosso Ergograph, than were individuals suffering from tuberculosis. When it was discovered that adrenalin excited the sympathetic nervous system, study of its effect upon the muscular system was for a time abandoned.

Recently, however, the study of the relation of adrenalin to muscular activity has been resumed. Three methods have been employed. They are, the older method, that of extirpating the glands, and the newer methods, those of splanchnic nerve stimulation causing the glands to secrete their adrenalin into the circulation and of injecting the adrenalin into the circulatory system or into the solution in which the muscle is contracting.

In 1892 Albenese (2) removed the glands from frogs and rabbits. Upon cutaneous stimulation, he observed that a stronger current was necessary to elicit a response after extirpation than before. That this increase was not due to the operation was shown by subjecting animals to more severe operations than decapsulation. He therefore concluded that the function of the adrenal glands was to destroy or to transform the toxic substances which, as a result of muscular or nervous work, are produced in the organism. His results were confirmed by Boinet (3) who reported that rats recently decapsulated were much more quickly exhausted in a revolving cage than were normal animals. A similar loss of muscular power was recorded by Biedl (4).

Founding his belief upon experiments done alone and with Langlois (5), Abelous (6) maintained that the suprarenal capsules, in frogs and guinea pigs, can modify, neutralize or destroy poisons which are produced in the course of muscular work or which accumulate in the organism after destruction of the adrenal glands. Adrenalin acts, he believed, as an antitoxin to fatigue. He observed that if the blood of an animal dying from decapsulation is injected into a normal animal true symptoms of fatigue result.

Oliver and Schäfer (7) in 1895 showed that adrenal extract has a bettering effect on the contraction of an unfatigued muscle. After injecting the extract subcutaneously into a frog and then excising the gastrocnemius muscle, they obtained a curve of contraction which was 33 per cent higher and 66 per cent longer than that of the corresponding muscle not supplied with the extract. A similar prolongation of the muscle curve was observed after the extract was injected intravenously into a dog. Analogous experiments were performed in 1915 by Kuno (8) and in 1916 by Takayasu (9), who obtained opposite results. Takayasu claims that adrenalin has a depressing rather than a bettering effect upon muscular contraction. He quotes Schäfer as saying of his own early experiments that he believed the results he obtained were due not to the action of adrenalin but to some impurities contained in the commercial extract which was employed. Schäfer also makes note of this belief in his recent publication, *The Endocrine Organs* (10). These results would seem to dispose of Joteyko's (11) arguments that adrenalin is a sarcoplasmic excitant.

Betterments in fatigued muscles after injections of adrenalin were observed by Boruttau (12) working on frogs and Dessy and Grandis (13) working on salamanders. Dessy and Grandis claim that the adrenal extract produces a beneficial effect on fatigued muscle either when injected subcutaneously or when added to the solution in which an isolated muscle is contracting. Cannon and Nice (14) were unable to confirm Dessy and Grandis' observations in frogs' muscles similarly exposed to the adrenal extract.

In a case of neurasthenia, Pantanetti (15) recorded a marked betterment in the total amount of work done after six subcutaneous injections of adrenalin.

The influence of fatigue upon the adrenalin content of the suprarenal glands in dogs was studied by Battelli and Boatta (16). In completely exhausted animals the adrenal content of the suprarenal capsules was decreased to less than one-sixth the normal amount. These

investigators believe that during fatigue adrenalin is set free to keep up the blood pressure which has a tendency to lower itself by dilation of the vessels in the active muscles. That the chromaffin system is thus exhausted by prolonged muscular effort was later confirmed by Carl (17) on strychninized frogs. Bernard and Bigart (18) made examinations of the histological structure of the adrenal gland before and after muscular fatigue. It was discovered that after prolonged muscular activity the gland contained numerous vacuoles. Bardier and Boone (19) confirmed them.

Carnot and Josserand (20) found that if 0.025 mgm. of adrenalin per kilo body weight was injected into the femoral vein of a normal dog there was an increase of 10 cm. of mercury in arterial pressure; if injected into the femoral artery (leg at rest) it produced an elevation in arterial pressure, of only 2 cm. of mercury. In one particular experiment upon a dog they injected 0.05 mgm. of adrenalin per kilo body weight into the femoral artery of a leg at rest and found that the blood pressure in the general circulation was increased 10 cm. of mercury. The opposite leg was then tetanized for fifteen minutes and at the end of that time 0.055 mgm. of adrenalin per kilo was injected into its femoral artery and the systemic blood pressure rose only 1.5 cm. of mercury. They claim that the adrenalin was neutralized or destroyed by the fatigue products.

Panella (21) observed a marked improvement with the use of adrenalin in the curve of contraction in heterothermic animals, frogs and toads, and also in homothermic animals, rabbits and guinea pigs, placed by special treatments (section of the bulb or profound narcosis), in a state of respiration, circulation and heat regulation somewhat like that of heterothermic animals. He believed that under normal mammalian conditions adrenalin has little effect because it is quickly oxidized or destroyed in the blood.

Radwánska (22) found that when the gastrocnemius muscle of a frog was wholly fatigued it could be made to contract again by injecting adrenal extract subcutaneously. He also found adrenin more effective in decapsulated than in normal frogs. Because the results were better when the muscle was stimulated through its nerve than when stimulated directly, be believed that adrenin acts upon the nerve endings. His belief was shared by Cannon and Nice (23) who thought the point of action of adrenin to be upon the nerve endings or neuromuscular junctions. They found that adrenin injected in small doses or secreted during splanchnic stimulation caused a marked improvement in the activity of the fatigued muscle.

A decrease in the irritability of the nerve in the nerve muscle of a frog as a result of the removal of adrenalin from the system by extirpation of the adrenal glands and a restoration of irritability upon injecting adrenalin into the same animal were demonstrated by Czubalski (24). He has assumed that the first phase of the action current is due to dis-assimilation or katabolism and the second phase due to assimilation or anabolism. Working upon this assumption he obtained evidence that the quick exhaustion of the muscles of decapsulated animals is due to slow and incomplete anabolism within the muscle. This process could be hastened by adrenalin.

In a series of papers published a few years ago, I (25) showed that adrenalin increased the height of muscular contraction when injected intravenously in small doses (0.2 to 0.5 cc. of a 1:100,000 solution), and that the same dose administered in the same manner would bring back to normal in five minutes or less the increased threshold stimulus caused by fatigue of the nerve-muscle or muscle, as would rest of one to three hours. I also showed by means of a comparative study of the effects of adrenalin and amyl nitrite that this improvement after fatigue was not due to a bettering of the circulation. Invariably the effect produced by adrenalin was greater although the effect upon the general blood pressure was the same. In some experiments the limb was perfused with warm Ringer's solution. If adrenalin was injected into this perfusion fluid a betterment in muscular contraction but always a decrease in the flow of the fluid (vasoconstriction) from the venous cannula was observed. The theory held by Radwánska that the action of adrenalin was on the nerve endings I disproved since I found that adrenalin has the same effect upon the muscles in which the nerve has been cut from nine to eighteen days as it has in normal muscles.

Recently Hoskins, Gunning and Berry (26) demonstrated that adrenin produces active vasodilation of muscle vessels and they believed that the betterment in the height of muscular contraction, which I demonstrated, was due, in part at least, to the betterment in circulation. In my experiments there had been three factors tending to bring about extreme dilation of the vessels in the active muscle. First, the nerves were severed and the vasomotor tonicity from the vasomotor center, therefore, inoperative; second, the rate of stimulation employed was favorable to dilation (27) and third, as Kaufmann (28) has shown, the fact that the muscles were active would presuppose actively dilated blood vessels. These circumstances led me to be-

lieve that in my former work any further dilation would have been so slight as to be negligible. Since I had not, however, separated the cutaneous circulation from the muscle circulation it seemed important that I repeat my work with the view to determining the condition of the blood flow through the muscle alone.

METHOD

In the earlier experiments the animals (cats) were anaesthetized with urethane (2 grams per kilo body weight by stomach), but later continuous ether anaesthesia was employed. By making a small slit through the skin on the outer side of either thigh, the anterior tibial nerve (peroneus communis) was isolated, cut and its distal end fastened in a Sherrington shielded electrode (29). The electrode was then held in place by fastening around it, with paper clips, the two flaps of skin.

Through another slit in the skin in the same leg the tendon of the tibialis anticus muscle was isolated from its insertion. It was then fastened to a muscle lever mounted upon a tripod base by a string passing about two pulleys. These pulleys were arranged so that the muscle pulled in its normal direction. One loop of cord about the hock and another around the foot just below the fastening of the tendon bound the leg to the board and made a very satisfactory nerve muscle preparation.

The strength of the stimulating current was 0.1 ampere in the primary circuit derived from a storage battery. The stimulating current in every case was a maximal break induction shock obtained from a glass knife blade key (30), which, propelled by a motor, made and broke the primary circuit. The rate ninety times per minute in these experiments was slow enough to produce vasodilation (27) in the vessels of the stimulated muscle. The muscle lever consisted of a piece of light straw 22 cm. in length from the axis to the tip of the parchment paper writing point. The tendon attached 3 cm. from the axis began at the moment of contraction to pull against an initial tension of 110 grams developed in a coiled spring. For each centimeter excursion of the muscle lever on the drum this was increased 5 grams. This spring was attached at the same position on the lever as was the muscle. Muscular contraction was, therefore, magnified about seven and one-third times.

The blood pressure, whenever recorded, was registered from one or the other carotid artery by means of a mercury manometer. A time marker which indicated intervals of five or of thirty seconds was placed at the atmospheric pressure line of the manometer. Thus, at any given muscular contraction, the height of blood pressure could be determined. Below the time marker was placed another signal magnet to indicate the time of the injection of adrenalin.

The rate of blood flow through the muscle was recorded on the kymograph paper in the early experiments by a simple key and signal magnet. Later an automatic drop recorder was substituted for the hand key. It consisted of a flat steel spring to which was soldered a platinum disc in contact with a platinum point adjustable by a thumb screw. The platinum point and steel spring were connected by copper wires to binding posts on the vulcanite arm which supported them. Each drop falling upon the spring broke the contact between the platinum disc and the point and the drop was recorded on the drum by the signal magnet. The recorded blood flowed through a cannula placed in the femoral vein. *All the branches* to the vein were tied off except the deep anterior tibial vein which comes from the tibialis anticus muscle and muscles of that region. The cutaneous vessels were tied and the limb was either skinned or mass-ligated above the hock. In the early experiments a necropsy was made after each series of observations to make sure that there had been a separation of the cutaneous and muscular circulations.

Usually adrenalin chloride but in many cases crystalline adrenalin in solution was injected into a cannula placed in the external jugular vein. The dilution of adrenalin with mammalian Ringer was made in all cases just after the operation so that the solution had no appreciable time for deterioration. This solution was kept at body temperature for injections.

Experiments were also performed in which the muscle was irrigated. The medium for irrigation, a warm (38.5 to 39.5°C.) Ringer's solution at a pressure of 60 to 70 cm. of water, ran through cannulae placed in the femoral artery and femoral vein after all the branches leading to and from these vessels were tied off except the two vessels of the deep anterior tibial region. The adrenalin, in most cases 1:100,000 but in some cases 1:1,000,000 and 1:1000 solution was injected into the running fluid close to the arterial cannula.

RESULTS

In no instance in my experiments did adrenalin in small doses (0.5 to 2 cc. of a 1:100,000 or 1:1,000,000 solution) produce dilation of the vessels in the limb in which the nerve was cut and stimulated at a rate favorable to dilation. In animals with the circulation undisturbed (except that the nerve was cut and stimulated) the vessels of the limb responded passively to adrenalin (0.5 to 2 cc. of a 1:100,000 solution) i.e., if the blood pressure decreased the rate of flow through the muscles decreased and vice versa. In the perfused limb with the nerve cut and stimulated vasoconstriction was obtained even with doses as small as 0.5 cc. of a 1:1,000,000 solution.

The action of adrenalin in intact muscles

Figures 1 and 2 are records made during one experiment and selected as typical of all the results obtained. The animal weighed 2.5 kgm. and was given 25 mgm. of hirudin intravenously as an anticoagulant just before the experiment and after the operation.

In figure 1 the blood pressure was 54 mm. of mercury. Upon injecting intravenously 0.5 cc. adrenin (1:100,000 solution) the pressure decreased to 44 mm. of mercury with a resultant decrease in the rate of blood flow through the muscle of 21 per cent and a concurrent increase in the height of muscular contraction of 55.5 per cent.

In other animals upon injecting 0.5 cc. of a 1:100,000 solution of adrenin no measurable change in the blood pressure but always an increase in the height of muscular contraction, in some cases as much as 35 per cent, occurred. The rate of blood flow remained unaffected. There is, of course, in all cases, a gradual decrease in rate and lowering of pressure due to the loss of blood from the recording vein.

Before figure 2 was recorded, 50 cc. of warm Ringer's solution was added to the blood that was lost by hemorrhage and the mixture was perfused back into the animal. As a result of the transfusion the blood pressure increased slightly (20 to 52 mm. of mercury). At *1* in figure 2, 2 cc. of a 1:100,000 solution was injected intravenously. The blood pressure rose 38 per cent and the height of muscular contraction was bettered 53 per cent. Since the same quantity of adrenin injected slowly at *2* produced no change in muscular contraction the betterment observed cannot be due to the injection of adrenin at *1* and *3*. Both the dilation of the vessels of the muscles and the increase in the height of muscular contraction were due to the increase

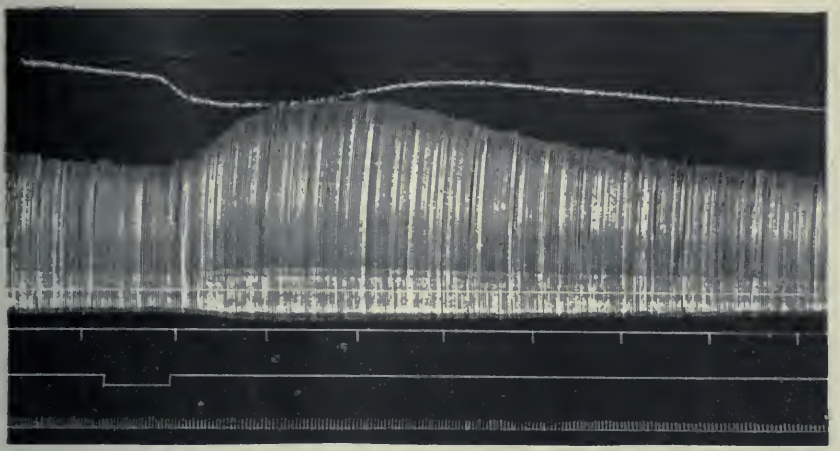

Fig. 1. In this and the following figure the upper curve is a record of blood pressure with mercury manometer, below it the record of muscular contraction. The lowest record indicates the number drops flowing from the venous cannula or limb circulation, above it is the signal record indicating the point of injection of adrenalin. Middle line time in thirty seconds. Hirudin was used as an anticoagulant. In this figure the time marker was placed 2 cm. below the atmospheric pressure level of the mercury manometer to allow room for muscular contraction. At the point indicated in the record 0.5 cc. of adrenalin (1:100,000) was injected.

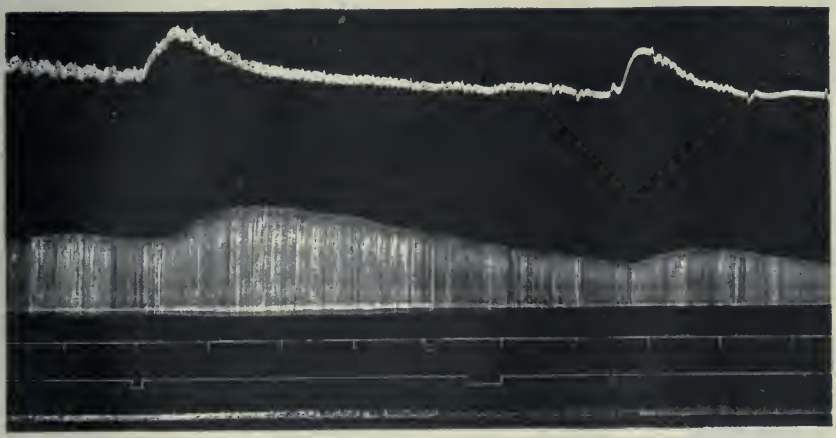

Fig. 2. Time marker 3.5 cm. below zero blood pressure. At the points indicated by the marker 2 cc. of adrenalin (1:100,000 solution) were injected.

in blood pressure. The increase in blood pressure is sufficient to account for the increased muscular efficiency (25).

The results obtained upon animals into which the lost blood plus Ringer's solution was perfused, were rarely satisfactory.

The action of adrenalin in perfused muscles

In the perfused muscles doses of adrenalin varying from 1 cc. of a 1:1,000,000 to 1 cc. of a 1:1000 solution produced a betterment in the height of muscular contraction with a decrease in the rate of flow of the perfusion fluid.

Figure 3 is a record obtained from an animal which had been given hirudin. Adrenin, 0.5 cc. (of a 1:100,000 solution) was injected at *1* and 2 cc. of the same solution was injected at *2*. As a result of the first injection there was a betterment of 17 per cent in the height of muscular contraction and from the second injection there was a betterment of 550 per cent with intermediary betterments, the whole lasting for six minutes. Here, as in most instances, the increase and subsequent decrease were gradual. After the first injection the rate of flow through the vessels decreased from seventy-four to thirty drops per thirty seconds and after the second injection the rate of flow decreased from thirty-five to less than one drop per thirty seconds. It is observed in this experiment as in many others that the maximal increase in the height of muscular contraction occurs simultaneously with the maximal constriction of the muscle vessels. These conditions are probably coincident and not interdependent.

When a muscle has ceased completely to respond to the original strength of stimulus it can be made to react again after an injection of adrenalin (see fig. 4). Here at *1*, 0.5 cc. of a 1:100,000 solution injected into the perfusion fluid produced a rise in the muscle curve of 26.6 per cent with no noticeable change in the circulation. At *2*, 1 cc. produced a rise of 32.5 per cent in muscular contraction and a decrease of 5 per cent in the rate of flow of the fluid through the muscle. At *3*, 1 cc. of the solution produced no noticeable effect upon the muscle. From point *3* to point *4* the muscle curve dropped until no contraction occurred. At *4*, 2 cc. of adrenalin were injected into the perfusion fluid. As soon as the adrenalin reached the blood vessels a marked vasoconstriction occurred, followed soon after by a slight muscular twitch which increased slowly until a contraction 6 mm. in height was obtained and then decreased to nil again. The length of time occupied by the entire recovery contractions was about six minutes.

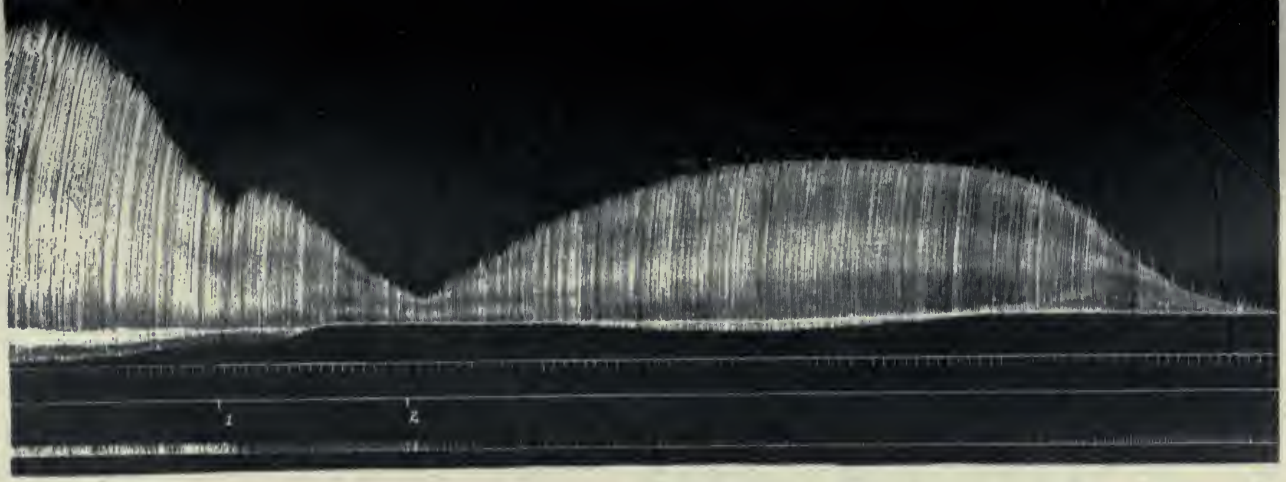

Fig. 3. Perfused muscle. At *1*, 0.5 cc. adrenalin (1:100,000 solution) was injected into the perfusion fluid. At *2*, 2 cc. were injected. Time in five seconds. The initial tension of the spring in this experiment was 140 grams with an increased tension of 7 grams per centimeter space on the drum. In this and the following figure the upper curve is the muscle record, below it the time record and the lowest line a record of the rate in drops of flow of the perfusion fluid through the muscle.

Fig. 4. A curve showing recovery of the muscular contraction in a completely fatigued perfused muscle. At *y*, the drum was stopped for five seconds. Adrenalin (1:100,000 solution) was injected, at *1*, 0.5 cc., at *2*, 1 cc., at *3*, 1 cc. and at *4*, 2 cc.; *x*, adjustment of the cannula. Time in thirty seconds.

Is adrenalin in large doses toxic?

Takayasu concluded that adrenalin in large doses was toxic in character and its effect resembled that of increased potassium salt concentration. Cannon and Gruber (31) remarked that in normal muscles adrenalin in large doses (1 cc. of a 1:10,000 solution intravenously) sufficient to cause a marked constriction of the arterioles, results in a lessened height of contraction and slowing or even a disappearance of the wavelike variations observed in rhythmically stimulated muscles. This muscular inefficiency they attributed to a deficient blood supply. The effect upon perfused muscles, of more concentrated solutions than that employed by Cannon and Gruber, is not different from that produced by more dilute solutions. Here may be given the results of one typical experiment. Adrenalin 0.5 cc. (1:100,000) was injected into the perfusion fluid. There resulted from this injection a betterment of 100 per cent in the height of muscular contraction. After this injection 0.5 cc. adrenalin (1:1000) was injected and there was observed a twenty-seven fold increase in the height of muscular contraction which required sixteen and one-half minutes for its development and return to the original height. Here as before marked vasoconstricton occurred.

The effect of adrenalin upon the make shock. In figure 4, the irritability was increased by adrenalin to such an extent that the make shock, which had been subminimal became submaximal at *1* and *2*. In two instances in which a 1:10,000 solution was employed the make shock contraction became equal to the break shock contraction.

In my experiments muscles exposed to the action of hirudin seemed to be more vigorous and to respond to adrenalin better than did normal muscles. According to Tatum (32) adrenalin has no action upon hirudin blood and hirudin plasma and vice versa. The question of the effect of anticoagulants upon muscular contraction is open for further investigation.

How is the specific effect of adrenalin in muscular fatigue shown?

All the curves presented in this paper except figures 2 and 4, *3*, show the specific effect of adrenalin on muscular fatigue. This effect is noted whether the limb is intact or perfused.

Figures 2 and 4, *3*, are given to show that at times adrenalin does not have this effect. Cannon and Nice (23) show a similar curve

(fig. 5, page 52) from a muscle of an animal undergoing splanchnic stimulation. In their experiment the blood pressure was kept at a fixed level by compression of the thorax. During that time, although there was undoubtedly a secretion of adrenalin caused by splanchinc stimulation, the muscle remained unaffected. At the moment at which the thorax was released, the blood pressure rose and with it the height of muscular contraction rose 466 per cent.

In an earlier article (33) I showed curves (figs. 6 and 7) in which adrenalin had the effect of lowering the blood pressure and the muscle curve 18.7 per cent in figure 6 and 17.3 per cent in figure 7. It might be argued in these cases that adrenalin may have had its specific effect upon the muscle but that it was obscured by the fall in blood pressure and decreased blood flow. Such is probably not the case as seen from *b* in both figures. An equal decrease in the height of muscular contraction and lowering of the blood pressure without the presence of adrenalin was brought about in figure 6, *b*, artificially by compressing the thorax. In figure 7 at *b* adrenalin was injected as at *a* but the blood pressure was maintained at a normal level by stimulating the splanchnic nerves with the adrenal glands tied off. The muscle curve was unaffected.

In the above described cases the only effect of adrenalin on the muscle curve must be a passive one. By lowering or raising the blood pressure it decreases or improves the muscle irritability.

The curves herein presented other than 2 and 4, *3*, show the specific effect of adrenalin. In figure 1 this is especially great for animals in which the circulation is intact. The injection of adrenalin brought about a decrease in the rate of blood flow but an increase in the height of muscular contraction of 55 per cent. In perfused limbs the increase is so great as to be almost unbelievable. Figure 3 shows an improvement of 550 per cent. In other experiments improvements of 2730 per cent were observed.

How does adrenalin produce its effect?

There has been much speculation as to the probable point of action of adrenalin. Although the question cannot be settled in this article it may not be amiss to discuss some of the possibilities.

1. *Does it act upon Langley's "receptive substance"?* Langley (34) has demonstrated a hypothetical "receptive substance" between the nerve endings and the muscle. In another paper (35) I showed that

the threshold stimulus of a denervated muscle was unaffected by curare and that adrenalin had no effect upon the curare threshold in this same muscle. I found, however, that the threshold of a curarized muscle with the nerve intact was affected by fatigue and that adrenalin counteracts this fatigue. If then curare acts upon the "receptive substance," fatigue and adrenalin must act at another point nearer the muscle than the "receptive substance."

2. *Is adrenalin a sarcoplasmic excitant?* It has been my observation that adrenalin does not lower the threshold of a normal, unfatigued muscle (25). Were adrenalin a sarcoplasmic excitant as suggested by Joteyko (11) it would probably do this and if it were an excitant at all it would certainly not be necessary to fatigue the muscle before adrenalin produced a marked result. This conclusion is further substantiated by Kuno and Takayasu.

3. *Does it neutralize, destroy or transform fatigue products?* One of the most reasonable explanations is this, that adrenalin has some effect upon lactic acid, rendering it less harmful to the active muscle. Various experimenters some of whom are Abelous and Langlois, Albanese, Carnot and Josserand, Joteyko and Gruber, have thought this a feasible explanation. This leads to the fourth possibility.

4. *Does adrenalin hasten the conversion of glycogen into sugar and does it assist in the reconversion of lactic acid into sugar?* It has been found that in a normal muscle with the circulation intact the same dose of adrenalin injected repeatedly brings about approximately the same reaction while in a perfused muscle a second or third injection has to be larger than the first to bring about the same response. This would seem to indicate that adrenalin acts upon some substance supplied through the blood or which is present in the muscle. Cannon and Nice (23) came to the conclusion that the increased muscular contraction cannot be due to hyperglycaemia because a typical rise could be obtained when the liver was removed. If an injection of adrenalin liberates sugar from the liver it does not seem improbable that the same injection could liberate sugar in the muscle. If that is the case, such liberation of sugar in the muscle might not be indicated in the blood and might along with the normal supply of sugar in the blood, supply the necessary energy for the increased muscular activity. This inference is in accordance with that of Cybalski (24) who believed that the decreased action current in the nerve muscles of decapsulated frogs and fatigued animals was due to the sluggish anabolic process and adrenalin is capable of accelerating this process,

but it is not in accordance with that of Wilenco (36) who maintains that the ability of the organism to burn sugar is decreased by adrenalin.

It must be admitted that none of these explanations is without its weak points and that none completely accounts for the various apparrently contradictory results obtained. At present a chemical study is being undertaken to determine more definitely the point of action of adrenalin.

SUMMARY

In the fatigued unaltered nerve muscle adrenalin may increase the height of muscular contraction by a twofold action, by improvement of the blood supply (vasodilation) and by its chemical action upon some substances in the muscle.

In a muscle in which the nerve is cut and stimulated, adrenalin in small doses, however administered, does not better the circulation and must therefore produce its effect of increasing the height of muscular contraction by its chemical (specific) action alone.

The following three processes which normally go on in the muscle may be greatly accelerated by adrenalin and it is not improbable that one or all of these will finally prove to be the way in which adrenalin produces its effects:

1. The conversion of glycogen into sugar.
2. The reconversion of lactic acid into sugar (transformation of fatigue products).
3. The oxidizing of lactic acid into carbon dioxide and water (destruction of fatigue products).

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THE EFFECT OF PHOSPHORUS POISONING ON THE CATALASE CONTENT OF THE TISSUES

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In phosphorus poisoning all the organs of the body are injured, the liver being the most affected. This organ first undergoes fatty degeneration with subsequent disintegration of the liver cells. It is supposed that phosphorus causes fatty degeneration by rendering oxidation deficient while the disintegration of the liver cells is supposed to be brought about by the increased autolysis. As a result of the investigations of Jacoby (1), Waldvogel (2), Welsch (3), Aberhalden and Bergell (4), Reiss (5), Wolgemuth (6) and others it is now generally accepted that the essential facts in phosphorus poisoning are an increased rate of autolysis and a defective oxidation in the tissues.

It has been shown in this laboratory that the catalase content is an index to the amount of oxidation in a tissue, being greatest where oxidation is greatest and least where oxidation is least. Furthermore, it was found that when oxidation was decreased in a tissue, as was indicated by a decrease in the catalase content, the tendency of that tissue to undergo autolysis was correspondingly increased. In starvation, for example, it is known that the rate of autolysis is greatly increased in all the tissues of the body except the heart and central nervous system. We found in starving animals that catalase, and hence oxidation, was decreased in those tissues in which autolysis is known to be increased and remained normally high in the heart which is not autolyzed during starvation. Furthermore, in thyroid feeding, it was found that the catalase content, and hence oxidation, was decreased in the fat, skeletal muscles and heart, with a corresponding increase in the rate of autolysis. In view of the fact that the autolyzing enzymes in common with all the ordinary enzymes are easily oxidized and destroyed and that autolysis can be increased in a tissue by decreasing oxidation, and vice versa, the assumption was made that there normally exists a balance between the autolyzing and oxidizing enzymes,

the idea being that when oxidation is increased in a tissue a larger amount of the autolyzing enzymes is oxidized and destroyed with resulting decrease in autolysis, and vice versa.

It is known that autolysis is more intense in the liver than in any of the other organs of animals suffering from phosphorus poisoning. If, according to the preceding hypothesis, the amount of autolysis in an organ is controlled by oxidation, then oxidation should be decreased to a greater extent in the livers of animals suffering from phosphorus poisoning than in any of the other organs. The object of this investigation was to determine if this is true. Cats were used in these experiments. Fifteen of these animals were placed in separate cages. Ten of them were fed daily 60 grams of salmon each, to which 2 mgm. of ordinary yellow phosphorus, previously dissolved in cod-liver oil, had been added; while the remaining five animals were fed the same amount of salmon without the addition of phosphorus. As a rule all of the animals ate the salmon to which the phosphorus had been added, for the first two days. After this some of the animals would eat a part, others all of the material, while some refused to eat any of it.

In the table after "cat fed phosphorus for three days" are given the data from cats that refused to eat the phosphorus after the third day while after "cat fed phosphorus for six days" are given data from animals that had been fed phosphorus for that length of time. When the animals were etherized, approximately 25 cc. of blood were drawn and the blood vessels, by the use of large quantities of 0.9 per cent sodium chloride, were washed free of blood as was indicated by the fact that the wash water gave no test for catalase. The liver and heart were then removed and ground up separately in a hashing machine. Since the blood of the animals that were severely poisoned did not clot it was used without further treatment, while the clotted blood from the less severely poisoned and normal animals was pressed several times through several thicknesses of cheese cloth and ground up in a mortar. The catalase content of the heart was determined by adding 1 gram of the ground material to 45 cc. of hydrogen peroxide, while 1 gram of the liver was added to 500 cc. of hydrogen peroxide in a bottle. A greater amount of hydrogen peroxide was used for the liver because of the greater catalase content of this organ. As the oxygen gas was liberated by the heart muscle, it was conducted through a rubber tube to an inverted burette previously filled with water, and that liberated by the liver to a large, inverted, graduated cylinder. The amount of oxygen gas liberated by the heart and liver

respectively was read off directly from the burette and cylinder where it had displaced the water. After this volume was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the ground material. In a similar manner the catalase content of the blood was determined by the addition of ten drops of blood to 500 cc. of hydrogen peroxide. A full description of the method may be found in a previous publication (7). It will be

After liver, heart and blood, are given the number of cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 1 gram of the heart, of the liver, and by ten drops of blood, respectively, of normal and phosphorus-poisoned cats

	CAT					AVERAGE AMOUNT OF OXYGEN
	1	2	3	4	5	
						cc.
Liver						
Normal cat	755	620	830	712	610	705
Cat fed phosphorus three days	500	640	602	355	600	539
Cat fed phosphorus six days	250	190	320	340	290	278
Heart						
Normal cat	213	198	266	213	220	222
Cat fed phosphorus three days	165	165	197	179	209	183
Cat fed phosphorus six days	160	162	165	187	140	162
Blood						
Normal	575	640	495	1070	590	674
Cat fed phosphorus three days	602	760	600	720	680	672
Cat fed phosphorus six days	380	420	580	400	630	482

seen that the average amount of oxygen liberated by 1 gram of the liver of the normal animals in ten minutes from 500 cc. of hydrogen peroxide was 705 cc.; that liberated by the liver of cats fed phosphorus three days, 539 cc., and that by cats fed phosphorus six days, 278 cc. of oxygen. The average amount of oxygen liberated by one gram of the heart of the normal cat in ten minutes from 45 cc. of hydrogen peroxide was 222 cc.; that by the heart of cats fed phosphorus for three days 183 cc., and that by cats fed phosphorus six days 162 cc. of oxygen. The average amount of oxygen liberated by ten drops of blood of the normal cats in ten minutes from 500 cc. of hydrogen peroxide was 674 cc.; that by ten drops of blood from the cats fed phosphorus three days, 672 cc., and that by cats fed phosphorus six days, 482 cc. of oxygen.

By comparing the data from the animals that had been fed phosphorus with that from the normal animals it is seen that the catalase content of the livers of the animals that had eaten phosphorus for three days was decreased 23 per cent and 60 per cent in those that had eaten it six days; that the catalase content of the heart was decreased 17 per cent in animals that had eaten phosphorus three days and 27 per cent in those that had eaten it six days; that there was practically no decrease in the catalase content of the blood of the animals that had eaten phosphorus three days while there was 28 per cent decrease in those that had eaten it six days. Since catalase is an index to the amount of oxidation in a tissue, being greatest in amount where oxidation is greatest and least where oxidation is least, the decrease in catalase in the liver, heart and blood of animals fed phosphorus is interpreted to mean that the feeding of phosphorus decreased oxidation in these tissues.

The livers of the animals that had been fed phosphorus three days presented the typical appearance of fatty degeneration with little or no indication of autolysis, while the livers of the cats that had been fed phosphorus six days showed extreme autolysis as well as fatty degeneration. The livers of these severely poisoned animals were literally in a state of falling to pieces as a result of autodigestion. From this it would seem that the amount of autolysis was inversely proportional to the amount of oxidation.

CONCLUSIONS

1. The catalase content of the liver, heart and blood is decreased in phosphorus poisoning, the decrease being greatest in the liver.

2. The fact that there was a greater percentage decrease in catalase, and hence in oxidation in the liver, than in the heart, for example, and the fact that autolysis is greater in the liver than in any other organ of the body would seem to lend further support to our contention that oxidation controls the amount of autolysis.

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THE NATURE AND PROPERTIES OF METATHROMBIN

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The literature concerned with metathrombin presents most widely divergent views as to the composition of the substance and contradictory experiments relating to its behavior. The belief of most later workers is that it is entirely absent from plasma but present in all sera. No one has succeeded in demonstrating the presence of metathrombin in plasma. Fuld (1) expressed the belief that thrombin in solution does not remain unaltered but passes over into an inactive form which on treatment with alkali undergoes hydrolytic cleavage with a new formation of thrombin. Morawitz believed at first that "β proferment" or metathrombin must be produced either by the calcium activation of prothrombin or at the time of fibrin formation, but later he (2) states simply in agreement with Fuld that the greater part of the thrombin formed during coagulation passes over into the inactive form of metathrombin. Weymouth (3) was led to believe that the substance is an antithrombin-thrombin compound. Melanby (4) recognized the existence of an antithrombin-thrombin compound in serum but denied that alkali-activation of serum could split this with the resulting liberation of free thrombin. He records his experiments which led him to the identification of metathrombin with thrombokinase. Collingwood and McMahon (5) support him in this view. During the progress of this work, Gasser (6) has recently offered further evidence in support of Weymouth's theory that metathrombin is an antithrombin-thrombin compound.

These experiments were undertaken at the suggestion of Dr. W. H. Howell to determine something of the composition and significance of metathrombin.

The method of alkali activation used for the detection of metathrombin consisted in the following procedure:

One-half cubic centimeter of the solution to be tested was incubated at room temperature with an equal volume of $\frac{N}{10}$ sodium hydroxide

for fifteen minutes and then neutralized with $\frac{N}{10}$ hydrochloric acid, Neutral Red being used as an indicator. Thrombin liberated by such activation was tested for by the addition of ten drops of a fibrinogen solution to the activated mixture. In all cases a control mixture was made which consisted of 0.5 cc. of the solution to be activated, to which was added ten drops of fibrinogen and a solution of 0.9 per cent sodium chloride, equal in quantity to that of the acid and alkali of the activated specimen. Such a control would reveal any thrombin present before activation.

In solutions in which free thrombin was suspected (in sera, for example), the solution was heated before activation at 60° to destroy this thrombin. If the presence of prothrombin was suspected in the solution to be tested, the solution was first oxalated in order to prevent any further liberation of thrombin from the prothrombin, and then heated to 60°C.

Repeated examinations of fresh and old oxalated plasmas (some of which were heated at 54° to remove the fibrinogen present and thus approach more closely the composition of serum) consistently failed to reveal any trace of metathrombin. It is regularly found in sera, however, as late as two weeks or more after coagulation. There are apparent, then, several possibilities as to the composition and time of formation of metathrombin.

First. Metathrombin might be formed as a result of one of the three main processes concerned in coagulation: (1) the action of the thromboplastic substance; (2) the calcium activation of prothrombin; (3) the combination of thrombin with fibrinogen to form fibrin. As has been stated, the last two possibilities were suggested by Morawitz.

Second. These processes leave in the plasma the following substances: antithrombin, prothrombin, free thrombin, calcium, thromboplastic substance in small amount and fibrin. If the fibrin be removed the remaining substances may be considered as the constituents of serum with which we are interested. In this serum there is present also metathrombin. It is well known that the thrombin of serum suffers a marked diminution within an hour after clot formation and then more gradually disappears, but the metathrombin persists for a much longer time. Pure thrombin, however, may be kept for long periods in solution without losing perceptibly its power of acting on fibrinogen. Alkaline, acid and neutral solutions of thrombin were kept for a week in the presence of calcium without the development of metathrombin. It is difficult to accept the suggestion that metathrombin

is formed by a change of some kind in the thrombin molecule. Experimental work indicates more strongly the occurrence of an interaction of substances. It is probable that the disappearance of thrombin from a serum may merely mark its combination with some other substance of the serum. From this viewpoint, metathrombin might be an antithrombin-thrombin compound, a prothrombin-thrombin compound, a thrombin-calcium compound, or a thrombin-thromboplastic substance compound. That metathrombin is not a prothrombin compound was determined by the alkali-activation of an oxalated serum that had been heated to 60°C. to destroy any free thrombin. Free thrombin was found after activation. Since this solution was calcium-free and thrombin-free to start with, the process of activation must have liberated thrombin rather than prothrombin. If the latter had been liberated it would have remained as prothrombin. It was determined also that alkali activation of isolated prothrombin does not yield free thrombin. The above mentioned possibilities in regard to the origin of metathrombin were each tested experimentally with the following results:

1. That the neutralization of antithrombin by thromboplastic substance is not attended by the formation of metathrombin was determined by incubating oxalated plasma (heated to 54°) with an equal amount of cephalin solution prepared after the method described by Howell (7). Alkali activation of the mixture gave no evidence of the presence of metathrombin. That neutralization of antithrombin by cephalin had taken place in the mixture was determined by antithrombin tests made on the mixture after incubation and at the same time on a control of plasma heated to 54°C., equally diluted with water and incubated for the same period. The cephalin plasma showed a marked decrease in antithrombin content in comparison with the water plasma.

2. The activation of prothrombin by calcium is neither attended by nor necessary for the production of metathrombin. This was determined directly by activating isolated prothrombin with a solution of calcium chloride and testing the thrombic power of the mixture at intervals up to forty-eight hours, carrying parallel tests for metathrombin. The thrombin yield was plentiful and did not decrease perceptibly in the period of the experiment, but at no time was there any indication of the presence of metathrombin.

More positive evidence of the fact that prothrombin activation by calcium is not necessary for the formation of metathrombin was

later obtained in confirmation of results published by Gasser (6) by incubating active thrombin with calcium-free plasma that had been heated to 54°C. and filtered from the fibrinogen precipitate. This mixture on standing developed metathrombin with a corresponding loss of free thrombin as will be described further on.

3. That the actual process of coagulation with the formation of fibrin is not responsible or necessary for the formation of metathrombin was clearly demonstrated by such an experiment as the last mentioned in which metathrombin was developed in a solution in the entire absence of fibrin formation. Further proof that fibrin formation alone cannot cause the production of metathrombin was obtained by clotting pure fibrinogen with pure thrombin; the serum from this coagulation showed no trace of metathrombin. Further, metathrombin was later found to be produced readily by the recalcification of fibrinogen-free plasma, which, of course, is parallel to the experiment of adding thrombin directly to fibrinogen-free plasma, since it has been seen that prothrombin activation plays no part in metathrombin formation.

4. That metathrombin is not a combination of thrombin left uncombined by fibrinogen, with the unactivated prothrombin of serum was determined by adding a solution of calcium-free thrombin to a solution of prothrombin. Activation of this mixture gave no evidence of metathrombin.

5. That the action of calcium on thrombin will not convert it into inactive metathrombin was determined by adding a solution of 0.5 per cent calcium chloride to a solution of pure thrombin. No metathrombin was developed. It was later found quite possible to produce metathrombin by addition of calcium-free thrombin to calcium-free plasma.

6. That metathrombin is not a thrombin-thromboplastic substance combination was determined by the addition of a strong cephalin solution to a pure thrombin solution. Metathrombin was not developed.

7. As this work progressed, the results led steadily to the conclusion that metathrombin is a substance formed by the union of antithrombin and thrombin. Such a union might conceivably take place either after all of the fibrinogen has been satisfied by thrombin, i.e., after a clot has formed, or else the two processes might go on side by side—part of the thrombin liberated from the prothrombin being taken up by fibrinogen to form fibrin and part by the antithrombin present in plasma to form metathrombin, which then becomes detectable in the serum. Later experiments and general considerations of the significance of metathrombin have led to the latter conclusion. The negative re-

sults obtained by experimentally testing other possibilities of the mode of formation of metathrombin served to confirm the theory of Weymouth that metathrombin is an antithrombin-thrombin combination. More direct evidence of the correctness of this theory was obtained by studying the mode of appearance of metathrombin in normal sera as well as by the experimental production of metathrombin in vitro by the recalcification of oxalated plasma and fibrinogen-free oxalated plasma; and finally by the direct addition of thrombin to the equivalent of an antithrombin solution.

The attempt was first made to determine at what point in the process of coagulation metathrombin becomes detectable. It has been the common experience of workers with metathrombin that the serum from normal coagulation apparently contains metathrombin in maximum amounts from the first. Their serum activations seem to have been made between periods of twenty minutes and several hours after coagulation. The attempt was made, therefore, to obtain a centrifugalized serum as soon after clotting as possible in the hope of finding it free from metathrombin. A cat was anesthetized and bled through a cannula from the carotid artery into a clean glass vessel. The blood was whipped with a wire brush to accelerate clotting. As soon as the entire clot had formed, half of the defibrinated serum was immediately oxalated (one part oxalate to eight parts serum). At once the oxalated and unoxalated portions were poured into separate small glass centrifuge tubes which had been packed in the brass centrifuge cups with ice-salt mixture. These cups with their contained tubes had been kept in the freezing mixture during the operation. They were now centrifugalized for seven minutes at high speed and the clear serum was drawn off. Part of the oxalated serum was heated at 60° for one minute to destroy the free thrombin, and alkali activations were made immediately upon the heated oxalated specimen, the unheated oxalated specimen and the unheated unoxalated specimen. Controls for each of these consisted in the same amount of serum diluted with a solution of sodium chloride, 0.7 per cent, in amounts equal to those used in the alkali activation, the same amount of fibrinogen being added in all cases. Activations were then made at intervals, using the unoxalated specimen which was allowed to stand at room temperature. Small portions of this serum were oxalated (one to eight) and heated at 60° one minute just before each test to destroy free thrombin and prevent further calcium activation of the serum prothrombin during alkali activation. Experiments of this type showed that if an activation were made within twelve or thirteen minutes

after clotting, no metathrombin could be detected. The table gives a record of such an experiment.

	ACTIVATED: + FIBRINOGEN	UNACTIVATED: + FIBRINOGEN
1. Serum oxalated immediately after clotting, heated to 60° 13 minutes after clotting	No clot, 24 hours	No clot, 24 hours
2. Serum oxalated and heated 60° 30 minutes after clotting	Membranous clot, 6 hours	No clot, 24 hours
3. Serum oxalated and heated 60° 5½ hours after clotting	Membranous clot, 20 minutes Gel, 40 minutes	No clot, 24 hours
4. Serum oxalated and heated 60° 20 hours after clotting	Firm clot, 11 minutes	No clot, 24 hours
Serum oxalated immediately after clotting		
5. Unheated 13 minutes after clotting	No clot, 7 hours Gel, 24 hours	Gel, 24 hours
6. Unheated 27 minutes after clotting	No clot 1¼ hours Membranous clot, 3 hours	Membranous, 1 hour, 30 minutes
7. Unheated 5½ hours after clotting	Good gel, 5 minutes, 10 seconds	No clot, 1½ hours Clot, 24 hours
8. Unheated 20 hours after clotting	Good clot, 3 minutes, 30 seconds	No clot, 50 minutes Clot, 24 hours
Unheated unoxalated serum		
9. 20 minutes after clotting		Membranous clot, 3 minutes
10. 5½ hours after clotting	Gel, 6 minutes	Gel, 1 hour
11. 20 hours after clotting	Gel, 4 minutes, 20 seconds	No clot, 50 minutes Clot, 24 hours

Experiments of this type show four interesting facts:

1. Metathrombin is not detectable under the conditions of the experiment in cat serum immediately after clotting, (cf. 1 in table).

2. Metathrombin is not formed suddenly in maximum amounts, but grows gradually in the serum. The effect of chilling may be regarded as merely the retarding of the normal process in which metathrombin is possibly formed more rapidly but nevertheless gradually.

3. The thrombin present in large amounts in the serum at first gradually diminishes in amount.

4. The formation of a clot after activation of a serum containing thrombin is no evidence of the presence of metathrombin. It has been stated that activation destroys any free thrombin present. There is no doubt that thrombin is weakened when subjected to alkali-acid activation but that it is by no means destroyed may be seen from a comparison of *1* and *5* in the above experiment. Both of these specimens were oxalated so that no further production of thrombin from prothrombin could be effected; *1* had its thrombin destroyed by heat and did not clot after activation; *5* unheated and containing free thrombin did clot after activation. This latter result must be attributed to the presence of free thrombin in the serum and not to the activation of metathrombin.

The same type of experiment was carried out using oxalated plasma instead of whole blood. Centrifugalized oxalated plasma was recalcified with a determined optimal amount of calcium, whipped, and fibrin removed and a portion immediately oxalated and heated as in the preceding experiment. From this cell-free serum it was possible to remove the fibrin without centrifugalizing and so the serum could be activated as early as three minutes after clotting. Even at this point metathrombin was invariably detectable. A typical experiment will serve:

	ACTIVATED: + FIBRINOGEN	UNACTIVATED: + FIBRINOGEN
Recalcified serum oxalated immediately after clot. Heated 60° 3 minutes after clotting	Membranous clot between 1 and 2 hours	No clot, 24 hours
Recalcified serum oxalated and heated 60° 2½ hours after clotting	Membranous clot, 37 minutes	No clot, 24 hours
Recalcified serum oxalated and heated 60° 20 hours after clotting	Membranous clot, 10 minutes	No clot, 24 hours
Recalcified serum oxalated immediately after clotting, unheated 6 minutes after clotting	Gel, 5½ minutes	Clot, 3 minutes, 15 seconds
Unheated, recalcified serum, oxalated 2½ hours after clotting	Gel, 5 minutes	Membranous, 2 hours
Unheated, recalcified serum, oxalated 20 hours after clotting	Membranous clot, 3½ minutes	No clot, 1 hour Clot, 24 hours
Unheated, unoxalated recalcified serum 7 minutes after clotting	Gel, 4 minutes	Clot, 1 minute, 15 seconds
Unheated, unoxalated recalcified serum 2½ hours after clotting	Gel, 3 minutes 10 seconds	Clot, 6 minutes
Unheated, unoxalated recalcified serum 20 hours after clotting	Membranous, 3 minutes	Membranous, 20 minutes

Metathrombin is then detectable in a serum obtained by recalcification of oxalated plasma earlier than in a serum resulting from normal coagulation. It is well known that an oxalated plasma recalcified with its optimum amount of calcium clots more rapidly than the same plasma allowed to coagulate unoxalated. The effect of recalcification is a more prompt activation of prothrombin with liberation of a relatively larger amount of thrombin in a shorter interval of time than occurs normally. The bearing of such an accelerated thrombin production upon a more speedy metathrombin formation will be seen from other experiments.

A further experiment was carried out with the object of determining whether metathrombin can be detected in unoxalated plasma, and of studying the normal period and mode of appearance of metathrombin. Birds' blood was used in this experiment. A large rooster was anesthetized and bled from the carotid through a paraffined cannula into paraffined tubes which were iced as in the preceding experiments. Part of this plasma was at once centrifugalized, the cell-free normal plasma drawn off and immediately activated, one specimen being heated to 60°C. and one unheated. Neither of these activated unoxalated plasmas or the unactivated controls gave clots during a period of twenty-four hours, indicating that metathrombin cannot be detected by alkali activation in circulating avian plasma.

A second portion of this plasma was not centrifugalized but was allowed to clot while cooled with its cell elements present. Portions of this clotting plasma were taken at intervals from the tube, oxalated, heated to 60° for one minute and activated. The table shows the results.

Plasma taken from carotid of rooster, 10.15 a.m.

10.15 a.m.
0.5 cc. plasma, oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. No clot, 24 hours.

10.35 a.m.
0.5 cc. plasma oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. No clot, 24 hours.

10.50 a.m.
Same procedure.

11.25 a.m.
Fibrin threads present.
Same procedure. Membranous clot, 4 hours.

11.25 a.m.
0.5 cc. plasma oxalated, heated 60°, unactivated, using NaCl 0.9 per cent to make equal dilution. 0.5 cc. fibrinogen added. No clot, 24 hours.

11.35 a.m.

Clot. 0.5 cc. serum, oxalated, heated 60°, activated, 0.5 cc. fibrinogen added. Membranous, 12 minutes. Gel, 15 minutes.

Same unactivated, using NaCl 0.9 per cent to make an equal dilution. No clot, 24 hours.

24 hours later. Same procedure (activating fibrinogen used here one-half as strong as that used above). Gel, 13 minutes.

Same procedure, unactivated, using NaCl 0.9 per cent to make equal dilution. No clot, 24 hours.

Clotting time:

Unoxalated plasma, 1 hour, 20 minutes.

0.5 cc. oxalated plasma, 4 drops 0.5 per cent $CaCl_2$ solution, 30 minutes.

0.5 cc. unoxalated plasma, 4 drops 0.5 per cent $CaCl_2$ solution, 3 hours.

0.5 cc. unoxalated plasma, 4 drops H_2O, 44 minutes.

0.5 cc. unoxalated plasma, 4 drops of Cephalin solution, 6 minutes.

It is seen from such an experiment as this that metathrombin is not detectable in birds' circulating plasma. However, in the slowly clotting blood there is a detectable trace of metathrombin before clot formation is completed. This is suggestive that the processes of metathrombin and fibrin formation go on side by side. It will also be noticed that the recalcified plasma clotted in a much shorter time than the normal plasma.

Experiments such as the three just mentioned demonstrate the fact that metathrombin makes its appearance gradually and its development is attended by a gradual diminution of the previously strong thrombic power of the serum. It is well known that if thrombin be added to serum it is readily inactivated. The possibility suggests itself therefore that this inactivation consists of a union of thrombin with antithrombin and that the gradual disappearance of thrombin from a serum marks the formation of just such a combination, the thrombin of which may be subsequently recovered by alkali activation—in other words, that metathrombin is a union of antithrombin and thrombin formed whenever thrombin is inactivated by antithrombin. If metathrombin is such a combination of antithrombin and thrombin it might be expected that the union would begin to take place in clotting blood by the side of the fibrinogen-thrombin union as soon as thrombin is liberated from prothrombin. The above experiments offer a certain amount of suggestive information in this regard. In cats' plasma allowed to clot unoxalated, and kept at a low temperature, metathrombin was detectable only some minutes after clotting had occurred. In the recalcified plasma under similar conditions it was detectable immediately after clot formation. Here, as stated above, the thrombin was more quickly liberated from prothrombin.

If metathrombin is a compound of antithrombin and thrombin, formed whenever antithrombin inactivates thrombin, it should be possible to produce it experimentally by direct addition of thrombin to antithrombin. A difficulty of this experiment lies in the fact that antithrombin has not been isolated from plasma. It has been shown, however, that there occurs no reaction between thrombin and either thromboplastic substance or prothrombin or fibrinogen which ends in metathrombin formation. The addition of thrombin to an oxalated plasma may be considered then, for experimental purposes, the equivalent of the addition of thrombin to an antithrombin solution, unless one assumes the presence in plasma of some as yet unknown substance with which thrombin combines. Further, it has been seen that coagulation is not necessary for the production of metathrombin. Therefore an oxalated plasma was heated to 54°, the fibrinogen precipitate filtered off and the plasma recalcified. This entailed the liberation of thrombin into the plasma which contained antithrombin. Tests made upon this mixture at first showed an absence of metathrombin and a high thrombin content, which relation after eighteen hours had become relatively reversed. As in the observations upon normal coagulation, there was seen here to be a gradual inactivation of the free thrombin with a corresponding appearance of metathrombin.

Further experimental evidence that the inactivation of thrombin by antithrombin is always attended by the production of metathrombin was obtained by using an oxalated plasma heated at 60° for five minutes. Such treatment destroys the fibrinogen and though the antithrombin is considerably weakened, it is not destroyed. This plasma approximates more closely a free antithrombin solution. With this plasma an experiment was made similar to that described by Gasser (6). Thrombin prepared by Howell's method (8) was dissolved in this plasma and incubated twenty-four hours at 37°. Thrombin and metathrombin tests showed a great decrease in free thrombin accompanied by the appearance of metathrombin. In two such experiments, antithrombin tests were carried out with the object of detecting a decrease in antithrombin content which might be expected if part of the antithrombin is combined by the thrombin. The heating of these plasmas at 60° for five minutes before the addition of thrombin together with the reheating at 60° for two minutes to destroy any free thrombin before each antithrombin test weakened the antithrombin so markedly that the results, while indicating a loss of antithrombin, were not very striking. One such test may be given.

Oxalated plasma heated at 60° for five minutes, incubated with dry thrombin.

0.5 cc. of this thrombin plasma added as soon as made to 0.5 cc. fibrinogen. Firm clot, 1 minute, 30 seconds.

0.5 cc. thrombin plasma 20 hours later, plus 0.5 cc. fibrinogen. No clot, 3 hours.

0.5 cc. thrombin plasma 20 hours later, heated 60° 1 minute, activated, plus 0.5 cc. fibrinogen. Flocculent fibrin, 30 minutes.

0.5 cc. thrombin plasma 20 hours later, heated 60° 1 minute, plus an equivalent amount of 0.7 per cent NaCl plus 0.5 cc. fibrinogen. No clot, 24 hours.

Antithrombin:

1 drop thrombin plasma immediately after mixing, heated to 60° 2 minutes plus 2 drops thrombin (15 minutes) plus 10 drops fibrinogen. Clot, 6 minutes.

Same, with 3 drops thrombin. Clot, 6 minutes.

Same, with 4 drops thrombin. Clot, 6 minutes.

1 drop thrombin plasma 20 hours after mixing, heated 60° 2 minutes plus 2 drops thrombin (15 minutes) plus 10 drops fibrinogen. Clot, 5 minutes.

Same, with 3 drops thrombin. Clot, 2 minutes, 30 seconds.

Same, with 4 drops thrombin. Clot, 2 minutes, 30 seconds.

Metathrombin, then, appears to be produced invariably in solutions that contain both free thrombin and free antithrombin. To test the possibility of its formation in the absence of antithrombin the following experiment was made. All of the known substances concerned in coagulation with the exception of antithrombin were mixed in the following proportions: 4 cc. active prothrombin solution + 4 cc. fibrinogen + 12 drops 0.5 per cent $CaCl_2$ + 20 drops cephalin solution. This mixture clotted and was allowed to stand twenty-four hours before removing the clot. The serum was tested for metathrombin.

0.5 cc. serum immediately after clotting plus 0.5 cc. fibrinogen. Membranous clot, 9 minutes.

0.5 cc. serum 24 hours after clotting plus 0.5 cc. fibrinogen. Membranous clot, 10 minutes.

0.5 cc. serum 48 hours after clotting plus 0.5 cc. fibrinogen. Gel, 8 minutes.

Activation of the serum at these intervals showed no trace of metathrombin. It is noticed that there is no diminution of free thrombin in such a mixture. As far as we know, this coagulation mixture contained every factor concerned in normal coagulation with the exception of antithrombin. It seems reasonable then to assume that the presence of antithrombin is essential for the formation of metathrombin in any solution. Weymouth reached the same conclusion by weakening the antithrombin of oxalated plasma by dialysis and then recalcifying. The serum showed metathrombin in subnormal amounts.

The above experiments indicate the following facts:

1. Metathrombin is not produced in solutions which lack either thrombin or antithrombin.

2. Metathrombin is readily produced in solutions containing both thrombin and antithrombin.

3. In such solutions the free thrombin gradually decreases in amount.

4. There is some evidence that in such solutions the antithrombin also decreases in amount.

It is evident that a combination of thrombin and antithrombin to form metathrombin might provide an efficient method, under certain conditions, for preserving the intravascular fluidity of the blood, as has been suggested recently by Gasser (6).

It was shown by Davis (9) that large amounts of thrombin might be introduced into the circulation without causing thrombosis. Apparently the thrombin in these cases must have been combined with antithrombin and so rendered inactive. In the plasma after such an injection one would expect to find detectable amounts of metathrombin. Accordingly these experiments were repeated with that object in view. It is evident that thrombin may be introduced into the circulating blood either in pure solution or by the indirect method of injecting thromboplastic substance which should cause the activation of prothrombin and the liberation of thrombin in the blood. The latter method was first used.

A cat weighing 2.6 kgm. was anesthetized; the carotid artery of both sides and the femoral vein of the right side were cannulated. The femoral cannula was attached to a burette containing an active solution of cephalin in 0.9 per cent solution of sodium chloride. The activity of this cephalin was determined by its addition to recalcified oxalated plasma immediately before the injection.

From the right carotid cannula 4 cc. of blood were run into a graduate containing 0.5 cc. sodium oxalate, care being taken to make all measurements exact. The graduate was inverted several times to insure thorough mixing of blood and oxalate. Thirty-five cubic centimeters of blood were then removed from the circulation and 15 cc. of cephalin solution were slowly run into the femoral vein from the burette, the entire injection consuming five minutes. Five minutes after the injection, 4 cc. of blood were received from the cannula in the left carotid into 0.5 cc. oxalate as before. The right carotid was then freshly cannulated and half an hour after the injection a third specimen of 4 cc. blood in 0.5 cc. oxalate was obtained. The recannulation of

the left carotid and the withdrawal of a final similar specimen one hour and forty-five minutes after injection, completed the experiment so far as the injection was concerned. The specimens of blood were centrifugalized, the clear plasma drawn off and tested as shown below. It was noted that no perceptible hemolysis had occurred.

Plasma before injection of 15 cc. cephalin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C
Plasma 1 hour, 45 minutes after injection = D

Metathrombin:

Portions of A, B, C and D were heated to 54° and the fibrinogen precipitate filtered off.

0.5 cc. + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A + 20 drops 0.9 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A, unheated, + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

The same series was carried using B, C and D with the same results. After forty-eight hours these activation experiments were repeated. In no case was there detectable a trace of metathrombin.

The antithrombin tests gave the following results:

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	20
3	A	10	15
4	A	10	10
5	A	10	10
2	B	10	25
3	B	10	15
4	B	10	10
5	B	10	10
2	C	10	60
3	C	10	30
4	C	10	25
5	C	10	20
2	D	10	20
3	D	10	15
4	D	10	10
5	D	10	10

A marked rise in antithrombin content is noted, followed by a return to normal. The rise appears to begin shortly after injection. In some instances the five minute specimen exhibited a fall in antithrombin content but in all cases the thirty minute specimen presented a marked rise. The return to normal occurs between thirty minutes and an hour and a half after injection. If the cephalin injected was neutralized by antithrombin we should expect a fall in antithrombin content, and, as stated, this could be detected in some instances in the five minute specimen.

It was believed that the failure to detect metathrombin in these experiments might have been due to a compensatory production of antithrombin which might thus render the cephalin valueless in aiding thrombin production from prothrombin. It has been shown by Delezenne (10), Nolf (11) and others that the liver is probably the seat of antithrombin production. Therefore a similar injection was made in an animal in which both the portal vein and coeliac axis had been ligated, with the object of removing the possibility of any antithrombin output from the liver during the experiment. Exactly the same methods were used in this case as were described in the preceding experiment. There was here a similar marked increase in antithrombin in both the five minute and the thirty minute specimens. Metathrombin was not detected.

Plasma before injection of 15 cc. cephalin = A
Plasma 6 minutes after injection = B
Plasma 26 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	35
3	A	10	25
4	A	10	20
2	B	10	45
3	B	10	35
4	B	10	25
2	C	10	1 hour, 30 minutes (imperfect clot)
3	C	10	55
4	C	10	35

Apparently antithrombin was thrown into the circulation from some source other than the liver, for at autopsy the ligatures were found to be intact. It was decided then to establish a head-thorax circulation in an animal with the object of removing the influence of all organs below the diaphragm. A cat was anesthetized, tracheotomy performed and artificial respiration established. The thorax was opened and the aorta and inferior vena cava were ligated just above the diaphragm. Ten cubic centimeters of an active cephalin solution were slowly run into the right external jugular vein. Specimens of blood were collected before and after the injection as in the previous experiments. The antithrombin in this case showed a marked increase in amount in the thirty minute specimen. That the output of antithrombin began very soon after the injection may be inferred from the fact that there was no detectable diminution of antithrombin in the five minute specimen.

Plasma before injection of 10 cc. cephalin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	*minutes*
2	A	10	20
3	A	10	15
4	A	10	10
5	A	10	10
2	B	10	20
3	B	10	15
4	B	10	10
5	B	10	10
2	C	10	50
3	C	10	35
4	C	10	25
5	C	10	15

Metathrombin was not detected.

That this antithrombin reaction could not take place outside the body was demonstrated by incubating whole oxalated plasma with similar preparations of cephalin at 37°, testing the antithrombic power before and after incubation as in the injection experiments. The addi-

tion of cephalin caused a diminution in antithrombin content which remained constant. There was no subsequent increase in antithrombin whatever.

To determine whether the cephalin itself was the stimulus for this increased output of antithrombin, an injection of 20 cc. normal saline was made into the femoral vein of a cat weighing 2.7 kgm. Fifty cubic centimeters of blood were drawn before this experiment to approximate the conditions of the cephalin experiments.

Plasma before injection 20 cc. normal saline = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	
3	A	10	No clot, 65 minutes
4	A	10	Clot, 50 minutes
3	B	10	Clot, 40 minutes
4	B	10	Clot, 20 minutes
3	C	10	No clot, 65 minutes
4	C	10	Clot, 55 minutes

This blood was of a very high antithrombin content before injection and the sole effect of the saline seems to have been merely a primary decrease in antithrombin power, followed by a return to normal.

Such experiments were unsatisfactory, however, from the standpoint of metathrombin, and it was decided to resort to the injection of pure thrombin. A very strong thrombin solution was prepared and dialyzed against water until the concentration in sodium chloride was about 1 per cent. An experiment was made upon an intact animal in all details similar to the femoral injections of cephalin described above.

Ten cubic centimeters thrombin solution were run into the femoral vein of a cat.

Plasma before injection of thrombin = A
Plasma 5 minutes after injection = B
Plasma 30 minutes after injection = C
Plasma 1 hour, 30 minutes after injection = D

Antithrombin

THROMBIN	INCUBATED 15 MINUTES WITH 1 DROP OF	FIBRINOGEN	CLOTTED
drops		*drops*	
2	A	10	No clot, 2 hours
3	A	10	No clot, 2 hours
2	B	10	No clot, 2 hours
3	B	10	No clot, 2 hours
2	C	10	Clot, 2 hours
3	C	10	Clot, 37 minutes
2	D	10	Clot, 1 hour, 10 minutes
3	D	10	Clot, 57 minutes

Metathrombin:

A, B, C, D heated to remove fibrinogen.

0.5 cc. A+ alkali activation +10 drops fibrinogen—no clot, 24 hours.

0.5 cc. A + 20 drops 0.7 per cent NaCl + 10 drops fibrinogen—no clot, 24 hours

Similar tests were made on B, C and D with identical results. No metathrombin was detected. It is to be noted, however, that the thrombin injected must have been inactivated, for the autopsy revealed no thrombi and the oxalated plasmas remained fluid. Heating to 53° gave a good precipitate of fibrinogen in all cases, further indicating that the fibrinogen had not been combined by the thrombin injected. It will also be noted that this blood was of extraordinary antithrombin content, and the effect of the thrombin injection seems to have been merely the lessening of this antithrombin by combination with thrombin.

There remained then the possibility that metathrombin is gotten rid of in the blood as rapidly as it is formed. To test such a possibility, a cat weighing 3 kgm. was used. The total volume of blood was roughly estimated at 150 cc.; one-third of the amount (50 cc.) was taken from the carotid artery, whipped with a wire brush to hasten coagulation, and the serum filtered off through gauze. This serum containing metathrombin was warmed to body temperature and injected back into the body twenty-five minutes after clot formation.

0.5 cc. serum before reinjection + 1 cc. 0.7 per cent NaCl = A

Oxalated plasma 5 minutes after injection of serum = B

Oxalated plasma 30 minutes after injection of serum = C

Metathrombin:

0.5 cc. A (heated 60° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. Found clotted, 1 hour, 15 minutes.

0.5 cc. A (heated 60° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B (heated 54° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. Scant fibrin, ppt. 24 hours.

0.5 cc. B (heated 54° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C (heated 54° 1 minute and filtered) + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C (heated 54° 1 minute and filtered) + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

Here then was an explanation of the fact that metathrombin could not be detected after cephalin and thrombin injections. Even if it were formed, it became quickly indetectable either by removal from the circulation or by the action of some factor existing normally in plasma. To exclude the possibility of body absorption of metathrombin from the circulation, a similar experiment was carried out using plasma outside the body.

Fifty minutes after clotting had occurred, centrifugalized serum was oxalated and heated to 60° for one minute to destroy free thrombin and prevent further activation of the serum prothrombin. The following mixtures were then made:

A = 3 cc. whole oxalated blood + 3 cc. serum; incubated 30 minutes at 37°.
B = 3 cc. centrifugalized oxalated plasma + 3 cc. serum incubated 30 minutes at 37°.
C = 3 cc. centrifugalized oxalated plasma heated to 54° and filtered + 3 cc. serum; incubated 30 minutes 37°.
D = 3 cc. 0.9 per cent NaCl + 3 cc. serum; incubated 30 minutes 37°.

Metathrombin tests made upon these mixtures after heating each to 54° and filtering are shown in the following table:

Metathrombin:

0.5 cc. A + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. A + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. B + 20 drop s0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. C + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

0.5 cc. D + alkali activation + 0.5 cc. fibrinogen. Firm clot, 3 hours.

0.5 cc. D + 29 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

It is seen that metathrombin apparently disappears when added to blood outside the body. Further the presence of blood elements plays no part in this disappearance of metathrombin, for centrifugalized plasma exhibited the same result as whole blood. The clot formed after activation of D shows that the metathrombin of the serum was not destroyed by mere dilution. It was possible, of course, that plasma in some way destroyed metathrombin, but there is nothing in plasma so far as is known except fibrinogen which is not present also in serum, and it is clear that the fibrinogen-free plasma exerted the same influence upon metathrombin as the other plasmas. A simpler explanation than that of a destruction of metathrombin by plasma suggests itself:

We have reason to believe that in plasma there is a certain amount of free or uncombined antithrombin. In serum, on the contrary, some of the free antithrombin has been removed by combination with cephalin and the remainder is in combination, loose or firm, with thrombin. When the serum is submitted to alkali activation the antithrombin of the metathrombin is destroyed and the liberated thrombin finds but little antithrombin to combine with and may therefore be detected immediately after the activation. When the plasma is submitted to the same process, some of the free antithrombin is removed but enough remains to insure a rapid combination with the thrombin liberated by the activation and this combination occurs so rapidly as to obscure the detection of the thrombin. If this reasoning is correct, it would follow that a plasma-serum mixture might reveal the presence of metathrombin if the plasma were first heated sufficiently to weaken greatly its content of free antithrombin, or if the mixture were submitted to a reactivation by alkali, on the ground that the first activation would weaken the amount of free antithrombin. The following experiments were therefore made.

Fifty minutes after clot formation, the centrifugalized serum was oxalated and heated at 60° for 1¼ minutes.

2 cc. oxalated plasma unheated + 2 cc. serum incubated 37° for 1 hour = A.

2 cc. oxalated plasma heated 56° and filtered + 2 cc. serum incubated 37° for 1 hour = B.

2 cc. oxalated plasma heated 60° 6 minutes and filtered + 2 cc. serum incubated 37° for 1 hour = C.

2 cc. oxalated plasma heated 70° five minutes and filtered + 2 cc. serum incubated 37° for 1 hour = D.

2 cc. 0.9 per cent NaCl + 2 cc. serum incubated 37° for 1 hour = E.

It is observed that the plasmas added to the metathrombin-containing serum were heated to various temperatures up to 70°, the critical temperature of antithrombin. It is known that heating even to 60° weakens antithrombin.

Metathrombin tests were made after each specimen was heated at 54° one minute and filtered.

Metathrombin:

0.5 cc. A + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. A + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. A activated, neutralized, reactivated, + 0.5 fibrinogen. No clot, 24 hours.
0.5 cc. B + alkali activation + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. B + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. B activated, neutralized, reactivated + 0.5 cc. fibrinogen. Membranous clot, 24 hours.
0.5 cc. C + alkali activation + 0.5 cc. fibrinogen. Good gel, 3 hours.
0.5 cc. C + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. D + alkali activation + 0.5 cc. fibrinogen. Good gel, 3 hours.
0.5 cc. D + 29 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.
0.5 cc. E + alkali activation + 0.5 cc. fibrinogen. Membranous clot, 1 hour. Solid, 3 hours.
0.5 cc. E + 20 drops 0.7 per cent NaCl + 0.5 cc. fibrinogen. No clot, 24 hours.

It is seen that the presence of metathrombin was detected in the mixtures the plasmas of which had been heated at 60° to 70° with a corresponding weakening of their antithrombin. Further, one activation failed to reveal metathrombin in the 56° plasma mixture (B) whereas a second activation of the solution (then weaker in antithrombin from the effects of the first activation and by reason of some of the antithrombin having combined with the thrombin liberated by this activation) showed the presence of metathrombin.

These experiments explain, possibly, the fact that metathrombin has never been demonstrated in plasma, as well as the failure to detect metathrombin in plasma after injections of cephalin, thrombin or metathrombin itself. The demonstration is in agreement with the injection experiments, as in these cases there was invariably a marked rise in the antithrombin content of the blood. The proof that metathrombin may be present in a solution and yet be "masked" by a high antithrombin content of the solution lends support to the view that metathrombin may be formed regularly in circulating blood as a protective mechanism as suggested above. The free antithrombin content of the plasma would render such small amounts indetectable by alkali

activation. At all events it is clear that the failure to demonstrate metathrombin in the plasma by alkali activation cannot be accepted as an argument against such a view.

CONCLUSIONS

1. Metathrombin is a thrombin-antithrombin compound. The following facts may be offered in support of this view:

a. The formation of metathrombin is not dependent directly upon any of the three essential processes of coagulation, the action of thromboplastic substance, the calcium activation of prothrombin or the formation of fibrin.

b. Metathrombin cannot be produced by the interaction of any known substances concerned in coagulation except thrombin and antithrombin.

c. Metathrombin cannot be produced in any solution from which either thrombin or antithrombin is absent.

d. Metathrombin is readily formed in solutions containing both antithrombin and thrombin.

e. In such solutions the thrombin gradually diminishes in amount.

f. There is evidence that in such solutions the antithrombin also diminishes in amount.

2. Metathrombin added to blood inside or outside the body cannot be detected by the method of alkali activation. The explanation offered for this fact is that the thrombin liberated by the activation is rapidly recombined by the free antithrombin of the blood.

3. On the basis of 2, it is suggested that metathrombin may be constantly forming in circulating blood although not detectable by the method of alkali activation; and that this process may serve to protect the blood from the coagulating effect of thrombin liberated within the circulation.

4. The injection of cephalin (tissue extract) into the external jugular vein (cat) causes a marked increase in the antithrombin content of blood kept circulating through the head and thorax only. No increase in antithrombin content occurs in whole blood to which cephalin is added in vitro at body temperature. This is offered as evidence that the abdominal viscera cannot be regarded as the sole source of antithrombin.

It is a great pleasure to me to thank Dr. Howell for his guidance in this work.

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THE CHANGES IN CLOTTING POWER OF AN OXALATED PLASMA ON STANDING

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Howell[1] has shown that in testing blood to determine the presence of a hemophilic tendency, it is more satisfactory to obtain the clotting time of the oxalated and centrifugalized plasma after recalcification than to depend upon the time of coagulation of the whole blood, since in the latter case small variations in conditions may make large differences in the figures obtained. In connection with this procedure and also as a matter of general interest, it was thought desirable to ascertain to what extent the coagulating property of an oxalated plasma undergoes alteration upon keeping, and the effect upon this property of temperature and of sterile versus non-sterile conditions. The following experiments were made at the suggestion of Dr. W. H. Howell with the object of testing these points.

It is evident that determinations of the clotting time of blood kept over a period must be made upon oxalate or fluoride plasma. In these experiments, therefore, the following method was adopted:

A cannula was introduced into one of the carotid arteries of an anesthetized cat, and the blood allowed to flow into centrifuge tubes containing one part of 1 per cent sodium oxalate for every eight parts of blood. The blood and oxalate were thoroughly mixed and then centrifugalized for twenty minutes. The cell-free plasma was pipetted off and divided into three parts, one of which was kept at 4°C. during the period of the experiment, another at room temperature and the third at 37°. The clotting time of these specimens was determined at intervals over a period of twenty-four hours by the method of recalcification. It is well known that if calcium be added to a plasma kept fluid by oxalate precipitation of its ionizable calcium the plasma will readily coagulate, the rapidity of coagulation being determined by the

[1] Arch. Int. Med., 1914, xiii, 76.

amount of calcium added. There is for every oxalated plasma a certain "optimum amount" of calcium, the addition of which will cause coagulation in the shortest time possible for that plasma under given conditions. If calcium be added in amounts under this optimum, the clotting time is appreciably slower, and the same is true for calcium in amounts above the optimum. In order to eliminate the errors arising from recalcifying with uncertain proportions of calcium, a series was carried for each test consisting of five clotting tubes, each of which contained 0.5 cc. of the specimen to be tested. To these tubes were added respectively three, four, five, six and eight drops of a 0.5 per cent calcium chloride solution.[2] The tube clotting first was assumed to contain the optimum amount of calcium chloride. For the cat, using this method, the optimum amount of calcium chloride ranged between four and six drops, for different bloods. One such experiment upon human blood showed an optimum of four drops. The amount of calcium necessary to exert this optimum effect upon coagulation remained fairly constant during the period of each experiment, the slight variations falling well within the limits of experimental error. It is realized, when one considers the very minute quantities of calcium which affect coagulation, that even variations in the size of the drops due to temperature changes during the period of the experiment may influence the clotting time.

The coagulation time of the recalcified plasma was found to lengthen markedly during a period of twenty-four hours. In most cases, the variations in the coagulation time of plasma kept at room temperature were negligible up to about four hours after the blood was drawn, when a marked lengthening occurred and persisted steadily, with the result that after twenty-four hours the clotting time was three to seven times as long as it was at the beginning of the experiment.

The temperature at which the plasma is kept was found to exert a definite effect upon the clotting time. The plasma kept at 4° exhibited a very much less loss of clotting power than did that kept at room temperature. Plasma kept at 37°, on the other hand, showed a very much greater loss than that kept at room temperature (fig. 1).

It was noticed in all cases that evidences of putrefaction were most prominent in the specimens kept at 37°—the ones which exhibited the most marked lengthening of the coagulation time during twenty-

[2] The solutions of calcium chloride used for this purpose should be prepared from the crystal or hydrated preparations rather than from the granulated or fused form.

four hours. This was suggestive of the possibility that the loss of clotting power might be the result of bacterial action. Accordingly, sterile plasma was collected in the following manner:

The required amount of oxalate was put into small-necked centrifuge tubes which were plugged with cotton and autoclaved. A clean

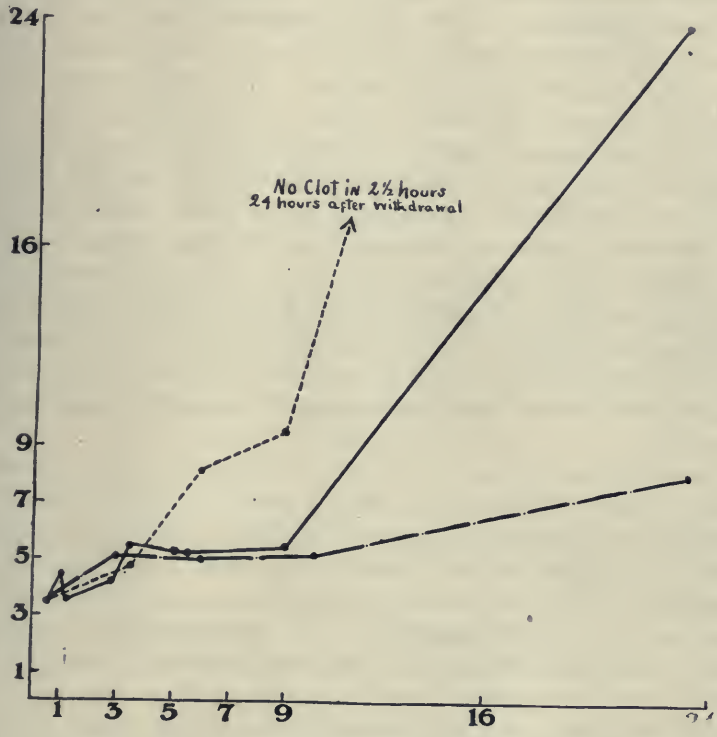

Fig. 1. Showing the loss of clotting power in a non-sterile plasma. One-half cubic centimeter specimens of the plasma were recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time (in minutes). Abscissae show time lapse (in hours) after withdrawal of blood from artery. Continuous line represents plasma kept at room temperature. Broken line represents plasma kept at 4°C. Dotted line represents plasma kept at 37°C.

operation laid bare the carotid artery of an anesthetized cat. The cotton plug was removed from one of the centrifuge tubes containing the sterile oxalate and a sterile rubber dam was quickly fitted over the mouth. This dam contained a small slit through which one end

of a sterile cannula was plunged, and the other end quickly and carefully inserted into the artery. When the required amount of blood had entered the tube, the cannula was withdrawn and a second sterile rubber dam replaced the one with the slit. The blood was centrifugalized. This method was found of value because it was quite impossible to prevent a cotton plug from being drawn into the tube during centrifugalization. After centrifugalization, the rubber dam was pierced by a long, sterile hypodermic needle and the clear plasma was drawn up into the sterile syringe. Two small flasks had been previously capped with rubber dams and autoclaved. The dam of each of these was now pierced, under sterile precautions, by the hypodermic needle and the plasma was delivered into each. When the needle was withdrawn, the elasticity of the rubber closed the hole to the exclusion of bacteria. Whenever a sample of plasma was needed for clotting time determinations, the dam of the little flask was punctured and the required amount drawn into a sterile syringe and transferred to the clotting tubes. One flask was kept at room temperature and the other at 37°. Plates were made daily from each flask. No colonies were found during the period of the experiment, which lasted five days.

The results of this procedure showed that no change whatever occurred in the clotting time of sterile plasma. The clotting time of the plasma at the end of one hundred and twenty hours was practically identical with the clotting time determined at the beginning of the experiment. This was true for the plasma kept at 37° as well as for that kept at room temperature. The slight variations during the period of the experiment were well within the limits of experimental error. After seventy-two hours, a portion of this sterile plasma was exposed to the air at 37°, and its clotting time at once began to lengthen steadily, so that forty-eight hours after exposure the addition of the optimum amount of calcium caused no coagulation in three hours. The unexposed plasma, however, remained constant in its clotting time (fig. 2).

An experiment was made with the object of determining the cause of the loss of coagulating power in exposed plasmas. Fresh oxalated plasma was heated to 54° and the fibrinogen precipitate filtered off. This fibrinogen-free plasma was then recalcified with the optimum amount of calcium, an active fibrinogen solution was added and the clotting time determined. The same test was made on the fibrinogen-free plasma kept at 37° for forty-eight hours. It is clear that any marked

difference in the clotting times so determined will indicate a change in the prothrombin content of the plasma. Another test made also upon fresh and forty-eight hour plasma consisted in the addition of an active thrombin solution to an unheated plasma. The clotting time of such a mixture will give a relative idea of the condition of the plasma-fibrinogen.

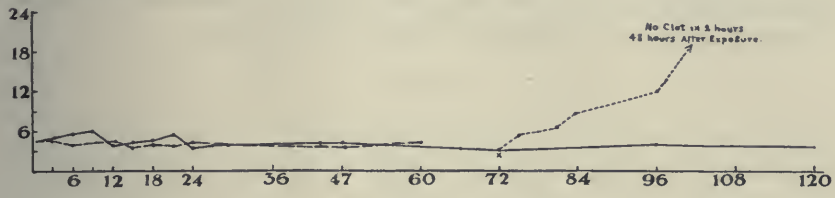

Fig. 2. Showing the retention of clotting power in a sterile plasma. One-half cubic centimeter plasma was recalcified at intervals with the optimum amount of calcium. Ordinates show the clotting time in minutes. Abscissae show time lapse (in hours) after withdrawal of blood from artery. The continuous line represents plasma kept at 37° (sterile). At *X* some of the plasma was exposed to the air at 37°. The change in clotting power of this exposed plasma is followed by the dotted line beginning at *X*. The broken line represents plasma (sterile) kept at room temperature during sixty hours.

The results were as follows:

0.5 cc. fresh oxalate plasma + 5 drops 0.5 per cent $CaCl_2$. Firm clot, 7 minutes, 30 seconds.
0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent $CaCl_2$ + 9 drops fibrinogen. Firm clot, 5 minutes.
0.5 cc. fresh oxalate plasma + thrombin 8 drops. Firm clot, 3 minutes.

Forty-eight hours later

0.5 cc. oxalate plasma + 5 drops 0.5 per cent $CaCl_2$. No clot, 24 hours.
0.5 cc. oxalate plasma heated 54° + 5 drops 0.5 per cent $CaCl_2$ + 9 drops fibrinogen. No clot, 24 hours.
0.5 cc. oxalate plasma + thrombin 8 drops. Poor floating clot, 10 minutes.

It is seen that there was a considerable alteration of fibrinogen during the forty-eight hours and a very marked destruction of prothrombin.

CONCLUSIONS

The coagulation time of non-sterile plasma lengthens steadily during the lapse of time after the blood is drawn. This loss of clotting power

is caused by bacterial action upon the prothrombin and fibrinogen and is not manifest in sterile plasma.

Coagulation determinations made on non-sterile plasma after a lapse of several hours from the time of obtaining the blood do not indicate the true coagulating power of the circulating blood. Low temperatures will greatly lessen the change that takes place, but an accurate test of the true clotting power of the circulating blood after a lapse of time, can be made only upon sterile plasma.

THE DIASTATIC ACTION OF SALIVA IN THE HORSE

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So many conflicting statements appear in the literature concerning the secretion and action of the saliva of the horse that the experiments outlined were undertaken in an effort to determine some of the more fundamental facts.

The following excerpts taken from the observations of various workers on the diastatic power of saliva will serve to illustrate the confusion that exists as to the action of ptyalin in the saliva of solipeds:

R. M. Smith (1) states that in almost all animals the diastatic action of saliva is less than in man with the possible exception of herbivora. He further states that the saliva of the horse will convert crushed raw starch into sugar in one-quarter of an hour. F. Smith (2) holds quite an opposite view declaring that "according to the writer's observations on the horse, saliva has no chemical action on the raw starch of its food." He also declares that it is doubtful if ptyalin exists in the herbivora.

Hofmeister (3) reports that "the mixed saliva of the horse has no effect on raw fiber prepared from hay. Such digestion of raw fiber in the horse occurs only in the small intestine."

According to Ellenberger (4) the secretions of the parotid and the submaxillary glands of the horse can convert starch into sugar but this diastatic action is much stronger in the saliva first secreted after a period of rest. In his later work with Scheunert (5), Ellenberger makes the rather conflicting statements that "the saliva of the horse (einhufer) has merely an insignificant action" while in another paragraph it is stated that "starchy foods chewed and swallowed by a horse with an esophageal fistula were sugar free when first caught but contained sugar after standing two minutes."

Goldschmidt (6) reports that the parotid saliva of the horse does not contain ptyalin but a zymogen which is transformed into ptyalin

during mastication. He believes that bacteria give the impulse for the change. He also finds that during precipitation with alcohol the zymogen is changed into active ptyalin.

Ellenberger and Hofmeister (7) state that "the parotid, submaxillary, sublingual and the buccal glands of the horse, sheep, etc., contain ptyalin which converts starch into sugar" and further that "the parotid is, in all species of animals, the richest in the ferment." In a later publication Ellenberger and Scheunert (5) seem to contradict the latter statement when they say "the action of the mixed saliva of the domestic animals is always more intense than that of the secretion of any one of the salivary glands by itself."

C. Roux (1871) is quoted by Hoppe-Seyler (8) as stating that "ptyalin is not found in the saliva of the horse."

Carlson and Crittenden (9) report their tests for diastase in the saliva of the horse to be negative in that mixed saliva in contact with boiled starch for twenty-four hours gave "practically no solvent action."

Colin (10) reports that the saliva of the horse "has not liquefied starch paste even after twenty-four to twenty-six hours of contact."

In the discussion of the saliva of the horse to be found in various text and reference books opinions of the authors are likewise in opposition. Mathews (11) says that "In the horse mixed saliva is said to have a very powerful diastatic action, whereas saliva collected from the parotid ducts is said to be inactive," while Foster (12) declares that in the horse the "amylolytic powers of either mixed saliva or of any one of its constituent juices are extremely feeble." Disselhorst (13) arranges the following in order of the ptyalin content: man, pig, horse, ox. Incidentally R. M. Smith (1) states that the "saliva of the horse is capable of converting cane sugar into grape sugar."

MATERIALS AND METHODS

In the tests that were made to determine the diastatic property of the saliva of the horse both extracts of the glands and freshly secreted mixed saliva were used as well as the pure secretion of the parotid and of the submaxillary. The difficulty of isolating the secretion of the sublingual prevented any experiments being made with that secretion alone.

Two glycerine extracts were used in the tests; one that has been designated as no. 1 being prepared two years before the tests were made; the other glycerine extract, no. 2, was prepared from fresh

glands, minced and placed in glycerine two weeks before the tests. Owing to bacterial decomposition but few tests were made with a water extract, 5 grams minced gland to 10 cc. of water.

Fresh mixed saliva was secured by either clamping open the jaws of the horse and then sponging or by permitting the animal to chew upon a slightly salted sponge. Isolated secretions were secured by placing a cannula in the duct from the gland from which the secretion was desired. Mixed fresh secretion was secured from different horses; the isolated secretions were taken from but one animal.

The tests charted in the table are merely representative tests; all tests made (exceeding 100) are not shown, although it may be stated that the general result was in every case similar to the test recorded. Constant checks (not shown in the table) were made by the use of human saliva under the same conditions; these were in every case found positive in the conversion of starch solution to reducing sugar. Blank checks (starch only) were also used as controls; these in every case were negative.

The shorter tests, up to four hours, were made by placing the tubes in the water bath at 40°C., in the longer tests the tubes were placed in an incubator at the same temperature.

A detailed discussion of the individual tests would seem to be unnecessary since the table shows the general methods used and the results obtained. Briefly these may be summarized as follows:

Freshly secreted mixed saliva from the horse was added to a 2 per cent solution of boiled cornstarch and incubated. At varying intervals a portion of the mixture was tested for reducing sugar by Fehling's test. In the earlier tests ten minute intervals were the rule; uniformly negative results led to the use of longer intervals for testing. All tests were negative up to four hours; most tests were negative up to six hours. All tests showed the presence of reducing sugar after eight hours, while the controls remained negative.

Tests made upon the isolated secretion of the parotid gave very similar results with apparently slightly accelerated action. However this shortened time was never marked, no more so than the variations found in the mixed saliva from the same horse at different times. Obviously it was not feasible to check the mixed saliva from the same horse simultaneously with its isolated parotid secretion.

Both the old and the recent glycerine extracts of the individual glands, as well as the glycerine extracts of all three glands, were uniformly negative in results in tests up to eighteen hours.

The tests made upon the water extracts were negative up to four hours; positive after six hours. Portions of the same glands were used in making the no. 2 glycerine extracts.

Several writers believe that the salivary glands of the horse secrete a zymogen which is later activated. From such statements the following is taken from Ellenberger and Scheunert (5) as being representative:

> Not all ferments are secreted in the active state by the cell; in many cases the action of chemical agencies is necessary to activate them; for example by the action of dilute acids or alkalies. In some cases even the oxygen of the air or oxygen carriers will convert the inactive proferment into an active condition. . . . . It is said that bacteria are kinase formers (p. 60).
>
> We know very little concerning the activation or the activators of proptyalin; it is possible that a kinase is furnished by other parts of the buccal cavity (cytoblastic tissue) or by the gastric mucosa, or that the activator is found in the air or the food (p. 319).

Attempts to activate the possible ptyalinogen which might have been present, were made with negative results. This was attempted by rendering alkaline and neutralizing; by acidifying and neutralizing; by passing air through the mixture; and by precipitating with alcohol (Lintner method). Activation through the action of bacteria during mastication (Goldschmidt, (6)) or through a possible kinase derived from the buccal mucous membrane (Mathews, (11)) apparently should have occurred during the process of securing the mixed fresh saliva. Carlson and Crittenden (9) state that in horses there is "certainly no activation in the mouth."

Experiments to test the possible action of the saliva of the horse upon cellulose were made with both hay and cotton fiber. Cured hay contains reducing sugar and it was therefore necessary to make the tests quantitative. No increase in sugar was found after twelve to twenty hours contact with either the freshly secreted saliva or the glycerine extracts. No test upon cellulose was made with the isolated secretions.

It seems improbable that the feeble diastase found to be present in the saliva of the horse can be of any importance in the animal's economy. It may be that this is but the lymph diastase as found by Carlson and Ryan (14) in the cat. Schafer and Moore (15) have shown that the salivary glands are not essential to life although Swanson (16) finds that upon extirpation of these glands there follows a modification of the quantity and of the acidity of the gastric juice. Colin

(10) states that when in the horse "the parotid ducts were opened to the exterior, in one month the animal lost one-seventh of its initial weight." This experiment was not repeated in the present series.

Negative results were always found in tests made concerning the action of fresh mixed saliva upon cane sugar, contrary to the findings of R. M. Smith (1).

As check experiments glycerine extract of the pancreas of the horse was found to rapidly convert starch solution into reducing sugar, the tests being positive in one minute.

A detailed description of the method followed in a single test will serve as an illustration of method used in all tests charted. For example in experiment 2 (see table) the procedure was as follows:

Five tubes containing 6 cc. 2 per cent boiled cornstarch were placed in the water bath at 40°C. To each of three tubes 1 cc. mixed fresh saliva of the horse was added. A fourth tube was left blank as a control, while to the fifth 1 cc. human saliva was added. Tests for reducing sugar were made at intervals. In three minutes the human saliva digest showed the presence of reducing sugar. Tests upon the horse saliva digest were negative up to four hours; doubtful trace found at seven hours; positive at eight hours. The blank control containing starch solution only was not tested until a positive reaction occurred in that containing horse saliva. When the control was then tested it was always found negative.

The following table summarizes the results obtained:

Action of fresh secretions on boiled starch

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
1	6 cc.	1 cc. MF	½, 1, 2 hours (−); 18 hours (+)
2	6 cc.	1 cc. MF	½, 1, 3, 4 hours (−); 7 (+?); 8 hours (+)
3	6 cc.	1 cc. PF	1, 2, 3 hours (−); 4, 5 (+?); 8 hours (+)
4	6 cc.	1 cc. PF	3, 4, 5 hours (−); 6, 7 (+?); 8 hours (+).
5	6 cc.	1 cc. SMF	1, 2, 3, 4, 5, 6 hours (−); 7 (+?); 8 hours (+)
6	8 cc.	2 cc. each MF and 1 per cent Na_2CO_3	1, 2, 3, 4, 5 hours (−); 8 hours (+)
7	4 cc. (paste)	1 cc. MF	No liquefaction after 2 hours; 18 hours (+)
8	4 cc. (paste)	1 cc. MF	No liquefaction after 2 hours; 18 hours (+)

Action on raw starch and cellulose

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
9	1 gm. powd.	2 cc. MF	4 cc. H_2O added; negative after 24 hours
10	2 gm. powd.	2 cc. MF	10 cc. H_2O added; negative after 24 hours
11	1 gm. powd.	1 cc. MF	4 cc. H_2O added; negative after 19 hours
12	Cotton	2 cc. MG 1	Negative after 24 hours
13	1 gm. hay	2 cc. MF	6 cc. H_2O added; no increase in sugar after 20 hours
14	1 gm. hay	2 cc. MG 1	Same as 13
15	1 gm. hay	2 cc. MG 2	Same as 13

Action of glycerine extracts

	2 per cent starch		
16	2 cc.	0.5 cc. PG 1	Negative after 1 hour
17	2 cc.	0.5 cc. SMG 1	Negative after 1 hour
18	2 cc.	0.5 cc. SLG 1	Negative after 1 hour
19	Repeated numbers 16, 17 and 18 with G 2; same results		
20	4 cc.	0.5 cc. PG 1 and SLG 1	Negative after 1 hour
21	4 cc.	0.5 cc. PG 1 and SMG 1	Negative after 1 hour
22	4 cc.	0.5 cc. SLG 1 and SMG 1	Negative after 1 hour
23	4 cc.	0.5 cc. each G 1	Negative after 1 hour
24	6 cc.	{1 cc. PG 1 and 0.5 cc. SMG 1 and 0.5 cc. SLG 1}	Negative after 15 hours
25	Repeated numbers 20 to 24 with G 2; results negative		
26	1 gm. powd.	2 cc. MG 1	Negative after 20 hours
27	1 gm. powd.	2 cc. MG 2	Negative after 20 hours

Attempts at activation

NUMBER	2 PER CENT STARCH	SECRETION USED	TIME TESTED AND RESULT (REDUCING SUGAR)
28	3 cc.	MG 1*, 2 cc.	Negative after 1 hour (*aerated)
29	3 cc.	2 cc. PG 1*	Negative after 1 hour (*aerated)
30	3 cc.	2 cc. SMG 1*	Negative after 1 hour (*aerated)
31	3 cc.	2 cc. SLG 1*	Negative after 1 hour (*aerated)
32	3 cc.	PG 2, pcpt.	Negative after 1 hour
33	3 cc.	SMG 2, pcpt.	Negative after 1 hour
34	3 cc.	SLG 2, pcpt.	Negative after 1 hour
35	3 cc.	MG 2, pcpt.	Negative after 1 hour
36	3 cc.	2 cc. PG 2*	Negative after 1 hour (*barely acidulated with 1 per cent HCl, and neutralized with solution KOH)
37	3 cc.	2 cc. MG 2	Negative after 1 hour (*same as 36)
38	4 cc.	2 cc. PG 2*	Negative after 1 hour (*6 cc. PG 2) mixed with 1½ cc. 1 per cent sodium carbonate; at end of 1, 2, 3 hours, neutralized and tested)
39	6 cc.	3 cc. PG 2*	Negative after 2 hours (*exposed to air in beaker for 12 days)
40	4 cc.	2 cc. MG 2*	Negative after 1 hour (*as in 38)
41	4 cc.	2 cc. PG 2*	Negative after 24 hours (*PG 2 mixed with equal amount 1 per cent HCl; neutralized with sodium carbonate at intervals of 1, 2, 4 and 24 hours; then tested)
42	4 cc.	2 cc. MG 2*	Negative after 24 hours *(MG 2 treated same as PG 2, number 41)

Additional experiments

43	5 cc.	2 cc. PW	1, 2, 4 hours (−); 6 hours (+)
44	1 gm. sucrose 4 cc. water	1 cc. MF	Negative after 24 hours
45	3 cc.; 2 per cent starch	0.5 cc. Panc. G.	Positive in one minute

Abbreviations: M, mixed secretion; P, parotid; SM, submaxillary; SL, sublingual; F, fresh or normal; G, glycerine extract; 1, old; and 2, recent extracts; W, water extract; pcpt., precipitated by alcohol.

SALIVARY SECRETION IN THE HORSE

It may be of interest to note some observations made concerning salivary secretion in the horse during the collection of saliva for the foregoing experiments. The facts noted were mere observations and in no sense quantitative or controlled experiments.

R. M. Smith (1) in speaking of the salivary glands of the horse says:

> Further, these glands are insensible to other stimulants (than mastication) such as salt, acids, etc. brought into contact with the mucous membrane of the mouth.

However, G. Colin (10) makes the statement that:

> The parotids secrete: First, when one puts some food in the buccal cavity while with the aid of a very simple apparatus the slightest movement of the jaws is rendered impossible. . . . . Fourth, they act, sometimes very feebly it is true, under the influence of salt. On the other hand they do not secrete when one forces the animal to masticate tasteless material.

While securing some of the mixed saliva used in the experiments outlined the mouth of the horse was held open by a dental speculum so that mastication was impossible. Nevertheless saliva could still be collected by means of a sponge or gauze; by placing salt on the sponge the rate of secretion was noticeably accelerated. Apparently acceleration also occurred when food was placed before the animal, thus confirming Ellenberger's (4) early observation that "the sight of favorite food will also cause a flow of saliva."

At other times the mixed saliva was secured by permitting the horse to chew upon a sponge which had been slightly salted. Under this procedure the secretion was more abundant than with either of the other methods. It would appear that if the ptyalin is normally activated within the mouth cavity during mastication it should have been so activated during the last mentioned method of securing the saliva.

The mixed saliva thus secured was a thin, translucent, sometimes slightly cloudy, alkaline fluid with a peculiar semi-spicy odor. Ferric chloride tests for potassium sulphocyanide were at all times negative which accords with the statement of Kingzett (17) that its presence in saliva is peculiar to man and with that of Ellenberger and Scheunert (5) that the saliva of the horse contains no potassium sulphocyanide. However, R. M. Smith (1) states that it has been detected in sublingual saliva of the horse although absent from parotid saliva.

SUMMARY AND CONCLUSIONS

1. The saliva of the horse, both the mixed saliva and the isolated secretions of the parotid and of the submaxillary glands, contains a diastase capable of converting starch into sugar. The diastase is extremely feeble, requiring at least five hours for the conversion of boiled starch.

2. The action of the diastase (ptyalin?) was not augmented by aeration, by acidifying or by exposing to the action of weak alkalies.

3. The saliva of the horse is inactive on cellulose and on sucrose.

4. The experiments showed no evidence of the secretion of a zymogen with a subsequent conversion into active ptyalin.

5. Salivary secretion may occur in the horse without mastication by stimulation with chemical substances, with an apparent augmentation through the psychic effect of the sight of food; the greatest flow occurs when the horse is permitted to masticate food material.

6. Potassium sulphocyanide is not found in the saliva of the horse.

The author gratefully acknowledges the assistance of Dr. A. M. Bleile in the preparation of this paper.

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THE RELATION BETWEEN THE THROMBOPLASTIC ACTION OF CEPHALIN AND ITS DEGREE OF UNSATURATION[1]

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Evidence has been presented to show that the thromboplastic action of the tissue juices is a property of the unsaturated phosphatide cephalin (1), (2). A solution of cephalin exhibits its most effective power to accelerate the coagulation of the blood immediately after its isolation from the tissue from which it has been prepared—brain, egg-yolk, liver or heart muscle. Cephalin exposed to the atmosphere or kept in a desiccator over calcium chloride gradually loses its thromboplastic action, and this fact obtains for impure cephalin, designated as "laboratory cephalin" (1) and for cephalin prepared in as pure condition as possible (2). Laboratory cephalin prepared two years ago and another specimen six months old now have absolutely no thromboplastic action although when freshly prepared from the tissue and for some time thereafter they exhibited marked power to hasten the coagulation of the blood. Cephalin is classified among the lipoids as an unsaturated phosphatide for it contains in the fatty acid group of its molecule an unsaturated fatty acid of either the linoleic, linoline or carnubic series—cephalinic acid (3). The object of this investigation was to determine if the gradual loss of the thromboplastic action of cephalin, as indicated by a decline of its power of acceleration of the clotting time, bears any relation to its gradual saturation as indicated by its iodine absorption number.

METHODS

Method of testing thromboplastic activity. The action of cephalin in accelerating the coagulation of the blood was tested in the following

[1] Investigated under the tenure of the Robert M. Girvin Fellowship of this department.

manner: Fresh serum and fresh oxalated plasma were prepared from the same animal.—By trial the amount of serum was determined which would cause clotting of a definite amount of plasma within a time convenient for observation. The thromboplastic activity of the solutions of cephalin (1 per cent in all experiments) of different degrees of unsaturation was tested by adding it to the mixture of serum and plasma and noting the acceleration in the clotting time. In all of the experiments the tests on plasma, serum and cephalin were compared with a control of plasma, serum and water. The tests were carried out according to the following example:

Control: Oxalated plasma, 8 drops; water, 3 drops; serum, 3 drops. Clot forms in from 5 minutes to 2 hours, usually the clot is imperfect.

Solution to be tested: Oxalated plasma, 8 drops; cephalin solution, 3 drops; serum, 3 drops. Using freshly prepared cephalin a solid clot forms in 1 minute plus or minus.

Determination of the degree of unsaturation

The degree of unsaturation of all specimens of cephalin tested was determined by the standard Hanus iodine absorption method (4).

Experiments with laboratory cephalin

"Laboratory cephalin" is an impure preparation of cephalin but very active thromboplastically. Its mode of preparation is as follows: A fresh pig's brain obtained from the slaughter house is freed as far as possible from membranes and blood and is then ground to a pulp in a mortar. The pulp is spread as thin as possible on a glass plate and dried in a current of warm air. When thoroughly dry the material is ground to a powder and is extracted for four or five hours with an excess of ether. The ether solution is filtered off in a closed space and is passed through the filter paper until the filtrate comes through clear. The ether filtrate is allowed to evaporate in a current of cool air to a moist residue. The residue is extracted twice with an excess of acetone for fifteen to twenty minutes and then twice with an excess of 95 per cent alcohol. Finally the residue is again treated with acetone, the acetone drained off and the material dried in a desiccator.

The cephalin thus prepared has not been thoroughly freed from other brain constituents, principally lecithin and cholesterin, as these substances are only completely separated from the cephalin by repeated precipitation of the ether solution by alcohol and by acetone.

I had on hand a preparation of laboratory cephalin isolated from the tissue six months previously, and through the kindness of Dr. Howell I was able to secure a specimen two years old. Both of these preparations had been freely exposed to the air since their preparation. The six-months-old specimen was further purified by dissolving it in a little ether, which was accomplished by adding a few drops of water and then agitating violently. The ether solution was centrifugalized and a small amount of insoluble material collected at the bottom of the tube was discarded. The clear ether solution was poured into four times its volume of absolute alcohol and the precipitate (cephalin) was shaken for a short time in the alcohol-ether mixture. The precipitate was collected by centrifugalization and dried in air.

The two-year-old preparation could not be completely dissolved in ether—being much more insoluble than the six-months-old preparation —so its iodine number was determined without any purification. This observation that cephalin loses its property of solution in ether as it ages is observed also when chloroform is used as a solvent.

The iodine number of the six-months-old laboratory cephalin was 37; that of the two-year-old specimen was 42. Both specimens were tested for thromboplastic activity with the following results:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Laboratory cephalin—six months old.....No clot in 1 hour
Laboratory cephalin—two years old.....No clot in 1 hour
Control.....Clotted imperfectly in 6 minutes

Not only had the old cephalin lost its thromboplastic activity but it even retarded the coagulation. This retardation of the coagulation may have been due to an acid reaction present in solutions of old cephalin. Solutions or rather emulsions of fresh cephalin are neutral in reaction. The solutions of both of these preparations were acid to litmus, the two-year-old specimen being more strongly acid to litmus than the six-months-old preparation. Possibly the cephalin had undergone a decomposition of its molecule which had freed a portion of the fatty acid content.

Experiments with Levene's hydrocephalin

Hydrocephalin is cephalin which has been reduced by the action of nascent hydrogen. The substance (5) was prepared by Dr. Levene

and through his courtesy I was able to obtain some of it. At the time of its receipt (January, 1916) it possessed no thromboplastic action and it possesses none now, as is shown in the following experiment:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; hydrocephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Hydrocephalin.....Imperfect floating clot in 1 hour, 30 minutes
Control.....Clotted solid in 4 minutes

The iodine number of the reduced cephalin (hydrocephalin) was 33. In solution it gave a slight acid reaction.

Experiments with cephalin prepared by the method of Levene and West

For the purposes of a previous investigation Doctors Levene and West had kindly supplied me with some cephalin prepared by the method described by the authors (6). The method yields cephalin in a high degree of purity. In the quantitative analysis made by the authors only 90 per cent of the original weight of material was obtained in the hydrolytic products. The authors therefore consider the possibility that the material contained some impurity. This specimen was tested when it was received one year ago and was found to possess marked thromboplastic action as is shown in the following experiment carried out at that time:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Cephalin—Levene and West.....Solid clot in 75 seconds
Control.....Poor clot in 1 hour, 30 minutes

Since then this sample of cephalin has been kept in a corked glass tube and in a desiccator, but not protected from the action of air and light. A one per cent solution of it now gives a slightly acid reaction and has lost its thromboplastic action completely as is shown in the following typical experiment:

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops
Cephalin—Levene and West, one year old
Imperfect floating clot in 1 hour, 30 minutes
Control.....Solid clot in 4 minutes

The iodine number of this preparation is now 42. (The degree of unsaturation was not determined when the specimen was received.)

We note from the above experiments that two specimens of old laboratory cephalin, the iodine number of one being 37 and that of the other being 42, had no thromboplastic action although when freshly isolated from the brain they had a strong action in accelerating the clotting of blood. Further, that a preparation of pure cephalin which one year ago possessed a marked thromboplastic action has no action whatever now, and its iodine number is 42. Finally, a preparation of pure cephalin which had been reduced by the action of nascent hydrogen does not now show any thromboplastic action. Its degree of saturation is indicated by its iodine number which was determined to be 33.

Experiments with freshly prepared cephalin

It was now proposed to prepare pure cephalin and to follow its loss of thromboplastic action and its degree of unsaturation, incident to age and environment, by tests made at regular intervals over a period of time. In order to determine what effect, if any, light and exposure to air may have in bringing about a saturation of the cephalin, a portion of the freshly prepared cephalin was allowed to be continually exposed to the light and air, and another portion was placed in a vacuum desiccator. This desiccator was kept in a dark cabinet and was only exposed to light and air for a minute or two every three or four days in order to withdraw a sample of cephalin. Upon removal of the sample a vacuum was immediately produced again within the desiccator.

Preparation of cephalin. Fresh pigs' brains obtained from the slaughter house are freed as far as possible from membranes and blood and then macerated to a pulp in a mortar. The pulp is spread as thinly as possible on glass plates and dried in a current of warm air. When thoroughly dry the material is ground to a powder and extracted with an excess of ether for twelve hours in a shaking machine. The ether solution, which contains the cephalin is grossly separated from the insoluble portion by first passing the material through a fine sieve and then more completely by centrifugalization. The clear reddish-yellow ether solution is poured off and concentrated by evaporation in a current of cool air. The concentrated ether solution is poured slowly into four times its volume of dry acetone with constant stirring of the white flocculent precipitate which results. The precipitate is

collected by centrifugalization, is partially dried in a current of cool air, is dissolved in enough ether to yield a thin solution and then centrifugalized for an hour at high speed. The ether insoluble mass which is thrown down is discarded. The ether solution is concentrated and poured into four times its volume of absolute alcohol and stirred. The precipitate is collected by centrifugalization, dried, dissolved in ether again and centrifugalized. The supernatant clear ether solution is concentrated and again precipitated with absolute alcohol. This process of dissolving in ether, centrifugalizing and precipitating with absolute alcohol is repeated four times. The last alcoholic precipitate is partially dried in a current of cool air, dissolved in ether and precipitated by four times its volume of dry acetone and shaken in a shaking machine for two hours or more in order to dry the cephalin so that it may be obtained as a loose powder. This final precipitate is collected by centrifugalization and dried in the air. The cephalin thus prepared is a yellowish red mass which pulverizes to a light yellow fine loose powder. One hundred and sixty-five grams of brain tissue yielded eight grams of cephalin.

This cephalin was immediately divided into two portions, one to be kept in a vacuum desiccator protected from the action of light and air and the other to be kept continually exposed to light and air. For convenience both of these portions were subdivided into 10 mgm. and 100 mgm. subdivisions. Every three days or so one of the 10 mgm. portions of exposed cephalin was tested for its thromboplastic activity. At the same time and with the same blood a 10 mgm. portion of the cephalin which had been kept in the vacuum desiccator was tested, both tests being controlled by noting the clotting time of the same mixture of plasma and serum to which no cephalin had been added. Either just before or immediately after the test for thromboplastic activity a determination of the degree of unsaturation of both the exposed and protected cephalin was made, using the 100 mgm. portions.

RESULTS

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops

November 27, 1916

Cephalin—freshly prepared. I. A. no. 79.....Solid clot in 49 seconds
Control.....Poor clot in 20 minutes

December 1, 1916

Cephalin—from vacuum desiccator. I. A. no. 74
Solid clot in 1 minute, 30 seconds

Cephalin—exposed to air and light. I. A. no. 68
Solid clot in 1 minute, 30 seconds

Control.....Sliding clot in 15 minutes

December 4, 1916

Cephalin—vacuum desiccator. I. A. no. 73.....Solid clot in 56 seconds
Cephalin—exposed. I. A. no. 68.....Solid clot in 58 seconds
Control.....Sliding clot in 11 minutes

December 7, 1916

Cephalin—vacuum desiccator. I. A. no. 72.....Solid clot in 58 seconds
Cephalin—exposed. I. A. no. 68.....Solid clot in 1 minute, 18 seconds
Control.....Sliding clot in 9 minutes

December 11, 1916

Cephalin—vacuum desiccator. I. A. no. 68.....Solid clot in 54 seconds
Cephalin—exposed. I. A. no. 64.....Solid clot in 1 minute, 20 seconds
Control.....Sliding clot in 15 minutes

December 14, 1916

Cephalin—vacuum desiccator. I. A. no. 67.....Solid clot in 41 seconds
Cephalin—exposed. I. A. no. 62.....Firm clot in 1 minute, 42 seconds
Control.....Imperfect clot in 9 minutes

December 17, 1916

Cephalin—vacuum desiccator. I. A. no. 64
Solid clot in 1 minute, 13 seconds

Cephalin—exposed. I. A. no. 57.....Solid clot in 2 minutes, 30 seconds
Control.....Sliding clot in 11 minutes

December 20, 1916

Cephalin—vacuum desiccator. I. A. no. 64.....Solid clot in 1 minute
Cephalin—exposed. I. A. no. 57.....Firm clot in 2 minutes
Control.....Sliding clot in 6 minutes

December 28, 1916

Cephalin—vacuum desiccator. I. A. no. 63:....Solid clot in 3 minutes
Cephalin—exposed. I. A. no. 56.....Imperfect clot in 8 minutes
Control.....Imperfect clot in 10 minutes

From the above experiments it is seen that the cephalin when freshly prepared from the brain had on November 27, 1916, an iodine number of 79 and possessed a very active thromboplastic action, but by exposure to light and air had become saturated to an extent indicated by its iodine number of 56 on December 28, 1916, and that it had lost its thromboplastic action to a marked degree. The material kept in the vacuum desiccator gradually became less unsaturated than it orignally was, but the saturation had progressed more slowly and not to the same extent as in the case of the exposed cephalin. Nor had it lost its thrompboplastic activity to the same degree as the exposed

cephalin. Evidently the amount of air admitted to the desiccator when it was opened for the purpose of withdrawing a sample (and there is the possibility of air leakage into the desiccator to be considered) was sufficient to bring about a certain amount of saturation of the cephalin.

Experiments with cephalin kept in vacuum tubes

Thus having found that storage in a vacuum desiccator—to which air must be admitted at intervals and which is always liable to air leakage—is not sufficient to maintain the original degree of unsaturation or of thromboplastic activity, another series of experiments was carried out with freshly prepared cephalin in which each of the subdivisions of the cephalin used in the tests were placed in small glass tubes, after which a very high vacuum was produced and the tube sealed. The tubes were wrapped in cotton and placed in a dark cabinet. The contents were tested for thromboplastic action and the degree of unsaturation at intervals and the results compared with identical determinations carried out at the same time and on the same blood with similar subdivisions of the same preparation of cephalin which had been kept continually exposed to light and air.

RESULTS

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops

February 16, 1917
Cephalin—vacuum tube. I. A. no. 59..Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 45.....Firm clot in 3 minutes
Control.....Jelly clot in 4 minutes, 30 seconds

February 19, 1917
Cephalin—vacuum tubes. I. A. no. 59..Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 42.....Firm clot in 4 minutes, 30 seconds
Control.....Imperfect clot in 6 minutes

February 23, 1917
Cephalin—vacuum tube. I. A. no. 51.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 33.....Sliding clot in 9 minutes
Control.....Sliding clot in 6 minutes

February 26, 1917
Cephalin—vacuum tube. I. A. no. 54...Solid clot in 1 minute, 10 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 5 minutes
Control.....Solid clot in 20 minutes

February 28, 1917
Cephalin—vacuum tube. I. A. no. 51.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 31.....Firm clot in 6 minutes, 30 seconds
Control.....Firm clot in 8 minutes
March 2, 1917
Cephalin—vacuum tube. I. A. no. 53...Solid clot in 1 minute, 20 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 4 minutes
Control.....Firm clot in 4 minutes
March 5, 1917
Cephalin—vacuum tube. I. A. no. 54.....Solid clot in 2 minutes
Cephalin—exposed. I. A. no. 30.....Poor clot in 10 minutes
Control.....Firm clot in 12 minutes
April 10, 1917
Cephalin—vacuum tube. I. A. no. 54.....Solid clot in 50 seconds
Cephalin—exposed. I. A. no. 31.....Sliding clot in 4 minutes
Control.....Firm clot in 4 minutes

It is noted in the above experiments that the cephalin sealed in the vacuum tubes had maintained its thromboplastic action and practically the same degree of unsaturation over a period of two months, whereas the cephalin exposed to the light and air for this period had gradually lost its ability to hasten the coagulation of the blood and its iodine number had dropped from 59 to 31. It will be recalled that the cephalin saturated by means of nascent hydrogen—Levene's hydrocephalin—possessed no thromboplastic action and its iodine number was determined to be 33.

It will be noticed that the iodine number of the cephalin kept in the vacuum tubes apparently varies slightly. The variations may be due to a slight variation in the amount of material in the tube, incident to placing the material in the tubes, which was accomplished with a small glass funnel and a camel's hair brush.

Although the cephalin used in the above experiment was prepared by the same method as that used in the previous series of experiments in which the material was kept in the desiccator, there was a marked difference in the iodine numbers of the two preparations. When freshly isolated from the tissue the iodine number of the first preparation was 79 and of the second 59. I assume that in the drying of the moist brain pulp the latter preparation became more saturated than the former. Also it is possible that some decomposition of the brain tissue may have taken place before it was thoroughly dried. One preparation of brain cephalin in which a certain amount of decomposition was known to have occurred gave an iodine number of 48 immediately after isolation from the tissue.

Experiments with cephalin exposed to ultraviolet light

In order to intensify the action of the atmosphere and light, cephalin in 10 mgm. and 100 mgm. portions was placed on small watch crystals and exposed to the action of the ultraviolet light rays from a quartz Cooper-Hewlet light and to the ozone in the surrounding atmosphere produced by the passage of the rays through it. This was done with the hope of bringing about much more quickly the same changes in the cephalin which are produced gradually by the action of ordinary light and air. These experiments were made with a very thromboplastically active preparation of cephalin having an iodine number of 64. Five portions of 10 mgm. each and five portions of 100 mgm. each on small watch glasses were placed on a stand 11 cm. under the Cooper-Hewlet light. After exposure for three hours two 10 mgm. and two 100 mgm. portions were withdrawn and tested for thromboplastic activity and degree of unsaturation. At the end of five and one-half hours two more specimens were withdrawn. The remaining specimen was exposed to the ultraviolet light and ozone for ten hours. The results upon the cephalin thus exposed were compared with similar determinations made upon a portion of the same preparation of cephalin which had not been exposed to the action of the ultraviolet light and ozone.

RESULTS

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops

December 13, 1916

Cephalin—not exposed to ultraviolet light. I. A. no. 64. Clotted in 1 minute, 10 seconds
Cephalin—exposed to ultraviolet light 3½ hours. I. A. no. 56. Clotted in 3 minutes
Cephalin—exposed to ultraviolet light 3½ hours. I. A. no. 41. Clotted in 4 minutes
Cephalin—exposed to ultraviolet light 5 hours. I. A. no. 50. Clotted in 3 minutes
Cephalin—exposed to ultraviolet light 5 hours. I. A. no. 49. Clotted in 3 minutes, 30 seconds
Cephalin—exposed to ultraviolet light 10 hours. I. A. no. 44. Not clotted in 1 hour
Control..... Clotted in 5 minutes

The combined action of the ozone and the chemically active ultraviolet light had brought about within ten hours a marked reduction

in the iodine number of the cephalin, together with a corresponding decrease in the thromboplastic action. The sample of cephalin which had been exposed for ten hours retarded the coagulation of the blood and gave a slight acid reaction.

CONCLUSIONS

1. The thromboplastic action of cephalin bears a direct relation to its degree of unsaturation, the greater the degree of unsaturation the greater the degree of thromboplastic activity.
2. Cephalin exhibits most effectively its power to hasten the coagulation of the blood shortly after its isolation from the tissues.
3. Cephalin which has become saturated beyond a certain degree, either by reduction or oxidation, completely loses its thromboplastic activity.
4. Cephalin in solution which has become saturated or partly saturated gives an acid reaction and retards the coagulation of blood.
5. As cephalin becomes saturated it gradually loses its property of solution in ether or chloroform.

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These are important questions.

One product may differ widely from another product identical with it in physical appearance and bearing the selfsame name upon its label. This lack of uniformity may arise from diversity in the process of manufacture. It may be due to the fact that the active constituents of the crude drugs entering into the medicaments vary from season to season and are modified by habitat, by climatic influence, or by differing modes of curing and storage.

Definite medicinal strength in a therapeutic agent is of the utmost importance. There is one method of assuring it. That method is by assay. That method is *our* method.

We standardize our entire output of pharmaceutical and biological products—chemically or physiologically, according to the exigencies of circumstance. *We were pioneers in standardization*, putting forth the first assayed preparation nearly forty years ago. We championed standardization when it was ridiculed by "conservative" manufacturers throughout the length and breadth of the country, holding then, as we hold to-day, that the essential value of a therapeutic agent lies in the definiteness of its activity.

The medicinal products of our manufacture are **uniformly potent and reliable**—*the same to-day, to-morrow, next week, next year.*

Home Offices and Laboratories,
Detroit, Michigan.

Parke, Davis & Co.