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THE QUANTAL PHENOMENA IN MUSCLE: METHODS, WITH FURTHER EVIDENCE OF THE ALL-OR-NONE PRINCIPLE FOR THE SKELETAL FIBER

FREDERICK H. PRATT AND JOHN P. EISENBERGER

From the Physiological Laboratory of the Medical Department, University of Buffalo

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Work on batrachian muscle already reported from this laboratory has shown that when electrical stimuli are applied to the surface of the sartorius over an area sufficiently minute, it is possible to bring under ob-

servation events which contrast strongly with the phenomena of gross contractility. The results as published may be summarized as follows:

The observations of Keith Lucas (1) that skeletal muscle, in simple contraction, responds in determinate, discontinuous gradients to relatively continuous change of stimulus intensity, have been verified (2). Like discontinuity has been found to obtain under continuous change of tetanic stimulation (2). The unit, or minimal entity, of function has been found by direct observation to be, as Lucas inferred, the individual fiber (3). The most conspicuous sign of fatigue in the locally excited muscle is successive abrupt elimination of individual fiber activity through rise of threshold (2). Conversely, the staircase effect is in similar case dependent to an appreciable extent upon accession to complete function of previously inactive fibers, through progressive fall of threshold (2).

The methods employed, heretofore only generally described, have naturally been subject to improvement and accretion in the progress of further work, and a stage is now reached where the technical results justify a more detailed account. With improvement in technique the graphic output has assumed an increasingly convincing character. The tracings reproduced, although introductory to a number of separate problems, are here presented as cumulative evidence of the independence on the part of individual contractile values toward change of stimulus or threshold.

PART I. METHODS FOR THE GRAPHIC RECORDING OF FIBER MOVEMENT

Principles of Method

The combined devices for recording are indicated essentially in the diagram, figure 1. The uncurarized preparation lies in a bath of Ringer's fluid. Upon the bared muscle surface impinges one end of a bent glass tube lying in a vertical plane parallel with the fibers. The interior of the tube, filled with Ringer's fluid, is continuous with the bath containing the preparation by means only of a terminal pore of less diameter than a single fiber. The other end of this active liquid electrode is led into a separate bath of Ringer's fluid in which is immersed the porous cup of an unpolarizable terminal. A similar terminal system is immersed at a convenient point in the solution bathing the preparation. The circuit is completed through a coil subject to alteration of position with respect to an appropriately varied magnetic field (primary coil or moving magnet). A fine globule of mercury rests upon the linear region

of the preparation made active by the unipolar stimulus at the pore. The globule is illuminated by a strong beam of light directed through a lens system, the vertically reflected rays being projected through a compound microscope to a photographic plate moving horizontally at right angles to the direction of fiber activity. The plate is also made to record the position of a second image, coördinate with the first, serving, as occasion requires, to record time periods or the extent of movement of the stimulating coil.

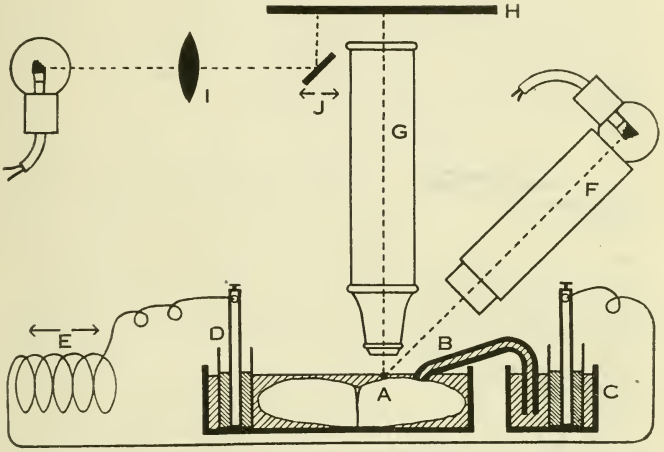

Fig. 1. Diagram to indicate essentials of the method. *A*, section of live preparation; *B*, active electrode, with pore in contact with muscle surface; *C*, bath of Ringer's fluid containing Zn-Zn SO_4 terminal; *D*, similar terminal in bath of Ringer's fluid covering preparation—the indifferent electrode; *E*, stimulating coil, movable with respect to magnet or primary coil; *F*, light-projector for illumination of mercury globule over *A*; *G*, recording microscope; *H*, photographic plate, movable at right angles to plane of drawing; *I*, lens system for projecting signal image on plate; *J*, mirror, movable with coil, *E*.

The Electrodes

The preparation and application of the pore-electrode were described in the first paper of this series (4). We shall review, however, certain points which have been modified in more extended practice. The electrode in present use differs from that first described chiefly in the form of the active, and in the mode of application of the indifferent, element.

Active electrode. In making the present bent form of pore electrode (fig. 2), soft glass tubing of about 2 mm. bore and 1 mm. wall is used, being cut to a length of about 3 cm. on completion. One end is first fused in a Bunsen flame until the closed lumen presents a perfectly sharp apex when examined with a strong lens. It has been found that with favorable material no drawing out (even by gravity) of the fused end is necessary to produce such an apex, which should converge at an angle as little acute as possible. An actually obtuse and seemingly blunt point may prove under the lens to terminate very sharply. While the end of the tube is still viscous it is allowed to bend at an obtuse angle to the shank, giving a very short arm directed obliquely downward when adjusted for use. After the end of the tube has been ground in a plane designed to lie horizontally on the tissue, and carried to within perhaps 0.01 mm. of the apex, the latter is opened by careful polishing of the facet. This should be accomplished strictly by polishing as distinct from grinding, as emphasized in Rayleigh's (5) study of the process. Even the finest grinding (removal by fine pitting) will mar the contour of the exposed pore and enhance later occlusion by particles retained by the jagged edge; whereas true polishing (approximate molecular removal) will effect a clean, circular opening sometimes of less diameter than necessary or practicable for use. The grinding, begun conveniently with the finer grades of emery or corundum cloth, may be completed with the finest grade of emery powder, the end of the tube being rubbed with a rotary motion over a plate of glass carrying the wet powder. The polishing is done in the main on a horizontal disk rotated by power, bearing a felt surface with rouge and water liberally applied. As soon as a facet of sufficient transparency is attained, the tube is placed under a high power of the microscope and the nearness of the apex to the surface ascertained by means of the fine adjustment used as a micrometer. Experience will decide as to whether to continue polishing or to repeat the last grinding operation. A number of such alternate procedures are often advisable, careful inspection being made after each polishing. It will finally be evident that only slight further polishing is necessary to touch the apex of the lumen and thus open the pore. At this stage, hand polishing is probably the safest procedure.

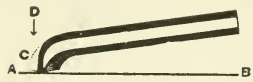

Fig. 2. Sagittal section of active electrode tube, actual size. *A*, *B*, surface of preparation in a direction parallel with fibers; *C*, region of wall ground away to facilitate observation or record from *D* of region close to pore.

If the pore has been broached with sufficient caution, a few particles of rouge within the lumen will be the first indication of the event. These will probably firmly occlude the orifice, and it may be necessary to immerse the tube in aqua regia for many hours to clear it. This final forcing of the polishing medium into the pore is, however, probably of distinct advantage in giving the opening a smooth margin.

A polished surface of glass, according to Rayleigh (5) is an absolute condition—not one of relative fineness of grain, as with ground surfaces. Polish once attained cannot be improved; it is in fact an "all-or-nothing" effect, the microscope meeting a blank surface up to the limit of visibility. It is therefore readily to be understood that the pore in present use, 7 μ in diameter, which is a particularly successful outcome of long continued hand-polishing, seldom becomes seriously plugged by foreign particles. The highest magnification shows a clean, circular pore in an unmarred surface. Since it opens, on the one hand, abruptly upon a slightly convex surface and, on the other, into a rapidly diverging conical cavity, an occluding particle, once dislodged from the pore, is at once free in either direction.

By preliminary shaping with a grinding wheel it is possible so to reduce the thickness of the glass on the distal side of the cone (fig. 2, *C*) that the pore will emerge very close to the edge of the facet and hence permit, if desired, observation or record of response almost at the point of excitation.

Indifferent electrode. In the method as originally described (4) the indifferent contact of electrolyte solution with tissue is located at the annular junction of the ends of two concentric tubes, the inner of which carries the pore. Both electrodal elements are thus carried together to the region of application, the theoretical advantages of which arrangement do not appear to concern the work in hand. Nor for present purposes does the plan of conveying the indifferent terminal to the tissue by means of a wick (3) seem to offer any advantage over the present method, which has the merit of simplicity combined with the greatest possible dispersion of current over regions not designated for stimulation. As already indicated, the porous cup with its Zn-$ZnSO_4$ elements (the form employed being, together with its counterpart in the active bath, the Porter boot-electrode) is introduced directly into the large bath of Ringer's solution which covers the entire preparation.

Principles of action. Figure 3 indicates what may be regarded provisionally as the mode of stimulation by this method. The electrolyte conductor is continuous between the baths through the pore, where

density of current will be abruptly increased. The region of high resistance is, however, intensely circumscribed, and the relative freedom of conduction elsewhere therefore permits the use of extremely attenuated currents. The region of the tissue lying directly beneath the pore (for example, a superficial fiber) will be in surface contact with a region of current density greater than at any other point in the preparation; the degree of localization of effect being modified by the distance of the pore from the actual receptive surface. Thickness of fascial investment would play the greatest part in such modification and would account for the ease with which small groups of fibers are excited, as contrasted with the relative difficulty of securing single fiber response. Following the conception expressed in the diagram, it is obvious that any increase of current strength above that which excites only one fiber (minimal stimulus) will tend to bring likewise into activity fibers immediately adjacent laterally and beneath (submaximal response); hence the "steps" observed on gradation of stimuli over a sufficient range (fig. 12, *a*). The following postulates, moreover, would appear to be justified.

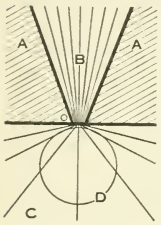

Fig. 3. Diagram to illustrate distribution of current density in pore region. *A*, *A*, section of active electrode tube, × 250; *B*, electrolyte solution contained in tube (active electrode); *C*, electrolyte solution of preparation bath (indifferent electrode); *D*, tissue (reduced to a circle of the diameter of a muscle fiber); *o*, pore. Density of current may be regarded as varying inversely with the distance between the radiating lines.

1. If the pore be cathodal with respect to the preparation bath, a stimulus of minimal value will, if the pore lie within the area of a fiber, excite that fiber alone. If it lie at the junction of two fibers both, if of equal threshold, will respond.

2. If the pore be anodal with respect to the preparation bath, the cathode for a given fiber impinged upon will be a lateral or deep margin where density of current is relatively low; and a considerably stronger stimulus must be used to excite. The use of a pole-changer in the stimulating circuit at once emphasizes the above assumption. Rarely do make-shocks have to be considered in any range of stimuli employed by us, if the minimal stimulus be a break-shock.

The Mechanical Stage

The mechanical stage (figs. 4 and 5) is for the purpose of carrying preparation, baths and electrodes so that any point on the surface of

the preparation may be brought under the objective and properly oriented without derangement of the active electrode with respect to the tissue to which it is applied. A detached revolving microscope stage is centered under the objective and fixed to the table. Upon this is cemented a disk of heavy plate-glass, forming a turntable of 28 cm. diameter. Above and movable upon the turntable is a square of similar plate-glass supported by four stout corks. To this is clamped an ordinary mechanical stage with double rack and pinion, its carriage, however, being greatly extended by a glass plate, 16 x 21 cm., which on account of the weight it must support has its overhang riding on a $\frac{1}{2}$

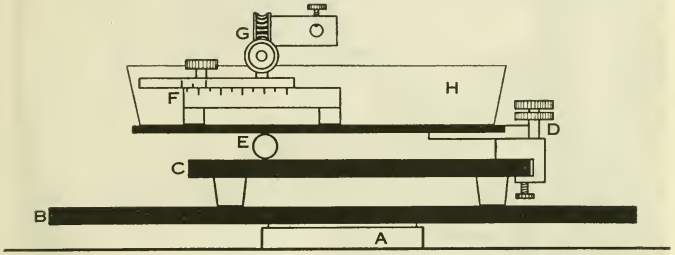

Fig. 4. Mechanical stage, front elevation, $\times \frac{3}{10}$. *A*, revolving microscope stage; *B*, plate-glass turntable; *C*, plate-glass support for attachment of *D*, mechanical stage proper, the glass extension of which rests upon ball-bearing at *E; F*, rack-and-pinion of electrode holder; *G*, worm-and-gear, carrying perforated extension with set-screw for electrode; *H*, container for preparation bath.

inch steel ball-bearing. This renders strain and friction negligible even with a heavily loaded stage.

The arrangement of the stage-load is shown in the photograph (fig. 5) taken from above. An electrode holder enables the pore to be lowered gently upon the preparation and carried laterally from fiber to fiber. Its adjustment mechanism consists of a worm-and-gear, carrying the electrode in a vertical plane, attached to a lateral rack-and-pinion. The electrode tube is further adjustable in a direction parallel with the fibers, by being passed through an opening in its support attached to the gear, where it is fixed by a set-screw.

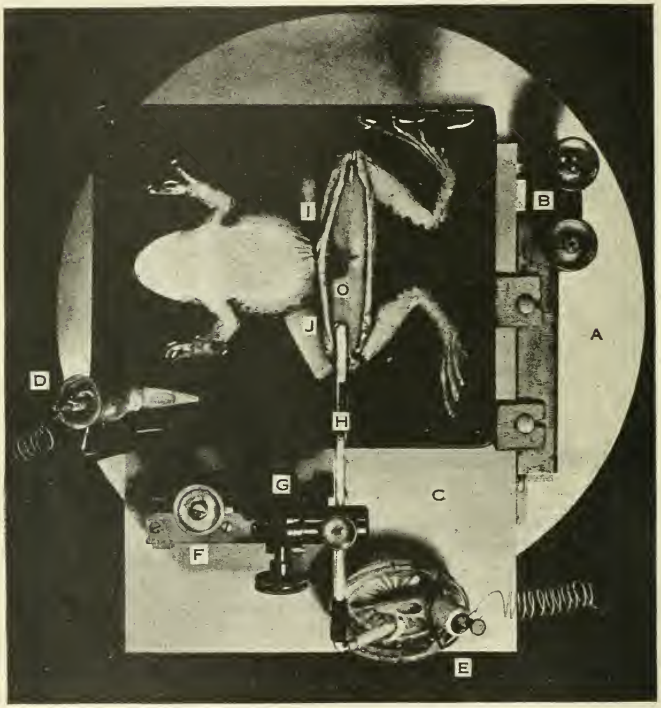

Fig. 5. Mechanical stage, loaded, photographed from above, × ½. *A*, turntable; *B*, adjustments of mechanical stage proper; *C*, extension of carriage; *D*, non-polarizable boot electrode (indifferent) immersed in preparation bath; *E*, boot electrode (active) in separate bath which also receives end of electrode tube; *F*, rack-and-pinion of electrode holder; *G*, worm-and-gear moving electrode, *H*, in vertical arc; *I*, *J*, plate-glass sinkers joined by cord over pelvis and supporting thighs horizontally. The pore end of the electrode is applied opposite *J* to the sartorius, and observations are taken at *O*, near the detached pelvic insertion.

The Mercury Mirror and its Illumination

Particles adhering to a muscle are moved in accordance with the distortion of the connective tissue investment by the fibers beneath. The convex spherical mirror furnished by a globule of mercury is a ready means of recording such movement by photography. It is our practice to spray the preparation (usually the bared satorius *in situ* of the pithed frog) liberally with mercury ejected from a very finely drawn glass tube, by quick and slightly sustained pressure on an attached atomizer bulb. It is usually possible on stimulating a fiber to find somewhere on its length a globule of requisitely small size, moving nearly in the direction necessary for recording. The field is then properly oriented by means of the turntable and the stage adjustments.

The source of illumination is a 15 c.p. tungsten nitrogen bulb supplied from the building current through a transformer. The rays are focussed upon the globule chosen as an index through a lens system (figs. 1, *F*, and 6, *H*) with adjustable support. Owing to the spherical form of the mercury mirror the angle of incidence is immaterial over a wide range, making it possible, while maintaining a brilliant image on the globule, to keep the background free from disturbing reflections (fig. 18).

For certain earlier records a segment of fine capillary tubing containing mercury was used as a mirror with distinctive results (figs. 19 to 22).

The Fibromyograph

The above term may be applied to the combined devices outlined mainly in figure 6. The purpose of the apparatus is to enable graphic records of fiber contraction to be made in a lighted room with choice as to speed of recording surface, to furnish a simultaneous graphic time-signal of desired frequency, or to indicate likewise graphically the changes in position of a secondary coil.

Plate-glide. This is built of wood in the form of a shallow trough without ends, and is mounted independently on three legs of heavy metal pipe above the mechanical stage and the microscope, the latter being carried on a rigid horizontal arm supported on a stand at the rear of the table, as previously described (4). Details of the plate-glide are shown in plan in figure 7, and in cross-section in figure 8. The plate-holder, of $6\frac{1}{2} \times 8\frac{1}{2}$ inches capacity, carries four grooved blocks bearing on two glass rods serving as tracks. On these the plate-holder may be drawn from end to end of the wooden bed by means of a cord attached outside to the traction mechanism and playing over a cylin-

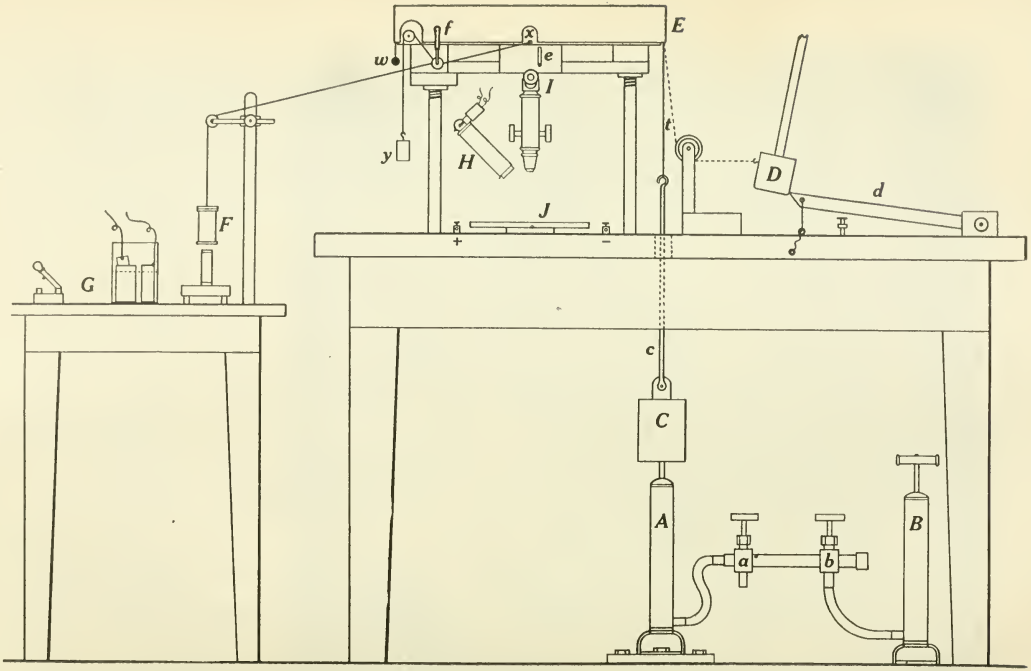

Fig. 6. Fibromyograph, × $\frac{1}{12}$. *A*, traction pump; *B*, filling pump; *a*, escape valve; *b*, filling valve; *C*, piston weight; *c*, traction rod; *D*, traction pendulum; *d*, trigger; *t*, traction cords; *E*, plate-glide, covered; *e*, lever of shutter; *F*, secondary coil; *f*, lever of coil-position recorder; *y*, counter-weight of same; *x*, signal-box pulley; *G*, space for battery, keys, etc.; *H*, globule illuminator (mechanically adjustable support omitted); *I*, demonstration ocular; *J*, turntable of mechanical stage; *w*, counterweight of plate-holder; +, −, terminals of secondary circuit.

drical glass guide. A cord from the other end of the plate-holder passes in a similar way to a counterweight. The bed is transversely divided at its middle by a rectangular groove, the floor of which is perforated by a slot directly over the eyepiece of the microscope. The groove serves at one end as a recess and guide for a small movable box, open above and at its outer end, containing lens and mirror. This is the signal-box, to be described later. A cardboard cover fitted over the plate-glide is used to exclude light during a record.

Traction piston. For slow traction of the plate-holder the descent of a weight over an escaping column of air is utilized. A cord extension of the heavily weighted piston-rod of a tire pump (fig. 6) is carried over a guide to the plate-holder, the table being bored for the purpose. The exit of this pump is led to a horizontal cylinder which has two connections: one with the outside air through needle-valve *a*; the other, through needle-valve *b*, with a second pump used for filling the first. The first or traction pump being filled and connected, and valve *b* closed, the descent of the weight will be delicately regulated by adjustment of outflow through valve *a*. By this means the length of the plate is transversed in a time ranging from 1 second to 30 minutes with sufficient smoothness and uniformity. At the lowest speed it is possible to record on one abscissa over one thousand separate and microscopically distinct contractions (figs. 36 and 37).

Traction pendulum. For rapid recording the plate-cord is uncoupled from the piston-weight and attached to a heavy and rigidly mounted pendulum (fig. 6) giving approximately a one second's single vibration when drawing the plate, and released by a suitable trigger. The moving plate-holder may be made to trip a stimulation-signal key sunk in the bed of the plate-glide (fig. 7, *G*).

Time-signal recorder (figs. 7 and 8). Mounted on a stand behind the plate-glide is a 15 c.p. tungsten lamp with compact filament, connected with a transformer and subject to adjustable resistances. Rays made parallel by a lens system attached to the back guard of the plate-glide traverse an opening in the guard and enter the signal-box already mentioned. Here they are converged by the lens mounted, with the mirror, in the box. The rays are then reflected vertically to their focus on the plate by the mirror, which consists in this instance of a fragment of coverglass with its back ground and blackened to leave a single reflecting surface. Interposed between the lamp and the first optical system is a celluloid diaphragm, the orifice of which is made with the point of a fine sewing-needle, rendering the image on the plate about the same

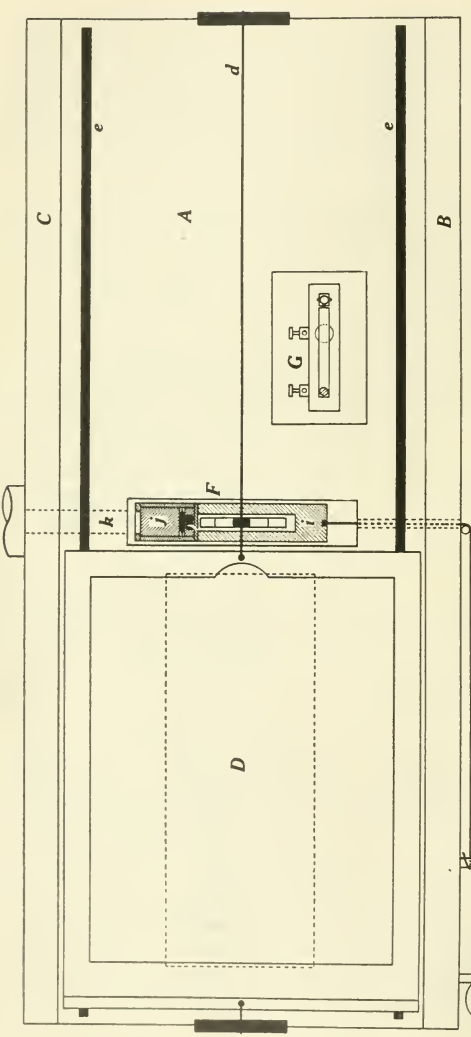

Fig. 7. Plan of plate-glide, $\times \frac{3}{16}$. *A*, bed; *B*, front-guard; *C* back-guard; *D*, plate-holder with $3\frac{1}{4} \times 8\frac{1}{2}$ in. plate; *E*, pulley actuated by lever of coil-position recorder, drawing equally and simultaneously on cord of secondary coil, *f*, of counter-weight, *g*, and of signal-box, *h*; *F*, signal-box groove, containing slotted strip, *i*, which is moved by cord *h* and carries signal-box, *j*, containing lens and mirror; *k*, perforation for beam of signal-light; *G*, stimulation-signal key, countersunk. The traction-cord, *d*, descends vertically over a glass cylinder. The plate-holder is counterweighted in a similar way at the left end of the bed. *e*, *e*, glass tracks for plate-holder.

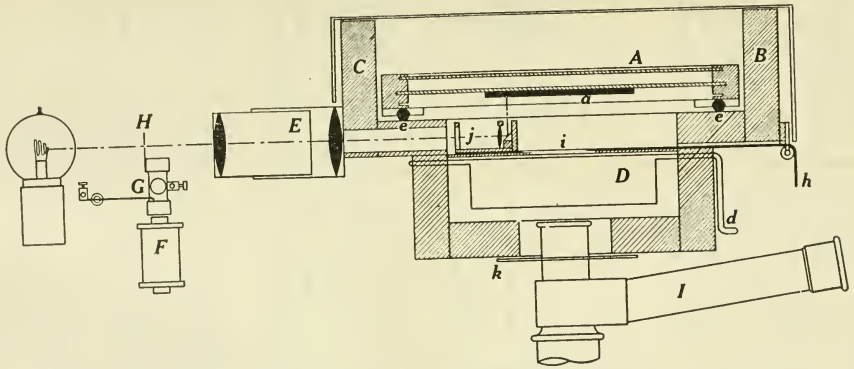

Fig. 8. Cross-section of plate-glide, × $\frac{2}{3}$. *A*, plate-holder; *a*, plate; *B*, front guard; *C*, back guard; *D*, shutter, actuated by lever, *d*; *E*, signal-light projector; *e*, *e*, glass tracks supporting plate-holder on grooved blocks. *F*, driving magnet of tuning-fork, *G*; *H*, diaphragm; *h*, signal-box cord; *i*, slot; *j*, signal box, projecting image defined by diaphragm, *H*, vertically to plate; *k*, flange; *I*, demonstration ocular (*Leitz*).

diameter as that derived from the mercury globule. The diaphragm is mounted on an electrically driven c'' fork (512 d.v.). Illuminated at rest, it writes an abscissa (fig. 14) subject to interruption by various means (fig. 15) or records the ordinate position of the signal-box if this be actuated (fig. 14). When vibrated by the tuning-fork it traces on the plate a typical sinusoidal curve (fig. 34).

Coil-position recorder. When it is desired to indicate on the plate the variations in position of the secondary coil, the diaphragm described above is kept at rest. The signal-box, by means of which a spot of light is made to trace its path on the plate, has been mentioned as capable of movement in its recess at right angles to the movement of the plate. The construction is shown in figures 6, 7 and 8. A pulley actuated by a hand-lever is fixed to the support of the plate-glide and connected in such manner that its movement clockwise will draw toward the front a thin strip of metal which rests upon the floor of the groove and is perforated to conform to the slot of the latter. The signal-box is attached to this strip and resists traction through the tension of a contained helical spring. The actuating process at the same time lowers the secondary coil of an upright inductorium over the primary (fig. 6). Both attached cords being wound on the axle of the actuating pulley as a drum, the extent of movement of box and coil is the same. Hence any departure of the spot of light from its abscissa will indicate increase of stimulus in absolute terms of coil-position (fig. 14). The cord actuating the coil, after several turns about the axle above mentioned, is led to a suspended weight. Thus a ready adjustment of initial coil position is permitted by reciprocal movement of coil and weight while the pulley is at rest. The rays projected to the lens of the signal-box are sufficiently parallel to enable the focus to be maintained on the plate throughout the limited range of movement provided (8.5 mm.).

Optical signal. For studying the latent period of the muscle cell it has occurred to us to utilize the image of the response-index (mercury globule) itself as a stimulation signal, so moving the image optically that sudden cessation of its movement shall be coincident with stimulation. In other words, the rays from (for the time being) stationary object to moving image are to form a magnifying signal-lever devoid of inertia and operating on the contraction-abscissa.

The essential of the device (fig. 9) is a small rectangle of plate-glass interposed between object and objective. This is set in a metal frame pivoted to the objective and continuous with a light armature extending horizontally to the rear. When the armature is drawn upward

about 3 degrees by a small electro-magnet likewise attached to the objective, the prism is tilted through a very small arc; the rays from the object suffer immediate refraction in a plane parallel to the ordinates, and the image on the photographic plate, thus set in magnified motion, will come to an abrupt stop at the instant of contact of armature with core. The armature, however, bears as a contact surface a short piece of platinum wire soldered horizontally. Attached to the core of the magnet with non-conducting cement is a second platinum wire at right angles to the first. These crossed wires are introduced as terminals into the primary stimulating circuit (quite distinct from the magnet or

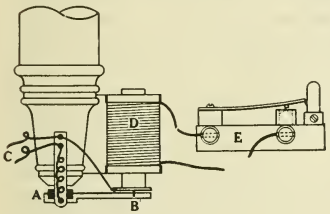

Fig. 9

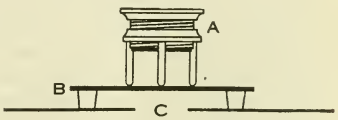

Fig. 10

Fig. 9. Optical signal, $\times \frac{3}{5}$. *A*, square of plate-glass, mounted in armature pivoted below objective; *B*, armature carrying Pt contact wire; *C*, terminals in primary circuit, one in metallic connection with contact at *B*, the other with similar Pt contact wire fixed with a non-conducting cement to core of magnet *D*; *E*, Pt-Hg signal key tripped by plate-holder in mid-swing of pendulum, actuating magnet *D*.

Fig. 10. Focussing table, $\times \frac{3}{10}$. *A*, adjustable-focus magnifier; *B*, transparent glass screen; *C*, opening of slot-groove in bed of plate-glide.

signal circuit) and cessation of image movement is brought about by and coincident with their contact. Thus in the tracings (fig. 34) the latent period is measured from the summit of the first rise, emphasized by a slight recoil, to the beginning of the make-contraction. The course of events thus is: 1, movement of plate-holder; 2, knockdown of signal key by plate-holder, closing magnet circuit by Pt-Hg contact; 3, movement of optical signal; 4, stoppage of optical signal by making of Pt-Pt contact in the primary circuit—the instant of this stoppage of the refracted ray being taken as the instant of delivery of an induced make-shock to the preparation. In the records here published the stimulation key was closed by the pendulum itself and not by the plate-holder.

Demonstration ocular. One of the greatest aids to the successful use of the apparatus is a demonstration ocular (figs. 6 and 8, *I*). In observing an area of locally excited muscle we are dealing not always with one, but often with several thresholds, depending on the number of fibers in the field of influence of the electrode. By the use of this valuable accessory the experimenter is able to check the events in the field from end to end of the graphic record, and thus to control the conditions of stimulation by means of the mechanical aids. Moreover his adjustments may be automatically recorded through the signal mechanisms already described. Success or failure of a record is thus often determinable without first having recourse to development of the plate. The shadow of the adjustable pointer furnished with the instrument, to be moved at will over the field, can be placed so as to write beneath the record an abscissa (figs. 27, *A-F*, and 30) which has proved useful as a base for measurements of contraction and detection of contractures.

Focussing table. This is a simple but indispensable adjunct (fig. 10). A transparent plate of glass is mounted on supports conveniently made from small corks. These are made of such a height that when the device is laid on the bed of the plate-glide, bridging the slot-groove, the lower surface of the glass will be in the plane to be occupied by the film of the recording plate. A magnifier is so adjusted that when laid on the glass the lower surface of the latter will be in exact focus for the relaxed vision of the operator. The image of the light-reflex from the globule, thus magnified, may then be brought into extremely accurate focus by the use of the microscopic fine adjustment without recourse to a focussing cloth or other means of excluding the light of the room. The demonstration ocular is permanently adjusted for focus. By its means any loss of definition during a record may be approximately rectified by the observer.

Rigidity. Every precaution must be taken to secure rigidity of the entire apparatus, especially since any disturbance of the liquid surface over the preparation will introduce errors of refraction. Hence there are a large number of clamps and braces, not shown in the figures.

Means of Stimulation

Stimuli for twitch. A Porter inductorium has its secondary coil detached and suspended solely by the actuating cord (fig. 6), which is attached to the center of a bridge crossing the distal end of the coil. Very fine extensible wires lead from the coil to a fixed support, being

carried thence to binding-posts screwed to the table near the mechanical stage. The fact has been emphasized in a previous report (2) that considerable latitude of technical error in stimulation is possible when dealing with the muscle elements, without obscuring the determinate character of the response. A dry cell and sparking contact in the primary circuit will always yield determinate (fig. 13) and often uniform (fig. 12) responses, it being of course characteristic of the all-or-none mechanism that it is impartial toward a relatively wide range of excitation strength. On the other hand, to obtain uniform results in the neighborhood of thresholds requires great nicety of stimulation, quite beyond the capacity of ordinary methods of circuit interruption. For uniform or smoothly graded *influence*, as distinct from effect, we have resorted to special means. The best twitch effects (figs. 24 and 29) have been obtained by a mercury-mercury key, still in process of development, in which a conducting mercury column is broken within a rubber tube by an outside pressure-mechanism, automatically controlled. A key of the Martin type is of course thoroughly adequate if high frequencies and extreme mobility are not required.

Magneto-inductor. For the production of uniform tetanizing stimuli, brief or prolonged, we have found nothing so convenient and reliable as an extremely simple induction machine of a form already briefly described (2). The principle is by no means new, being a direct return to that employed by Pixii (6) who, in 1832, applying the new-made discoveries of Faraday, was the first to use the rotating magnet for producing currents in stationary coils.

Figure 11 shows semi-diagrammatically the details of the apparatus, in which uniform rotation of a light permanent bar-magnet is effected by an hydraulic centrifuge driven by a water-column of constant height. The column is provided by a 0.75 in. standpipe fed by the tap and overflowing at constant level beneath a 1 in. air vent into a waste-pipe. The motor is mounted for present use so as to discharge directly into a sink; the standpipe, attached to a chimney on the roof of the laboratory, receiving and feeding through two lengths of rubber hose led through the upper part of an adjacent window. The waste-pipe discharges into a building vent-pipe in the roof.

The two finely-wound coils influenced by the magnet are opposed and in series, and so connected that the circuit carries the jointly induced alternating current directly through the electrodes. The position of one coil, as well as that of its core, is adjustable with reference to the magnet, and is used in roughly approximating the threshold

stimulus. The core of the opposite coil is fixed; but the entire coil glides smoothly on glass ways over a metric scale. Fineness of gradation is attained by belting this coil over a system of pulleys so that one revolution of a large hand-wheel covers 2 cm. on the scale. This coil further registers its position on an empirically graduated quadrant through a 1:4 magnifying lever, enabling the observer controlling the wheel to read at a distance the coil-position in millimeters.

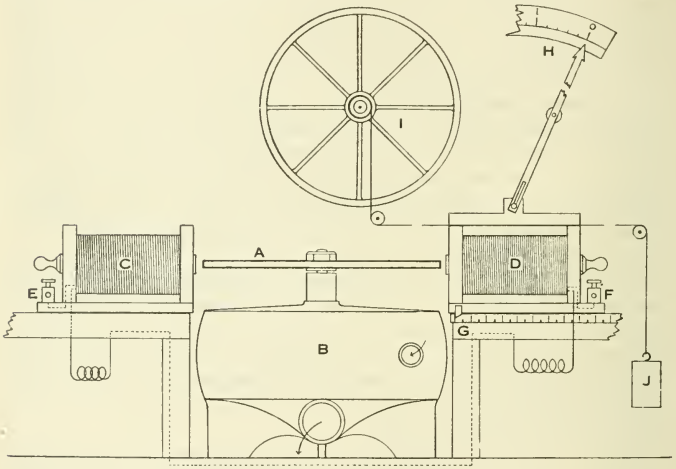

Fig. 11. Magneto-inductor (diagrammatic, × ¼). *A*, bar-magnet, 150 x 12 x 6 mm., rotated by hydraulic centrifuge, *B*, under constant pressure from standpipe. *C*, coarse adjustment coil; *D*, fine adjustment coil; *E*, *F*, terminals in electrode circuit; *G*, metric scale traversed by coil *D*; *H*, quadrant empirically graduated in terms of scale *G*, through × 4 magnifying lever. *I*, hand-wheel (relatively reduced) actuating coil, *D*, over reduction pulley; *J*, counterweight. The movement of coil *D* may be automatically recorded by connection of signal-box pulley (fig. 6, *x*) to an appropriate point on lever.

This apparatus, in conjunction with the pore-electrode, has been found to work with great uniformity. Experiments can be carried on all day without removing the electrode from the fiber beneath. It is particularly useful in exploring the muscle surface for favorable responding elements, determining threshold, and demonstrating contractions over the ocular-micrometer scale. Applied as a continuous tetanizer

it elicits clean-cut quantal effects (fig. 15) and admits of continuous and wide variation in stimulus-intensity (figs. 16 and 19). A simple metallic key makes and breaks the circuit without discoverable spark. A reduced record of stimulation-strength can be made on the plate by attaching the coil-lever near its fulcrum through pulleys to the signal-box, thus automatically registering the position of the coil relative to the magnet (fig. 16).

After trials with many different forms of stimulation, we have found this simple alternating current Pixii machine, placed in direct circuit with the electrodes, apparently the ideal mode of high-frequency excitation. The water column employed gives sufficient speed of rotation to completely tetanize the superficial fibers of the frog's sartorius (about 30 cathodal waves per second). By the employment of the bar magnet with its widely separated poles (15 cm.) instead of Pixii's horse-shoe form, the lines of force sweep through the coils abruptly on a relatively wide radius, yielding sharply-peaked waves of potential which are effective as stimuli with a current of extreme tenuity. The apparatus is kept permanently set up, and is ready for immediate service on opening the tap feeding the standpipe.

The coarse-adjustment coil, being always near the magnet, serves through its preponderating influence to maintain the form of the waves of potential, while the fine-adjustment coil, actuated over a wider range, alters their value slightly and with extreme delicacy. A telephone introduced into the circuit gives a pure musical tone and indicates clearly the continuity of the upward and downward gradients of excitatory influence. The exact form of these gradients is as yet immaterial. The ends to be achieved for present purposes are uniformity and reliability for any given coil-position, and a constant and self-maintaining driving mechanism, with absence of all sliding and brush contacts.

Choice of Plates and Methods of Reproduction

The form of photographic recorder described commends itself to a large extent in the fact that the tracings are made in a lighted room. This is possible from the protection afforded the plate-holder, first, by the bed and side-guards of the plate-glide; second, by the cover which is lowered over the device when the slide is removed from and restored to the plate-holder. For slow records with the traction piston we have found the slowest or "process" plates amply sensitive; but for the pendulum records a very rapid plate is necessary. The plates ($6\frac{1}{2}$ x $8\frac{1}{2}$ in.) are

cut lengthwise in two before use, giving strips of the right width to use in a standard projection lantern. For this form of demonstration the negative alone is of course as good as the derived positive. Enlarged positives are readily made by similar projection upon plates or developing paper (fig. 13). Direct prints often yield a record similar to a kymographic tracing, and are reproducible by line-cut (fig. 15). On account of the faintness of the more rapid portions of curves as well as the tendency of the partially illuminated field to leave a strip of "fog" as a bakground, the half-tone or similar process is usually required, as in most of the tracings here rendered.

Conduct of an Experiment

The following description sets forth the routine procedures as at present developed. A number of the tracings, however, were secured by methods differing in detail from those outlined in the text. Such differences, proper to various stages of the work, are indicated in the descriptions accompanying the figures wherever the departure is sufficient to require comment.

Preparation of sartorius. Although any parallel-fibered muscle preparation, if not too thickly invested, is adaptable to this method, the greatest convenience and most satisfactory results have been attained with the uncurarized sartorius, in situ and circulation, of a leopard frog of average size. This muscle, peculiarly adapted to the present work through its structure and thin investment, is also chosen advisedly on account of the mass of data already obtained through its study in the past, affording in the literature a safe basis of comparison. With respect to the disuse of curare, it is remarkable what liberties may be taken in this intensely localized form of stimulation without eliciting effects toward which the inhibitive action of curare is usually directed. Although a nerve-free region of the muscle is usually selected for application of the electrode, this is done largely for reasons other than the absence of nerve filaments,—comparative freedom of the fiber extremities from linear movement, primarily. Numerous experiments have been performed with the electrode on the mid-region of the muscle (fig. 13). Under a certain limit of intensity the stimulus, effective for superficial fibers, fails to influence the nerve elements; beyond this limit, however, a sudden dispersion of response reflects unmistakably an indirect stimulation. The hypothetical advantage therefore accrues of employing a tissue with all its susceptibilities chemically intact.

Brain and cord are destroyed as bloodlessly as possible; but if bleeding occurs from the pithing puncture it has been found best not to check it artificially on account of an apparent resulting motor nerve irritation. Suspension of the frog by the jaw for a few minutes until heart action is vigorous favors the capillary circulation in the leg muscles. The frog is placed in its container (a 4 x 5 in. developing tray), ventral surface uppermost, where it is anchored by two glass sinkers, 5 mm. thick, connected by a cord passing over the pelvis (fig. 5); the sinkers being turned underneath the thighs to support them in such a way that their ventral surfaces lie horizontally. The thighs are abducted slightly more than at right angles to the trunk. The skin of both thighs is now incised in a line which meets over the symphysis and extends in the median line of each thigh to beyond the knee. The skin is turned back by carefully dividing the delicate tissue forming the lymph compartments on the ventral surface of the thigh near the pelvis and along the rectus muscles. These muscles, when detached from the pelvic origin, retract with little if any bleeding. The sartorius muscles will now lie exposed to view very nearly in a horizontal plane, favoring ready observation under the microscope from end to end. It has been found a decided advantage, where more nearly isometric contractions are not required, to incise the superficial sartorius fibers or even the whole muscle at the iliac origin. The resulting retraction not only renders the fibers more distinct (fig. 18) but greatly favors contraction in a straight line.

As soon as possible after exposing the muscles the tray is placed on the mechanical stage and the bare tissue sprayed with mercury so that the globules lie on the average about 1 mm. apart. The preparation is at once flooded with Ringer's fluid just sufficient in amount to cover the entire muscle surface. More elevated portions of the frog are covered with filter-paper in contact with the solution, which favors maintenance of peripheral circulation and prevents movements induced by drying.

The preparation made as above can be used for experiment at any time subsequently during the day, and is in fact best left undisturbed for a time to permit conditions altered by the operation to regain equilibrium.

Setting up of electrode. The pore electrode is kept in distilled water when not in use. In preparation for use the electrode and connecting tubes are filled with Ringer's fluid. To test whether the pore is clear, an atomizer bulb or hypodermic syringe is attached to the large end of

the electrode tube, the pore end dried with lens paper, and a slight air pressure applied. If a droplet of liquid does not appear at the pore, even under heavier pressure, the obstruction may be removed by one of the following methods, to be resorted to in sequence:

1. Apply considerable force after attaching the pressure tube to the pore end.

2. Completely fill the electrode and empty it abruptly through its large end by the same movement used in shaking down a clinical thermometer.

3. Place a drop of concentrated nitric acid for a few moments on the face of the electrode. Wash off and irrigate by pressure applied at the other end.

4. If the pore is badly obstructed the electrode can be filled with acid and pressure exerted preferably at the pore end. Irrigate thoroughly.

It is suggested that pepsin-hydrochloric-acid solution would be of service in certain types of obstruction.

To prevent siphonage during attachment of the tubes connecting the electrode with its bath, it is convenient to plug them in several places with cotton, avoiding the imprisonment of air. Glass-rubber joints are best not immersed on account of possible short-circuiting.

Application of electrode. The electrode must only gently touch the surface of the tissue; heavy pressure appears to inhibit activity of the surface fibers, permitting only of deeper stimulation. Insufficient pressure, on the other hand, allows the fibers to withdraw from effective contact on contraction, often resulting in a rhythmic series of contractions in response to a tetanizing current. At the right pressure any slight movement under the electrode due to contraction appears to introduce no variable factors. The proper tension is best gauged empirically on stimulation.

Field of response. Observation through the demonstration ocular under low power (e.g., Zeiss AA $\times$ 2) reveals the sartorius fibers with an apparent width of about 1 mm., with several mercury globules in the field of a diameter averaging about that of the fibers. Capillaries with circulating corpuscles are readily seen in various parts of the field. The electrode has been applied to a region where the linear sliding movement of fibers is not likely to be marked; such as at the tibial end, especially when the pelvic insertion has been incised. On stimulation with single shocks or briefly-held tetanic currents, the muscle is explored by means of the mechanical stage for a favorably responding region. Almost

invariably the fascial investment shows some movement in any field first observed, and the preponderating extent of movement on one side or the other leads the observer unerringly to the field of actual contraction. Here a fasciculus or small group of contiguous fibers, or even a single fiber, may be recognized in activity, the globules which happen to rest upon the active region showing preponderance in extent and straightness of movement over those which lie adjacent. If reduction of current to minimal strength fails to yield a sufficiently localized contraction, a slight shifting of the electrode laterally (to an adjacent fiber) or vertically (altering tension) will often bring a single fiber into activity. For most puposes, however, all that is desired is a clean-cut, straight contraction whether from one fiber or from several fibers acting as a unit. Observation at any region where the fibers curve or dip downward to their attachments, shows nearly always a curvilinear or aberrant contraction on stimulation (fig. 27), this being the resultant of asymmetrical elastic tensions opposing shortening. In nearly every instance a globule of mercury resting directly over an active superficial fiber will move exactly with the apparent fiber movement. Such a globule is selected as the recording mirror, and any neighboring globules within the field of the slot are removed with a hair cemented to the end of a glass rod. A good field for recording should have the following characteristics:

1. Globule about 30 μ diameter, highly illuminated throughout its range of excursion, which should cover several quantal steps.

2. Movement in a straight line and horizontal plane; the latter in order to maintain sharpness of definition.

The small electric lamp used for illuminating is a nitrogen-filled bulb with a short coiled filament. The projector throws upon the field a parallel sided image of the filament and this is aligned so that the globule is well within the bright area throughout its movement. The last adjustment made in the demonstration-ocular field is directed toward aligning this area with the direction of contraction as finally corrected by means of the turntable during the focussing process.

Focus. Accurate focussing of the image reflected from the mercury globule is essential for a good record. In using the magnifier shown in figure 10 it is necessary to maintain a relaxed accommodation in its adjustment and use, and to check this by frequent reference to a scratch or other mark on the under surface of the glass table. If the focus of the demonstration ocular has been set to conform with the plate image, the eye lens requires no further adjustment for a given observer.

Owing to its greater depth of focus, however, it is inadvisable to use it in making corrections except during a record, when focussing from above is precluded. As a rule it is seldom necessary to make the latter corrections. The focus of the image reflected from the signal-box is permanently adjusted and needs only occasional verification. The needle-hole diaphragm, when vibrated by the tuning-fork, traverses the narrow image of the lamp-filament; hence care must be taken so to align the signal system that the diaphragm transmits light of equal intensity at the extremes of oscillation.

Signals. In using the tuning-fork, it is vibrated electrically until clear and steady in tone. The driving current is then broken and the pendulum record taken during the residual vibrations, which give a curve (fig. 34) undisturbed by the interference waves liable to occur from the vibration of supporting apparatus during the electrical actuation. If a time record of longer intervals, such as seconds, is required for slow records, the intensity of the lamp may be altered periodically by cutting out with a clock circuit a certain amount of interposed resistance, giving an abscissa broken into periods (fig. 15). Ordinate alignment of signal image with globule image is done at the time of focussing by bringing the latter into the required position with the mechanical stage. The alignment is checked for error photographically at the beginning of each record, as later described. The optical signal for recording the instant of a make-shock requires only connection, on the one hand, with the primary circuit; on the other, with the magnet or trip-key circuit. The trip-key, closed by the plate-holder in mid-swing of pendulum, is of course to be opened before each record. During a slow record the optical signal may be cut off from the stimulating circuit and the magnet operated by a hand-key to denote on the response curve the occurrence of various procedures recorded in the notes, such as modes of stimulation, application of drugs, etc. The signal was employed thus in figure 17, but is readily visible only on magnification of the original plate.

Traction of plate. The plate-moving devices, as are all others, are readily operated by the observer without assistance. In either piston or pendulum traction the plate should be started with the cord at full tension to avoid sudden jar. In using the piston, the escape valve (fig. 6, *a*), previously adjusted for the desired speed, may be connected to a long rubber tube, the outlet of which is released at the proper moment by the seated observer at the microscope.

Management of plate-holder. To load the apparatus, the plate-holder is placed in position on its tracks, with traction cord and weight-cord attached, and the cover is laid over the plate-glide, the end of the cover over the plate-holder being raised so as just to rest on the ends of the guards. The plate-holder is steadied with one hand while with the other the slide is quickly drawn; the cover now being immediately dropped in position. A low strip of felt at the end of the bed, against which the end of the plate-holder impinges, shuts out light sufficiently during this operation to safeguard "graflex" plates when the room is moderatly lighted. The traction-cord is not hooked to the pendulum or traction-rod until everything is in readiness for a record. The shutter (fig. 8, *D*) is closed throughout the above preparations, and it should be seen that the flange (fig. 8, *k*) encircling the tube of the microscope is raised against the under surface of the bed after all focussing is completed. The end of a record is indicated by the arrival at the edge of the bed of a knot in the weight cord. This cord is used for returning the plate-holder to its original position, where the slide is replaced after lifting the edge of the cover. If a zero abscissa is required, a second exposure is made without stimulation. The two recording images are sufficiently separated to enable as many as six exposures to be made on one plate by carefully measured actuation of the mechanical stage between records (fig. 34).

Record for correction of ordinate position. Unlike the optical signal, the time and coil-position recorder is subject to error of coördination between response image and signal image. Such error is rendered determinable by taking a stationary exposure at the beginning of each plate record (fig. 14). This is done by stopping the plate just as it begins to cover the slot, opening the shutter and turning on the two recording lights. If the signal record is to be one of coil position, the actuating lever is moved through its entire quadrant, thus tracing a reference line for all positions of the signal image (fig. 24).

Order of procedure. The following points summarize in sequence the events of a typical experiment:

1. Preparation made, mercury globules applied and muscles flooded.
2. Pore tested and electrodes set up.
3. Illumination adjusted.
4. Recording area selected by trial stimulation.
5. Stimulation intensity range adapted to threshold.
6. Field oriented for aligment of direction of contraction and coördination of globule image with signal image.

7. Focus adjusted at plate level; shutter closed.
8. Electrodes, keys and signals circuited.
9. Traction mechanism set.
10. Plate-holder inserted and connected. Slide drawn.
11. Response verified and illumination of field corrected.
12. Traction cord connected to traction mechanism.
13. Plate drawn to slot. Shutter opened. Exposure made for record of signal-image alignment.
14. Traction released; observer at the microscope. Record taken and shutter closed.
15. Traction cord uncoupled; plate-holder returned to position of loading.
16. Abscissa drawn, if required, by repeating exposure under traction without stimulation.
17. Slide inserted and plate-holder detached from cords.

The amount of time required for the above series of operations is inconsiderable. When, in 1916, the first tracings were obtained by the photographic method, a full day was the time regarded as necessary for the attainment of a result. It is now possible, though not always advisable, to carry out the entire procedure up to a completed negative well within an hour. This shortening of time is the result of practice, improved technique and the fact that the bulk of apparatus can be permanently set up, ready for instant use.

PART II. FURTHER EVIDENCE OF A QUANTAL PRINCIPLE

On the Use of Terms

The accompanying tracings have one characteristic in common: change in extent of contraction is for the most part conspicuously abrupt; and the transition is introductive of a new level which itself does not materially alter except abruptly—such gradual changes as make the latter a qualified statement being without known exception independent of alteration in stimulus intensity or threshold level. Recognition of these gradual changes thus serves to emphasize the dual character of muscular gradients—the continuous, and the abrupt or discontinuous. The discontinuous mode of gradient formation, reflecting as it must the accession or elimination of unit energy values, has been termed *quantal* by one of us in a previous paper (2). It is the most conspicuous character of activity revealed in a skeletal muscle by intensively localized stimulation; change of level is in steps. It is con-

Fig. 12. June 3, 1918; E. *R. pipiens.* Sartorius; tibial and pelvic tendons cut. Electrode (pore 7 μ) near pelvic end. Record taken near middle of muscle. Break shock every 2 sec. produced by pendulum of Powditch clock opening Pt-Hg contact in primary circuit. Dry cell, slight spark at pendulum contact. Rheocord and rheostat in primary. The first 20 twitches were recorded without change of stimulus. Thence the primary resistance was successively raised and lowered on the rheostat, introducing three limited series of which *a* shows perfect di-quantal gradients. Elsewhere there are variations out of grade to be ascribed to the crude method of interruption, but all are strictly determinate in terms of *a*. For the final series the resistance was continuously decreased to 0 on the rheostat, introducing a third quantal, the series being completed with uniform stimulation at this level. An initial continuous fatigue at the beginning of the tracing is emphasized by delayed plate movement.

This record is fifth in a series extending over several hours, of which figure 32 is the first. The threshold has risen to an extent represented by a range of 4 cm. on the rheocord. Continuous fatigue is apparent on comparison. Fatigue leaves all-or-none capacity untouched.

Contact print from original plate, actual size. Microscopic magnification, × 13.5.

venient, therefore, to call the step-making mechanisms *quantals* since each manifests an energy value proper to itself—a physiological quantum. The term may, moreover, be carried over without ambiguity to designate the visual or graphic result of such determinate energy discharge, and we have found such usage greatly conducive to clearness and brevity in the description and discussion of experimental records.

A quantal may obviously be highly multiple, as in the ventricle, or simple, as in the striated fiber; or again, as in the "steps" of many of these records, double (from concomitant action of two fibers), triple, quadruple, etc. Compounding of quantals in varied degree is reflected in the graded character of skeleto-muscular reactions in response to varied stimuli and varied thresholds, the two effects being, as has been shown (2), reciprocal. The term all-or-none (all-or-nothing) as applied by Bowditch to the heart is therefore in the case of skeletal muscle strictly applicable to the single fiber only, or to the whole muscle after it has begun to be supramaximally excited. The skeletal *musculature* must be, however, as is the heart, quantal at all times and in any part taken; being constituted throughout, as is the heart, of all-or-none elements. Hence, were the heart at any time to suffer its assumed complete interconductance to become a function of stimulus or threshold it must still remain quantal by virtue of its elements. These conceptions may be summarized in a series of postulates:

In response to changing stimulus intensity or changing threshold level,

1. The fiber of skeletal, as of cardiac, muscle is all-or-none.

2. The normal cardiac musculature as generally considered is, owing to interconductance, likewise all-or-none; not so the skeletal musculature, owing to insulation of its quantals.

3. Both cardiac and skeletal musculatures, however, are quantal. The latter resembles the former in integral activity when the range of stimuli is supramaximal; the former resembles the latter in fractional activity if gradients of interconductance be assumed.

4. A quantal may appear as the single fiber system (absolute minimal contraction); as an intermediate multiple fiber system (submaximal contraction); or as a tissue system (maximal contraction, heart-beat).

The Twitch of Fibers in Response to Varied Intensity of Stimulus

Biedermann (7) has emphasized by a quotation from Fick a conception of extreme importance in the critique of such data as are here considered:

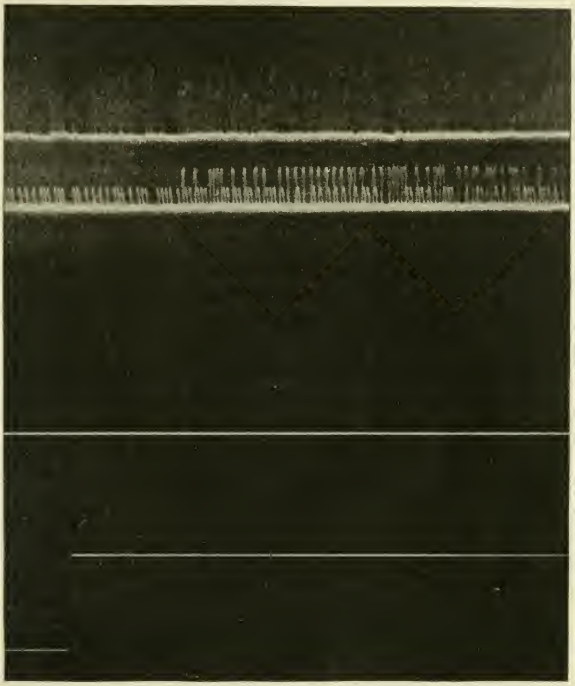

Fig. 13. Sept. 18, 1918; A. Tendons cut, electrode at middle of sartorius, record from tibial end. Break shocks by pendulum interruption, 1 per sec. The fluctuations in height of twitch reflect the crudeness of stimulation, the primary circuit being broken unevenly. The lower series was taken first and is subject to alteration in position of secondary coil as recorded in the lower signal record. The upper series was produced by stimuli of considerably greater intensity, as shown by the upper signal record, possibly exciting nerve filaments. Both are subject to similar adventitious irregularity of stimulation; the seeming turbulence of the upper series being, however, as measurement reveals, dependent upon the greater number of quantals involved as contrasted with the lower series, where the field of choice is limited to two. Transitory contractures appear in the upper series.

Plate record enlarged × 5. Micr. mag., × 10. Total: × 50. The plate contains about 2600 contractions.

This maximal limit is usually but little above that at which the first just perceptible contraction was yielded. The entire process of this greatest contraction and extension is known as a *maximal contraction*. It may be described in Fick's words by saying 'Each impact of excitation discharges either a maximal contraction or no contraction at all; it is only in a limited interval of the scale of excitation (often hard to find on account of its narrow proportions) that *sub-maximal*, so to say, imperfect, contractions are given."

If the range of muscle activity as a function of stimulation strength be so narrowly limited, the same limitation would presumptively apply to the fiber unit. Assuming this, for the occasion, to share in all the apparent qualities of the muscle as a whole, we would expect to find a preponderating region of fiber response where, with respect to stimulus, a completely all-or-none régime would obtain—the maximal region, or region subject to maximal and supramaximal stimuli. The truth stated by Fick is one of common experience; the graded region of gross muscular response may, in highly irritable preparations, be easily overstepped and missed on the application of stimuli too coarsely graded. There is no reason to suppose that the single fiber, toward which very attenuated stimuli must be directed in order to localize the action, would reveal its assumed graded character with any greater readiness; in fact, it would be reasonable to assume considerable difficulty in so delicately grading the stimulus above subminimal that the region of continuity would reveal itself. That is, gradations of stimulus, fine for the tissue, might in similar increments be exceedingly coarse for the cell. The validity of this assumption was apparently recognized by Lucas (8) in the following words:

If any continuous gradation of the contraction of a single skeletal fibre can occur, it lies completely within a range of stimulus far smaller than that required to bring a whole muscle from rest to maximum activity.

Our own evidence against the assumption is partly negative, but cumulative. In the course of several years' observation we have never met with what we could regard as a region of continuity in fiber action dependent on change of stimulus, whether as the result of such change consciously applied, or of any adventitious change of the sort that must inevitably occur in electrical experiments. Attention has been called in a previous paper (2) to the significance of "crude" stimulation, in this respect—that in such crudeness lies a source of delicate as well as coarse variation. Hence the effect, in response, of such variations has been closely scrutinized. The contraction effect is variant; but all

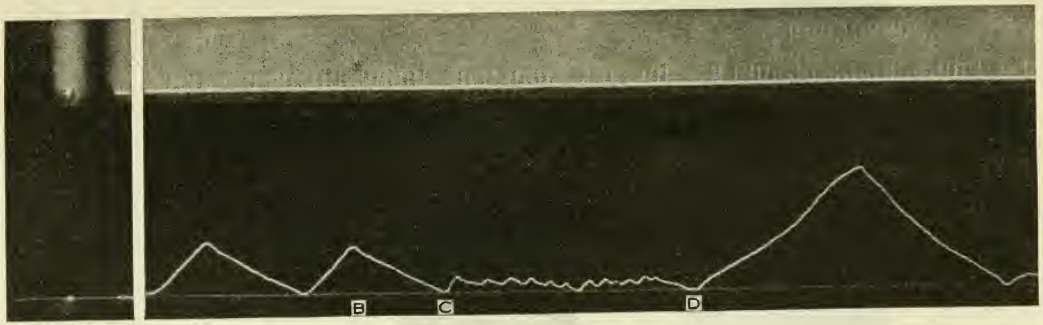

Fig. 14. Nov. 1, 1918; A. Pelvic insertion of sa torius divided. Tibial end stimulated. The same preparation as shown in figure 5. Break shocks, 1 per 2 sec., of considerable uniformity as compared with those employed for the records in figures 11 and 12. Daniell cell; primary circuit interrupted by Hg-Hg key actuated by magnet circuit controlled by second's pendulum of Bowditch clock. Detached portion of figure shows, above, mercury globule; below, signal image; plate at rest: the two images exactly aligned. The lower tracing records on the plate the actual changes in position of the secondary coil. The stimuli first increase in strength to a maximum and then subside, bringing into action a series of quantal twitches. The second series, *B*, shows a lower maximum. From *C* to *D* the threshold is explored by attempting to maintain the liminal stimulus. At *D* a new series is begun and carried to the extreme maximum of signal movement. The result shows that the maximum *B* is in reality an intermediate quantal which failed previously to differentiate. Thresholds are well maintained. The minimal threshold is roughly indicated by the trend of the exploration curve, *C D*. A strong light is required to resolve details.

Micr. mag., × 10; total, × 28.

variates are subject to precise repetition, and can be recalled at will by appropriate adjustment of current. Such determinate responses to relatively indeterminate stimuli may be regarded as characteristic of all tracings where especial precautions were not taken, as is the case in figure 12.

Fig. 15. March 23, 1918; A. The time is recorded in seconds by cutting out resistance from the signal light by means of a pendulum interrupter. The first part of the tracing shows several twitches produced by inductorium break-shocks. After the sixth contraction the electrodes were switched into the magneto circuit already adjusted for minimal brief tetani. The record now shows tetani of about 1 sec. duration followed by a prolonged tetanus, all with uniform stimulus. The tetani vary as do the twitches, giving one or both of two definite quantal values.

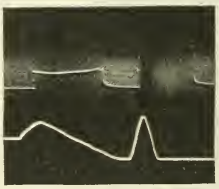

Fig. 16. Oct. 28, 1918; A. The lower curve records change in position of the fine-adjustment coil of magneto, a rise indicating approach to magnet and hence increase of stimulus (alternating current, about 30 cathodal waves per sec.). The upper tracing is of tetanic response, showing quantal composition. The first tetanus hesitates momentarily in its development (shown by a white dot close to the abscissa) at a low, probably minimal, quantal. This is apparently eliminated at the end, before the higher value subsides (shown by a slight notch). The second tetanus shows in its development three distinct quantal stages, the first of which is the maximum of the first tetanus. Continuous gradients, independent of stimulus-change, appear in both tetani.

Any region of continuity in the fiber, if it exist, must, as in the muscle, lie near the liminal value. Hence, as a more positive method of detection, we have resorted in many experiments to what may be termed *threshold exploration*, where the stimuli applied are consciously and repeatedly varied in both directions through the threshold at different

rates. Such a series of explorations is seen in figures 14, 17 and 29; and may in fact be recognized in all tracings where the stimulus is shown to linger in or traverse the threshold region (fig. 25), including those in which threshold has been the chief variable factor (figs. 23 and 32). The results are uniform. Stimulation change can create or destroy; it cannot alter.

Fig. 17. Oct. 31, 1918; B. Break-shocks and brief magneto tetani produced automatically by Porter clock. *A*, twitches, 1 per sec. At *B* the optical signal was actuated three times (just visible on abscissa) to denote transition from twitch to tetanus. *B C*, brief tetani, 1 per sec., magneto coil in constant motion up and down the scale (fig. 11). *C D*, constant tetani, with similar movement of coil, fast and slow, as indicated in the varying duration of tetanus. *D*, optical signal. Following *D*, break shocks, as at *A*, failed to restore twitch. The signal record applies to twitches only. Direct print from plate, micr. mag., $\times 10$. The tetani though apparently minimal all exceed the twitches in height, and vary in height with duration, except when prolonged. Their quantal character is seen in their common form and especially in their identical behavior toward diminishing stimuli—sudden elimination at a common rate of relaxation.

The Determinate Character of Tetani

Our work began with the study of tetanus, owing to the necessity of employing microtometer-scale readings in the earlier determinations. A fiber region was tetanized just long enough to read the excursion of an index-spot and, to exclude vitiation of results by possible staircase or fatigue effects, the excitation was seldom repeated oftener than once in 30 seconds. Such results have been plotted in a previous report (2). A graphic record of similar origin is shown in figure 21, where measurement of separate ordinates will show a determinate series. Other series of brief tetani are shown in figures 17 and 27, in the latter of which the duration of each tetanus is limited to the transit through a mercury contact of a platinum wire attached to a pendulum, cutting the alternating current into essentially twitch values.

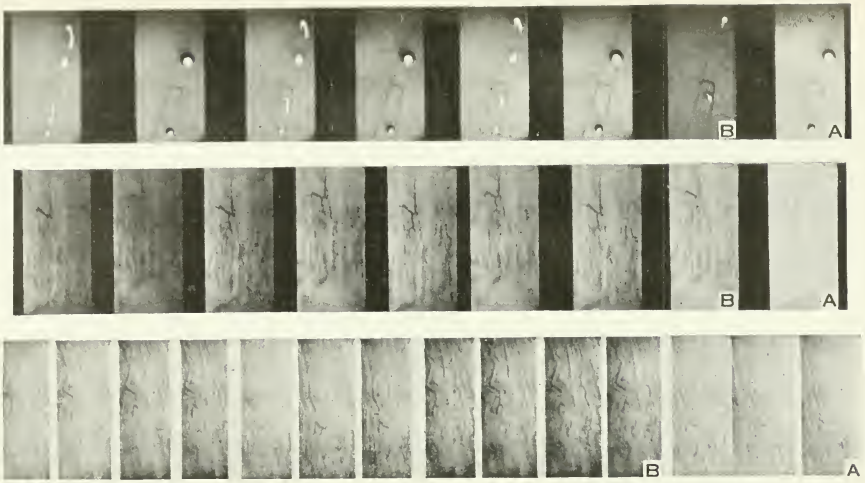

Fig. 18. Aug. and Sept., 1917. Surface of sartorius at rest and in local tetanus; stationary exposures. All three series read from *right to left*. Contact prints from original plates; micr. mag., × 13±. *A*, uncontracted; *B*, contracted surface. Electrode, 7 μ.

The upper series shows alternate rest and movement of two mercury globules, the stimulus (alternating magneto current) lasting 1 second. Comparison of *A* with *B* shows immobility of one capillary arch in the presence of marked displacement of neighboring arches.[1] The stimuli were varied in strength.

The middle series shows alternately the excursion of a hooked vessel, giving an accurate index of the stress exerted by a straightening fiber to its right.[2] Stimuli varied in strength, each lasting 4 seconds.

The lower series shows two groups of three exposures, surface at rest, each followed by a group of four exposures, surface contracted. Tetanic stimuli of equal intensity. The determinate nature of the contraction is here shown by the uniformity of pattern presented by the crumpled fibers on either side of the straightened active fiber.[3]

[1] For details of this field, cf. Eisenberger (3), pp. 52–53. [2] Ibid., pp. 54–55. [3] Ibid., pp. 53–54.

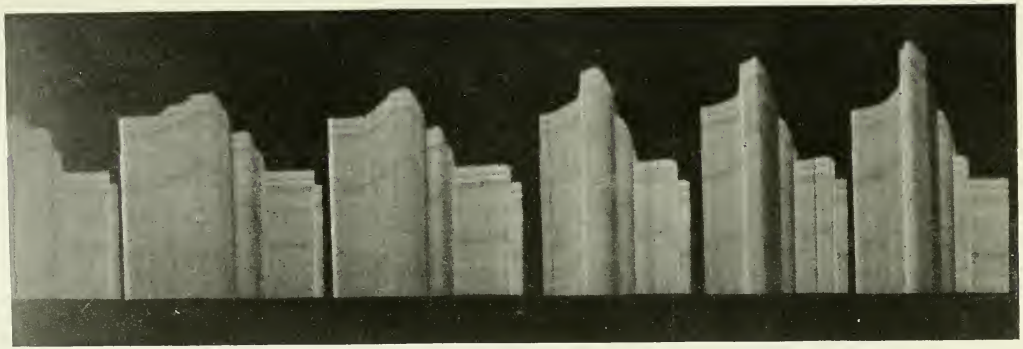

Fig. 19. Aug. 28, 1916; A. The record reads from *r'ght to left:* the index mirror in this and the three succeeding figures was a column of mercury sealed in a capillary tube laid parallel to the contracting fibers of sartorius. For each series ("elevator" curve) a tetanizing stimulus (magneto) was applied with initial strength below threshold, and the coil continuously approached and then continuously withdrawn with reference to the magnet, covering 30 mm. and completing the cycle in about 3 sec. The plate was drawn by clockwork. Each curve begins at a level or step which represents the 0 abscissa, the duration of which varies with the time necessary for starting the coil slide mechanism. Each new level, always abruptly assumed, is complicated by continuous gradients (staircase or fatigue, both conspicuous in step 2). A repeated fall of threshold (quantal staircase) is seen in the high level at which the stimulus of initially subminimal value leaves the contractile state when cut off.[4] Contact print from plate. The faint, narrowly spaced abscissae are traced by the graduations of a micrometer scale in the ocular. Electrodes, about 50 μ for figures 19–22.

[4] For evidence of the localized character of activity in this preparation, cf. Eisenberger (3), fig. 6 and p. 52.

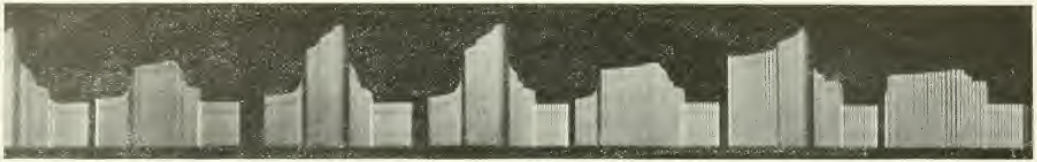

Fig. 20. Aug. 30, 1916. Method the same as in figure 19. Coil of magneto was moved continuously for each tracing, covering a distance of 60 mm. in each direction, over a time of 5 sec. The mercury column records itself at first in distinct lines, owing to irregular clockwork traction. The first quantal rise of the first and second tracings becomes the second of subsequent tracings, owing to the differentiation of a constituent quantal which persists as the minimal. Apparent fluctuation of threshold is reflected in the presence or absence of the higher quantals in the members of the series. Unlike figure 17, the final activity tends to regain the original abscissa. This and the two following records are to be read from *right* to *left*.

Figures 20, 21 and 22 are contact prints, slightly reduced; micr. mag., $\times$ 13 $\pm$.

Fig. 21. Record of brief tetani taken after that of figure 20. The first two lines (right) are the mercury column at rest, a stationary exposure being made of each with a 30 sec. interval. The subsequent images are subject to similar conditions; but the strength of tetanizing stimulus (duration, 1 sec.) increased 3 mm. of coil movement for every image in the series after the first up to a maximum, and then decreased by the same steps; covering approximately the same range as in each series of figure 20. Allowing for slight fluctuation of zero level, the quantals are well determined, though not always graded. That they are the same quantals elicited by the continuous method of grading stimuli may be seen by comparing the steps with those of figure 20, and also in the superimposed figure 22. The doubling of the image is due to a slight lateral movement of the active muscle surface, revealing the resting position of the column for each test to the left of the contraction position.

Fig. 22. Printed from the superimposed plates of figures 20 and 21. In spite of the wide difference in the time factors of stimulation, there is apparent identity of the main quantal values.

To return to figure 21, comparison is now to be made with figures 20 and 22. In figure 20 the stimuli, instead of being discontinuous, are continuous, up and down, over successive brief periods. The same quantals as in figure 21 reappear, but with marked continuous increments or decrements between steps. Such, however, prove to be less disturbing to the quantal hypothesis than might seem; for they frequently run counter to the gradient of stimulation obtaining at the time, as is graphically registered in figure 16. Moreover the superimposed curves in figure 22 show that the initial step-values persist.

That tetanic contractions are quantal is shown even more clearly in figure 15, where the corresponding twitch-values, empirically obtained, are present for comparison. The question of the existence of a tetanic increment over the corresponding twitch-value is a problem which awaits future consideration. It is sufficient for present aims to point out the indubitable fact of the quantal *construction* of tetanus, especially emphasized in the two curves of figure 16, where a certain hesitancy in the development of the limbs of the curves discloses the measured constitution.

The precision with which tissue patterns and contours reëstablish themselves on repetition of a tetanizing stimulus is shown in three series of stationary exposures (fig. 18).

The Quantal Factor in Fatigue

It has been pointed out (2) that study of the fiber confirms the recognized principle of *threshold elevation* in the fatigue process; and that, moreover, the mere occurrence of such elevation must, in a quantal system, lead to a form of fatigue discontinuous in character. As thresholds rise above the stimulation level, the fibers having these thresholds must, if all-or-none, cease abruptly to function. This condition is present in figures 23, 31 and 32, and is to be repeatedly detected in others of the series. Nothing could be more inevitable *a priori;* and it becomes a very familiar phenomenon in the course of work with muscle elements.

Quantal Facilitation in the Staircase Effect

In figure 13 the reverse process presumptively occurs, and in figure 25 more convincingly,—an effect almost invariably met with when a preparation begins to respond after prolonged rest. The threshold suffers an initial fall, bringing into activity fibers erstwhile or otherwise

Fig. 23. July 26, 1918; C. Quantal fatigue. Break shocks, 1 per sec., rise in strength as indicated, introducing a series of uniform twitches, which persist for a time under uniform stimuli. After abrupt cessation of contractions a rise in stimulus fails to restore response. The only evidence of fatigue is rise of threshold leading to elimination of the quantal effect. The earlier contractions fail to appear in the cut.

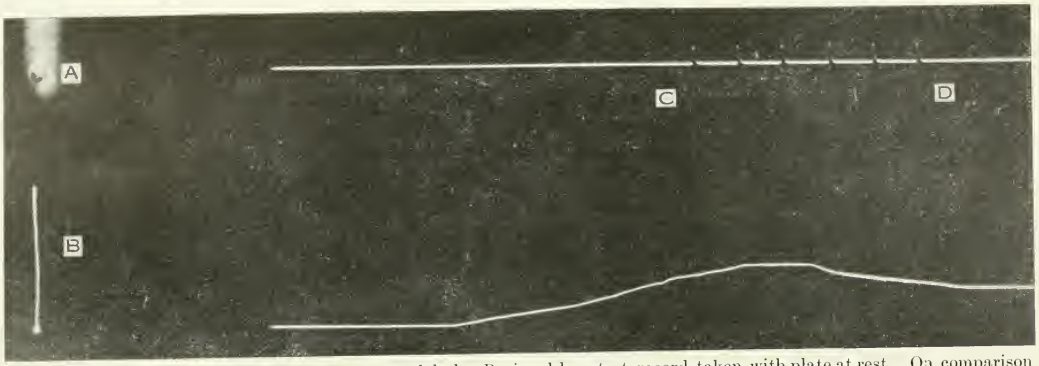

Fig. 24. Dec. and Jan., 1918–19. *A*, mercury globule; *B*, signal-box test record taken with plate at rest. On comparison with the position of *A*, there is found to be a left-handed error of about 1 mm., which is to be applied in determining thresholds. The departures of the ordinate from the vertical are here insignificant. As the stimulus (break-shocks, 1 per 2 sec., mercury tube key) rises, a series of uniform twitches, *C D*, is induced, which ends as the stimulus again crosses the liminal point. Micr. mag., × 10; enlargement, × 3; total, × 30. To be compared with the extended record, figure 25.

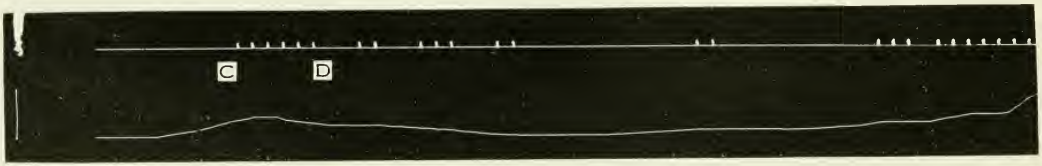

Fig. 25. Contact print from same plate as figure 24. *C D*, the region similarly lettered in figure 24. The contraction curves, all of which are identical with *C D*, have been filled with white under a lens for emphasis. It will be noted that as the stimulus falls from *D*, the twitches reappear at a lower level of stimulation; and the threshold continues to fall, as evidenced by several recrudescences. As the end of the record approaches, the threshold rises, and is finally restored to its first position. The process in other words has exhibited quantal staircase effect of one term followed by relative quantal fatigue. This is an especially convincing demonstration of the all-or-none law. Both stimulus and threshold have slowly and continuously changed and have discovered no intermediate energy system in any liminal region.

idle. This, too, would seem *a priori* inevitable; for when threshold, at first elevated, subsides to stimulation level an all-or-none system must enter with full energy discharge.

Continuous Gradients in Fibral Response: A Contrast

Even a cursory inspection of the tracings will bring immediate conviction that discontinuity is not the sole mode of functional change in muscle. Figure 30 is an extreme example of graded change within quantal limits. Every gap in response introduces a staircase effect; every persistent series, a continuous fatigue. Yet change of stimulus, which indeed must here be applied in order to parallel rising threshold,

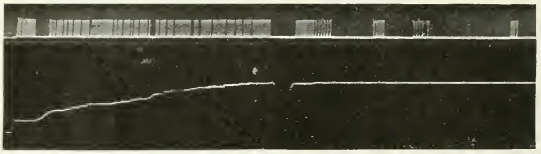

Fig. 26. July 25, 1918; B. Break twitches, pendulum interrupter, stimuli 1 per sec. Secondary coil covered 20 mm. of primary at minimum distance (highest stimulus). The stimulus was raised slightly whenever a gap occurred in the series (rising threshold), the coil, with signal, being returned to zero position near middle of record, then raised and held as indicated. The series shows repeated elimination of the responding quantal (quantal fatigue), with a continuous staircase effect after each gap. Recrudescence occurs in groups in the latter half of record. Slight contractures are present. Contact print.

is impotent to alter these changes. Moreover, in spite of extreme alteration in the individual quantal capacity, the determinate nature of every step is relatively preserved, as reference to the description of the tracing will show. Thus, as expressed by Adrian (9) for the nerve fiber,

> the all-or-none relation between disturbance and stimulus holds good for refractory as well as for normal tissue.

One familiar character of fatigue, in the muscle working as a whole under frequent stimuli, is conspicuously absent in our tracings. This is contracture, so-called—the progressive elevation of the base-line associated with delayed relaxation. Long continued twitch-series at one second intervals are repeatedly obtained with horizontal bases (fig. 31).

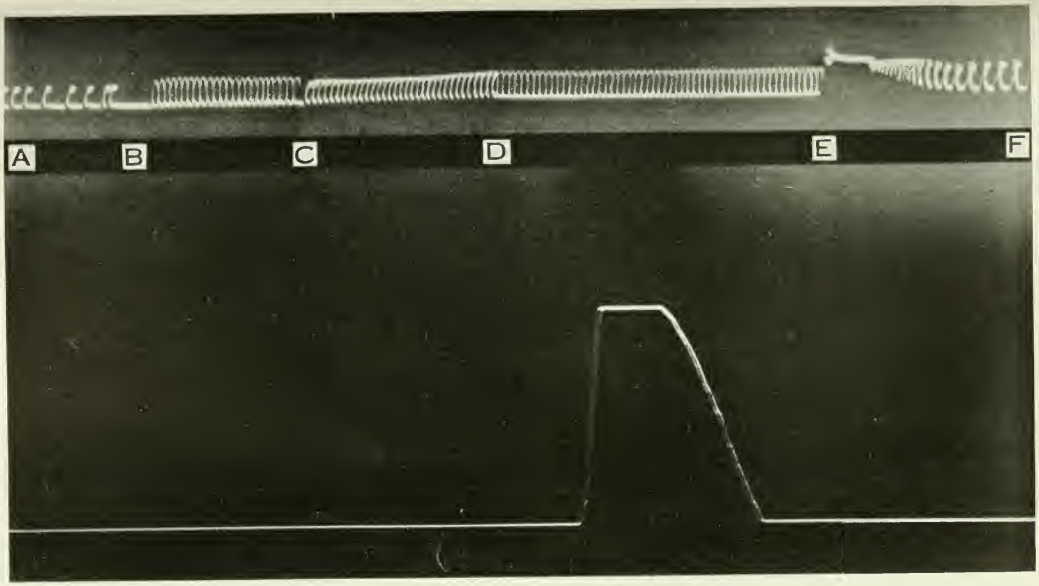

Fig. 27. July 26, 1918; B. From the latter half of a record showing in its earlier portion marked continuous staircase and fatigue. *A B*, brief tetani; *B C*, twitches, 1 per sec.; *C D*, brief tetani, 1 per sec.; *D E*, twitches repeated; *E F*, continuous tetanus held at uniform stimulation strength, breaking spontaneously into tetanic oscillations, becoming rhythmic at *F*. The twitches were produced by pendulum break-shocks; the brief tetani were cut in from the magneto circuit by same pendulum. The signal record indicates stimulus (secondary coil position) for twitches only. The abscissa *A F* was drawn by the shadow of the demonstration needle in ocular. Micr. mag., × 10; enlargement from plate, × 4.5; total, × 45.

Continuous staircase introduces twitch-series, *B C*. The tetani, *C D*, induce staircase and contracture. The twitch-series, *D E*, with its stimuli covering the entire range of the signal-box at two different continuous gradients, is conspicuously all-or-nothing. The tetanic oscillations, *F*, may be regarded as an example of quantal fluctuation (2). The quantal involved is a multiple one; at least several fibers are in action together throughout.

Occasional apparent exception is found, as in figure 36, *C*; but on the whole the fiber appears to be relatively immune to the delayed return so characteristic of batrachian muscle as a whole. The significance of this awaits solution.

On the other hand, very marked contracture is frequently to be observed in tetanus, if one may express by that term the relatively slow increment that succeeds the quick rise (figs. 17 and 19). It is sometimes of long duration; sometimes merged into fatigue. Its contractural character is to be seen in the fact that if degraded by the loss of a quan-

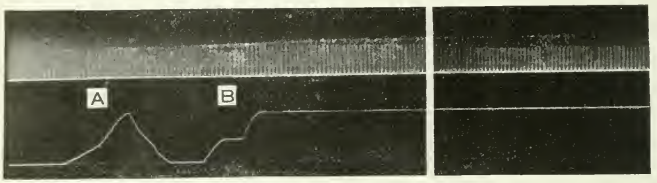

Fig. 28. Nov. 1, 1918; B. Large *R. pipiens*. Right sartorius, pelvic extremity cut. Tibial end stimulated just above tendon. Electrode pore, 7 μ. Daniell cell in primary circuit. Twitches, 1 per sec. (break shocks), Bowditch clock working Hg–Hg tube-key. Contact print; first and last parts of record, 134 intervening contractions removed. Micr. mag., $\times$ 10.

The stimuli being in the threshold region (quantal fluctuation), the succeeding continuous rise and fall of stimulus introduce quantal gradients, with an apparent continuous fatigue at *A* (cf. fig. 29). The twitches fail abruptly (apparent quantal fatigue) just before *B*, and are restored by again raising the stimulus, which brings in another continuous decrement at *B*. Subsequently, for the remainder of the record, a slow continuous fatigue is seen in the quantal maintained by the maximum stimulus, with occasional spontaneous reversion to the minimal contaction which is seen likewise to have shared in the fatigue.

tal (figs. 16 and 32) the amount of the increment tends to be assumed for the time being by the next lower step or, if the degradation be complete, by the base-line. Figure 15 is notable from the virtual absence of such increment.

Fiber Recording as a Differential Method

The reward of reduction to components in any complex system is a new instrument of analysis. One of us (3) has shown how, by the induction of fatigue in a multi-quantal system, the successive elimination of elements will reveal finally one, and one only, whose threshold still lies

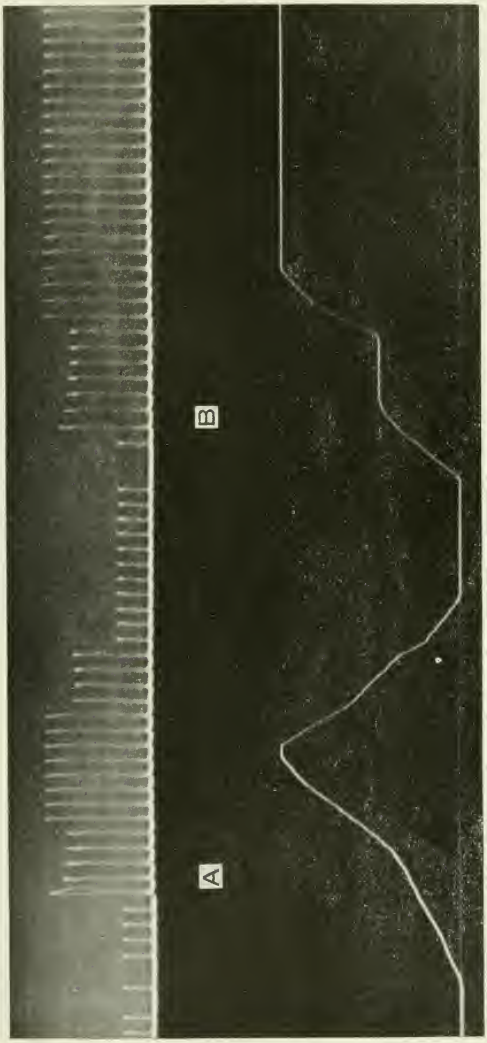

Fig. 29. Portion of figure 28, × 3½; total × 33+; with corresponding lettering

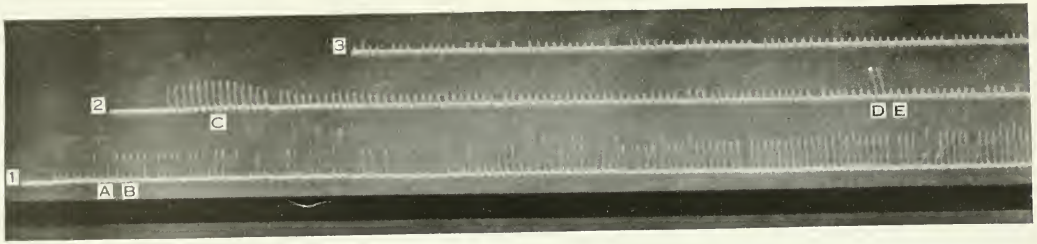

Fig. 30. Aug. 12, 1918; A. Intact sartorius; electrode at junction of tibial and middle thirds; recording globule 2 mm. from margin of rectus abdominis. Break-shocks, 1 per sec. by pendulum. The series *1*, *2* and *3* consist of about 400 contractions each, and were taken in immediate succession. The lower dark band is an abscissa drawn by the shadow of demonstration needle. Micr. mag., × 10; enlarged × 3.5; total, × 35. First part of record only is reproduced.

Continuous fatigue is very marked. Study of the entire record shows an eventual progressive decrement of quantal *B* throughout, which is represented finally by the short contractions of series *3*. Every gap, however, leads to a temporary recovery, the extent of which varies with the length of the gap. Thus in series *2*, the group *C* is a recovery effect due to the short period of rest incident to returning the plate and adjusting for a new record line; it shows typical continuous staircase and fatigue, with a subsequent tendency to low undulations. That quantal characters are preserved is to be recognized as follows: Let *b* represent the quantity of the dominant contraction, *B*, and *a*, that of *A*, which is a higher quantal of which *B* is evident one constituent. If *E* actually represents the degraded quantal *B*, *D* may be suspected of being a recrudescence of *A*, but with only the constituent *B* diminished by fatigue. Denoting the quaantities of *D* and *E* by *d* and *e*, respectively, the relation above suggested will be

$$a - b = d - e$$

the assumption being apparently verified by actual measurement. (The summit of the first *D* has been accentuated. Measurment is best made from the second.) Further evidence tending to identify *A* and *B* with *D* and *E*, respectively, is 1, the recovery at *C* of the quantal to the original height at *B;* 2, the characteristic backward trend of *A*, from lateral strain, reappearing in *D*. The strength of stimulus during the record was accommodated to the rising threshold.

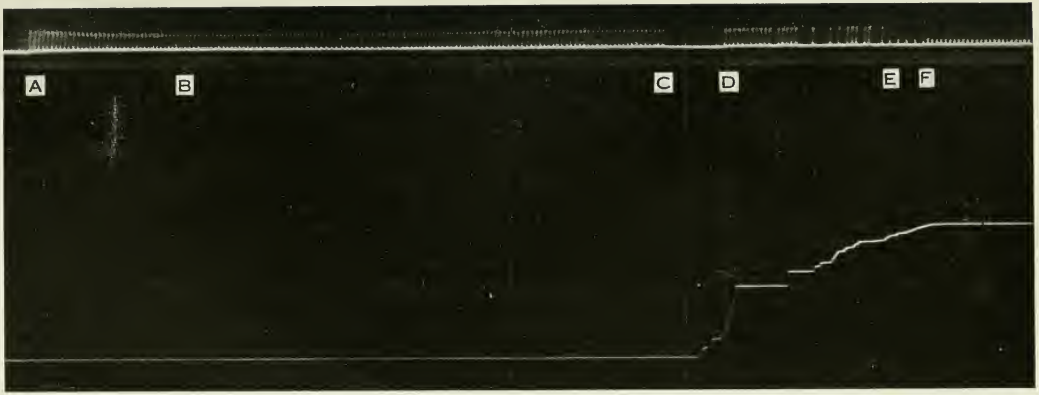

Fig. 31. July 26, 1918; A. Sartorius; circulation visible in superficial capillaries; 7 μ electrode. Minimal break-shocks, 1 per sec., by pendulum interrupter. Micr. mag., $\times$ 10; enlarged, $\times$ 3.5; total, $\times$ 35.

The abscissa formed by the edge of the dark field is drawn by the end of the slot. *A B*, introductory fatigue with slight contracture, merging into a slowly developed staircase; uniform stimuli, as indicated in lower curve. At *C* the stimulus is no longer effective, and the quantal is eliminated until rising stimulus restores it at *D*. Subsequently it becomes necessary to continue the increase of stimulus in order to retain activity. Finally a new quantal is reached at *E*, representing an outlying or deeper fiber, and added to it as the contraction, *E*. From *E* to *F* only alternate shocks are responded to; and the response is a new minimal. At *F* the higher, compound quantal again appears; but at once gives place to a complete and even series of the new minimal. It is apparent that the first quantal composing the series *A*–*E*, long showing signs of failure, is at last completely eliminated, and that the additional factor in *E* and *F* now appears alone. This may be expressed by the equation, $e - d = m$; e being the submaximal quantity (E, F), d the old minimal (A, B, C, D), and m the new minimal differentiated as the result of quantal fatigue. The deduction is supported by measurement of the several contractions in the region *E F*.

Fig. 32, June 3, 1918; A. See legend of figure 12 for experimental details. Pendulum break shocks, 1 per 2 sec. Crude stimulation; no deliberate change in intensity up to long gap near end, when rheocord slider was dropped 10 mm. decreasing primary resistance. At end, brief tetanus, followed by continuous tetanic fatigue curve (magneto). Contact print; micr. mag., × 13.5.

The series of twitches shows an introductory continuous fatigue, the submaximal value reverting to, and at length permanently replaced by minimal, which at length falls out by quantal fatigue (rise of threshold). Increase of stimulus only partially restores the series at end. The brief tetanus and the maximum level of the continuous tetanus evidently represent the submaximal quantal. The same minimal (now tetanic) is at length differentiated; this, finally, is itself abruptly eliminated.

Fig. 33. July 18, 1918; A. Latent period. Sartorius; pelvic attachment cut superficially; capillary circulation active. Electrode (7 μ) at tibial end; record near pelvic end; 5 exposures; 4 minimal contractions, 1 submaximal. The time curves (tuning fork moving signal diaphragm, 512 d.v. per sec.) correspond in ascending order with the contraction curves (make-twitches). The first summit denotes stoppage of optical signal by Pt-Pt primary make-contact. The stimulus was not altered except for the upper and last contraction (submaximal). Contact print; micr. mag., $\times$ 10.

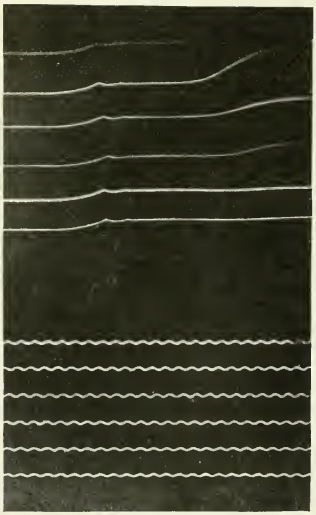

Fig. 34. July 19, 1918: D. Latent period. The description of figure 33 applies to this, except that the stimulus was increased in ascending progression. At least three different quantal values are registered.

beneath the level of stimulation. Such a process is now shown graphically in figures 31 and 32, where it will be seen that the process is by no means dependent upon fatigue of a continuous character. The remainder in such a series may or may not be the contraction of a single fiber. If irreducible, except by extinction, it is probably, in accordance with the first postulate on page 6, of either single or double quantal constitution.

That every quantal is a law to itself is clearly shown when fibers long in activity are compounded in action with others brought in by change of stimulus or threshold. The effect is a resultant which, in accordance with the dominance of one or the other, may exhibit staircase, fatigue or a virtual level. The chief example of this is seen in figure 37, which will presently demand special consideration.

The Latent Period

Reference to figures 33 to 35, included largely for the method, will show that there is here opened a fruitful field of enquiry. Biedermann (10), in his discussion of the latent period, reflects the generally accepted view that the period is, within the submaximal range, an inverse function of the stimulus intensity (Tigerstedt). The fact, however, which he also points out, that varying *maximal* stimuli do not control the length of the period (Helmholtz, Tigerstedt), introduces an interesting problem. It will be seen that no evidence is forthcoming from our results pointing to graded latent periods for a given quantal. It will also be seen that far greater refinement is necessary to make them of more than negative value. If the actual excitation process be of sufficient duration to contribute to the period as a measurable quantity, the all-or-none character of the response concomitant must have the simplifying value of a constant and hence negligible factor in the problem.

Muscle Phenomena, Real and Virtual

The object of this paper has been primarily to introduce the details of a new method; and secondarily to furnish as firm a basis as possible for an application to future problems of a conception of muscle activity which renders the gross phenomena, so long studied, necessarily subject to a certain amount of re-interpretation. Such re-interpretation, we believe, must take cognizance of two very distinct modes of altering mechanical effect. To what extent are gross muscular gradations to be

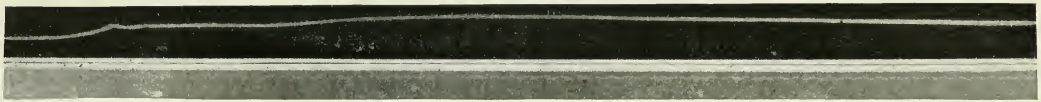

Fig. 35. The same preparation as in figure 34. Six minimal contractions superimposed, the time-curve applying to the first one recorded. The stimulus was increased for each response, but kept within minimal range. The apparent all-or-none effect is negatived as evidence by the failure of the optical signal exactly to synchronize, thus leaving in doubt the synchronism of the contractions.

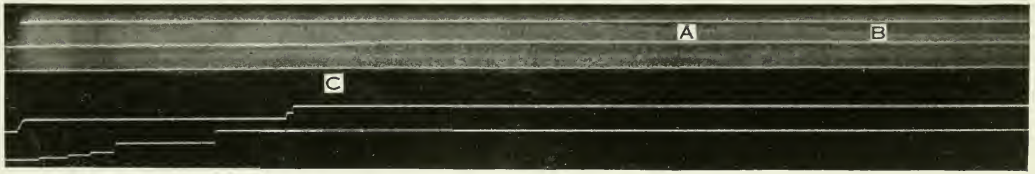

Fig. 36. Sept. 16, 1918; A. Sartorius, deeply severed at both extremities. Record taken 3 hours after completion of preparation; capillary circulation stationary. Electrode (7 μ) about middle of muscle; record near pelvic end. Approximately 4000 twitches (pendulum break-shocks, 1 per sec.) are traced on this plate over a combined recording period of more than an hour. The two records of secondary coil position are continuous, and apply to the two lower contraction series, the upper series continuing with the maximum stimulus recorded. Contact print; micr. mag., $\times$ 10.

Marked continuous gradients are present (staircase and fatigue). Contracture is little in evidence except at *C* in the middle series. At *A*–*B* a remarkable differential effect occurs, apparently spontaneous (cf. fig. 37), the stimuli for this portion of the series remaining unchanged so far as known and recorded.

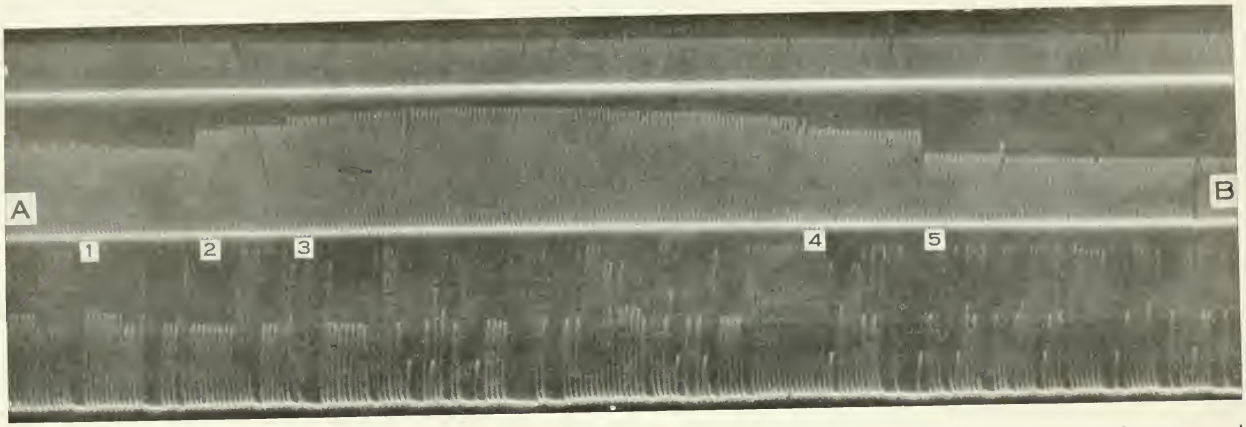

Fig. 37. Enlarged portion of the three series of figure 36, *A*–*B*. In the lower series the stimuli are sufficiently fluctuant to encroach upon several quantal thresholds. The upper series shows continuous fatigue of the dominant quantal. From *A* to *B* the chief events are as follows:

1. Continuous fatigue of dominant quantal, with initial fluctuations, probably of quantal character.
2. A higher quantal undergoing staircase increment.
3. A third quantal, with initial staircase merging into fatigue.
4. Abrupt return to the second step, but with continuity of fatigue gradient.
5. Resumption of fatigue gradient of dominant quantal.

The differential significance of this record is discussed in the text.

interpreted in terms of continuity; to what extent in terms of discontinuity? Until these precincts, which certainly appear to overlap where the tissue is studied in the gross, can be defined it is unsafe to reason from molar to molecular events.

Even staircase and fatigue effects, as already shown, may be described in strictly quantal terms. These may further be superimposed on continuous gradients in such a way that only extreme reduction to terms can separate the two distinct types of response. The tracing *A*, *B* in figures 36 and 37 emphasizes this very sharply. The series taken together is a staircase, merging into a fatigue. But it is quantally composed; each quantal having apparently its own state of irritability and contractility. Analysis shows that the whole is subject to the slow degradation of a dominant quantal reflecting long continued activity, carrying the others as they appear. But its dominance is utterly obscured temporarily by the idiosyncrasies of the less fatigued elements, which manifest their own gradients quite independently. A muscle capable of differential activity of this sort would indeed prove deceptive toward any attempted description.

Apparently the sufficiently complex web of muscular behavior must be approached on wholly new lines of analysis, directed toward the disentanglement of the actual from the seemingly continuous processes. It is not to be wondered, therefore, that so many phases of muscular function, though elicited with ease, have remained in their essence obscure and baffling. Such problems need restatement to yield new points of attack.

SUMMARY

A complete account is given of the method for photographically recording the contraction of muscle fibers on excitation with the pore-electrode. By the use of the apparatus an experimenter is enabled to watch through a demonstration ocular the movements of the recording mercury-globule mirror on the surface of the muscle, while these are being automatically recorded, and at the same time register on the plate, by turning a lever, the actual extent of movement of a secondary coil. Thus the continuity of stimulus variation is shown in a curve coördinate with that which reveals the quantal or discontinuous character of contraction gradients.

Full details of principles and construction are shown in the drawings, and a number of graphic records are reproduced, illustrating the different phases and capabilities of the method.

On these records further and more complete evidence is based with respect to the all-or-none behavior of the skeletal muscle cell in simple contraction, tetanus, and staircase and fatigue effects; and the significance of latent periods as obtained by the method is briefly discussed.

Emphasis is laid on the opportunity for analysis of mechanical effects which the method furnishes, since the continuous gradients can thereby be readily differentiated from the discontinuous—functions which must always tend to confusion or obscurity in gross muscular action.

BIBLIOGRAPHY

(1) Lucas: Journ. Physiol., 1909, xxxviii, 113.
(2) Pratt: This Journal, 1917, xliv, 517.
(3) Eisenberger: This Journal, 1917, xlv, 44.
(4) Pratt: This Journal, 1917, xliii, 159.
(5) Rayleigh: Nature, 1901, lxiv, 385.
(6) Pixii: Annal. de Chimie, 1832, l, 322.
(7) Biedermann: Electro-Physiology (Transl.), London, 1896, i, 69.
(8) Lucas: Journ. Physiol., 1905, xxxiii, 137.
(9) Adrian: Journ. Physiol., 1913, xlvi, 412.
(10) Biedermann: Electro-Physiology (Transl.), London, 1896, i, 72–73.

ON THE FUNCTIONAL CORRELATION OF THE HYPOPHYSIS AND THE THYROID

JOHN A. LARSON

From the Rudolph Spreckels Physiological Laboratory of the University of California

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INTRODUCTION

Workers have for a long time noted certain resemblances in structure between the hypophysis and the thyroid gland. Thus there is a substance present in the pituitary which resembles the colloid material in the thyroid. Again in addition to marked similarities of function in relation to metabolic processes these two endocrine glands play an almost identical rôle in respect to growth. The extirpation of either gland slows or checks developmental processes in general, and especially interferes with the functions of the sexual organs and the nervous system.

If the extirpation of one gland causes changes in the structure and function of the other, there is ground for suspecting that there exists normally a functional reciprocity between the two. For instance, if the hypophysis should undergo hypertrophy as a result of thyroidectomy a natural inference would be that the pituitary had taken over some part, if not all, of the functions of the thyroid. Such an inference would be strengthened if one gland could be substituted for the other.

The literature upon the results of hypophysectomy is very meager owing to the inaccessibility of the gland and the difficulties encountered in its removal.

Smith (57) and Allen (1) working independently, found that hypophysectomy in frog embryos produced changes in the thyroid. The glands in most cases were only about one-third as large as those of the controls and showed atrophic conditions histologically.

Pardi (39) experimented to determine whether modifications of the structure of the thyroid could be caused by pituitary extracts or by other extracts, spleen and liver, as well. He found changes of structure which indicated colloid hypersecretion but that practically the same alterations followed the injection of the various extracts.

Cushing (15) reports in six dogs a hypertrophy of the thyroid after hypophysectomy. Blair Bell (6) states that after extirpation of the pituitary

> One anticipated finding hyperplasia in the thyroid, for Cushing is very definite on this point, but in no case was any change to be discovered.

While after complete removal of the anterior lobe no changes could be seen, he found in one dog following partial extirpation of the anterior lobe that the thyroid was enlarged but normal histologically. Houssay (28), in the dog, reports an excessive accumulation of colloid and sometimes degeneration of the cells in the thyroid following the removal of the hypophysis. Aschner (3) found that the extirpation of the pituitary in young animals causes increase of colloid in the thyroid.

From the above results it is apparent that hypophysectomy causes marked changes in the structure and appearance of the thyroid. On the other hand the effects of thyroidectomy upon the pituitary are even more pronounced.

Rogowitsch (49), in 1886, found enlargement of the pituitary following thyroidectomy in rabbits and dogs. He also reported an increased production of colloid by the cells of the anterior lobe with indication of its passage into the blood vessels. In rabbits he found a doubling of the weight of the hypophysis. He was induced by these results to formulate the theory that after thyroidectomy the pituitary takes over the function of the thyroid gland, and as a result of its increased activity endeavors to compensate for the loss of the thyroid secretion. In the herbivora where there is but little apparent effect of thyroidectomy the pituitary is large and the compensation is sufficient to meet the requirements, but in the carnivora the pituitary is small and the animal dies as a result of thyroid deficiency. Later the discovery of the true relationship of the parathyroids to the thyroid accounted for the different symptoms exhibited by the herbivora and carnivora. However, now, evidence began to accumulate substantiating the results of Rogowitsch.

Hofmeister (26) found hypertrophy of the hypophysis in thyroidectomized rabbits, the gland having nearly doubled its weight in the course of twelve weeks. Stieda (59) described hypertrophy of the anterior lobe brought about by an increase in the number of "Hauptzellen," with the formation of vacuoles in them. He was unable to find any changes in the eromophil cells or formation of colloids. Degener (16) found the pituitary to be three times the normal size in the rabbit one

hundred and seventy-nine days after thyroidectomy. Simpson and Hunter (56) found an increase in weight of 15 per cent in lambs and 20 per cent in the adult sheep after thyroidectomy. Gley (21) reported the normal weight of the pituitary in the rabbit as 0.02 gram. This increased to 0.10 gram at the end of a year (five times the normal), after thyroidectomy. Leonhardt (34) found an increase of one-half in the hypophysis of the rabbit after one hundred and twenty-six days. Lucien and Parisot (36) always found an increase in the weight of the hypophysis in the rabbit. Cimoroni (12), in the dog and rabbit, found a distinction between the nature of the hypertrophy of the pituitary which resulted from castration and from thyroidectomy. Torri (61), in the same animals, reported similar changes. Parhon and Galstein (40) always found a hypertrophy. Traina (62) found little or no increase after fifteen days. Hoskins (27), in his experiments upon amphibia, found what he designates physiological hypertrophy of the anterior lobe of the pituitary after thyroidectomy. This explains the gigantism and infantilism noted in the thyroidless larvae. The failure to metamorphose was due either to the loss of the thyroid or to the abnormal secretion of the anterior lobe. The hypophysis of the female was larger than that of the male. This might account for the fact that the ovary differentiates earlier than the testes. Rogers (48) reports an enlargement of the anterior lobe in Rana pipiens following thyroidectomy. Alquier (2) saw in the dog an increase in volume and signs of hyperactivity. Kamo (30) reported a hypertrophy of the hypophysis in puppies one and a half to five months after thyroidectomy, the pituitary being enlarged to two to five times the normal size. The chief change was in the anterior lobe. An enlargement was also noted in the adult dogs after operation. He also reported that parathyroidectomy caused no changes in the anterior lobe, but an increase in volume of the pars intermedia in puppies and an augmented deposit of colloidal substance in the adult animals. Thaon (60) perceived hypertrophy and structural changes in the young ram after forty days.

Trautmann (63) studied the effects of thyroidectomy in goats and reported a hypertrophy of the pituitary. In a histological examination he detected vacuolized areas and other evidences of what he considered to be degenerative changes. Therefore the hypertrophy may be of a pathological nature and not indicative of physiological activity.

Klebs (32) found hyaline globules in the blood vessels of the nervous part of the pituitary, struma-priva dogs, and therefore assigned the origin of the disturbance to this organ.

Boyce and Beadles (9), in cases of myxedema, reported an enlargement of the pituitary and an increase of colloid in the posterior part of the anterior lobe. Large cysts filled with colloid were seen here. The posterior lobe was atrophied.

Herring (25) worked with cats, dogs and rabbits. The changes were most marked in the rabbit. He found that the anterior lobe was apparently unaffected but that there was increased activity of the cells of the pars intermedia and a probable increase in the number of these in animals surviving the operation for a considerable period. He noted that the changes were most marked in the nervous portion of the posterior lobe and in the laminae constituting the floor of the third ventricle. Here colloidal bodies of a hyaline or granular nature were very numerous. There were also localized proliferations of neuroglia. According to Herring, the extensive production of colloid was merely an exaggeration of a normal process.

Degener (16) found that the hypertrophy affected all parts but most of all the pars anterior in which sometimes colloid vesicles develop similar to those of the thyroid. Halpenny and Thompson (24) described enlargement of the pituitary with an increase of the colloid vesicles in the pars intermedia. Kojima (33) reported that in an animal killed thirty-four days after thyroidectomy the pars anterior was less compact in structure than in the normal rat, and a larger number of vesicles were visible over the whole section. These vesicles varied in size and shape. Many were filled with a hyaline substance, which stained faintly red with eosine; others were empty; there were also a number of large, swollen-looking cells, the cytoplasm of which was opened in appearance. Numerous small vacuoles were noticed. The ordinary oxyphil and basiphil cells are remarkably few in number. The pars intermedia was relatively thickened. These changes were already visible twenty-three days after thyroidectomy and they were not removed after feeding the animals for some days with one gram of ox-thyroid per rat per diem.

A. E. Livingston (35) found that thyroid feeding prevented the increase in the size of the pituitary which would otherwise be caused by thyroidectomy.

Thus, to recapitulate, practically all investigators agree that the pituitary increases in size as a result of thyroidectomy and that the gland contains an increased amount of colloid material. Opinion, however, differs as to where in the gland this colloid is found. According to Simpson and Hunter, the pituitary colloid is not identical with that of the thyroid and contains no iodine or but slight traces.

On the clinical side there is also evidence of disturbances in the hypophysis as a result of thyroidectomy. Nièpce (37), in five cretins, found all the pituitaries to be enlarged. Pisenti and Viola (43) found much colloidal substance in the pituitary in cases where the thyroid was the seat of a fibrous struma. Dolega (18) reported that in a cretinoid skull the pituitary fossa was relatively larger than in the normal skull. Boyce and Beadles (9), observed hypertrophy of the gland in cases of myxedema, with increase of colloid in the posterior part of the anterior lobe, a doubling of weight. Schönemann (54), from the study of the pituitary and thyroid glands of one hundred and twelve cases in Kocher's clinic, found support for the hypothesis of Rogowitsch. Comte (13), in one hundred and eight cases of thyroid disease, found a hypertrophy of the hypophysis when the thyroid showed atrophic changes. Calderara (10) reported the same in a case of myxedema and the presence of much colloid in the pituitary. Bourneville and Bricon (8) found enlargement of the pituitary in cretins. Eichorst (19) found in cretins and cases of myxedema a hyperfunction of the pituitary. The gland was larger and hyperemic. There were often hemorrhages and later a growth of connective tissue and cyst formation. Finally the pituitary became smaller and smaller. Wells (64), in a case of scleroderma where the thyroid was greatly atrophied, found the hypophysis to be twice the normal size and this increase seemed to be due to the large accumulation of colloid material.

On the other hand, in some cases, no change in structure could be detected and in a few cases the pituitary was below normal. Coulon (14), in five cretins, found the pituitary to be normal or below the average size. Ponfick (45), in a case of myxedema, found a degeneration of the pituitary.

Thus the preponderance of clinical evidence points to hypertrophy of the hypophysis as a result of thyroid deficiency.

Simpson and Hunter (56) experimented on the assumption that the iodine-containing body in the thyroid represents the active principle or internal secretion of the gland. Therefore if there were a compensatory activity on the part of the pituitary as a result of thyroidectomy they expected to find some iodine. The failure to detect iodine is considered as evidence against the theory. They found in the sheep that removal of the thyroid does not lead to the appearance of iodine in the pituitary and that therefore there is no vicarious relationship between the two glands. Their assumption is based on the statements of Baumann (5), Halliburton, Candler and Sikes (23), that there is no iodine

in the human pituitary. On the other hand Schnitzler (53) did find positive evidence of iodine in the human pituitary but in very small amount. He therefore assumed that the iodine is present as iodothyrin and brings forward his result in support of the view that the thyroid and pituitary may act vicariously. Wells (64) also found a small amount of iodine in the human pituitary. As compared with the thyroid the proportion was about 1:50. Pellegrini (41) studied the relation of the thyroid iodine to pituitary functioning. According to the abstract (the original was not accessible to me),—

> No relation was discovered between the volume of the hypophysis and the amount of thyroid iodin. The same is true as regards microscopic evidence of functional activity, relative size of the lobes of the hypophysis or frequency of occurrence of the various cellular elements. There was noted a marked lack of pigment in the neural lobe in cases in which the iodin was decreased.

The failure of Simpson and Hunter to detect iodine in the pituitary after thyroidectomy does not necessarily invalidate the idea of a functional correlation. The autacoid supplied by the hypophysis although perhaps not containing iodine may be able partially or wholly to take the place of the thyroid hormone.

In addition to the experimental data it may be mentioned that considerable clinical evidence exists of the correlations between the two glands. Thus Falta (20, p. 323) calls attention to the fact that after extirpation of a part of an adenoma in a case of acromegaly, there was an enlargement of the thyroid. Again, Benda (7) is quoted as saying that in Basedow's disease the glandular hypophysis is small. Josefson (29) reported associated hyperplasia of the hypophysis in a case of congenital struma of the thyroid.

It is interesting to note that pathological processes may occur spontaneously in the two ductless glands. To illustrate, Rosenhaupt (50) reported a case of sarcoma of the anterior lobe of the hypophysis, in which there was also a tumor of the same nature in the thyroid gland. Again indications of hyperthyrosis may occur with corresponding pathologico-anatomical alterations of the thyroid gland. In multiple ductless glandular sclerosis the sclerotic process usually affects the hypophysis and the thyroid. A slight degree of thyroid gland insufficiency may occur in hypophyseal dystrophy. Also the hypophysis may degenerate in the later stages of Basedow's disease. Again Falta (20) states

The pathologico-anatomical finding in the thyroid gland in acromegaly almost always shows something abnormal. When hyperthyrosis has existed, there is found the picture of a Basedow's struma. Otherwise there is seen almost always connective tissue proliferation, such as is found also in other organs in acromegaly, or colloid degeneration in combination with, eventually, high-grade sclerosing and atrophy of the parenchyma. Gaussel found a thyroid gland that was normal.

Thus a summary of the literature clearly establishes the fact that the removal of either the hypophysis or the thyroid gland causes definite changes in the structure of the other.

In the case of thyroidectomy the consensus of opinion is that the hypophysis undergoes hypertrophy. As to which portion enlarges the most, the anterior lobe is indicated wherever a specific region is mentioned. In making any statement, however, regarding hypertrophy of the pituitary, the extreme variations normally occurring in animals of the same species and of the same size must be taken into consideration. For instance, some of the glands of the ox often exhibit two or three times the proportions found in others. This fluctuation merely emphasizes the necessity for accurately controlled investigation.

Hypertrophy of the hypophysis following thyroidectomy has by many investigators been considered evidence of a vicarious relationship between the two glands. Assuming that the hypertrophy of the hypophysis is physiological, the next question to arise is that of the extent to which the pituitary can function for the thyroid. That the substitution, if it exists, is incomplete may be shown by the myxedematous symptoms and often the death of the animal following thyroidectomy. Since the compensatory effort on the part of the pituitary substance attempting to perform a double function in the absence of the thyroid is insufficient, the administration of hypophyseal extract should prove beneficial. Through the adjuvant action of the added hormone the pituitary might now perform its double task.

THE EFFECT OF ADMINISTRATION OF THE ANTERIOR LOBE OF THE HYPOPHYSIS TO THYROIDECTOMIZED RATS

In order to ascertain the effects of direct administration of the anterior lobe to thyroidectomized animals it was essential to find a species in which the injurious effects of parathyroidectomy were at a minimum. It is usually impossible to extirpate the thyroids completely without injury to the parathyroids.

The rat was finally chosen, as this animal is particularly favorable for these experiments and is not subject to tetany as an early result of removal of the parathyroid. The only apparent modifying factor is that of age. I have found that rats weighing less than forty grams invariably die as the result of operation, convulsions being observed in some cases.

The rats selected were approximately of the same size, age and strain, and wherever possible, from the same litter. The importance of these details in the selection of controls cannot be over-estimated. Growth curves of normals obtained in one can not properly be compared with those of experimental animals at another season. The thyroids and parathyroids were completely removed under ether anesthesia. After some preliminary operation work to ascertain the simplest technique and the most favorable age, the rats were divided into four groups. Two series were operated and fed fresh liver and anterior lobe of the ox respectively in addition to the normal diet. Two other groups were control animals and fed liver and anterior lobe; the amount of liver administered corresponded to the quantity of hypophysis substance.

The rats were all kept in individual cages in order to insure a uniform consumption of the glandular food. An excess of food was furnished so that the rats had access to as much as desired. The ordinary diet consisted of swill, barley, cooked rice and fresh cabbage, but no water as the animals seemed to thrive best without it. Besides the special glandular materials, a small quantity of salt was kept in each cage. As for the dosage of anterior lobe administered, the average amount during the first three months varied from one-half to one gland per rat, and later, two to three glands administered at least three times a week and more frequently when the glands could be obtained. But in every case the same amount was fed to each rat of the two series. The dosage used is larger than that usually reported as allowance was made for a destruction of some of the active principle in its passage through the alimentary tract before absorption.

In an attempt to determine the maximum amount of glandular substance utilized, all of the excess of glands after each feeding was administered to two individuals, one in each of the pituitary groups. The number of glands varied from two to eighteen per rat, but each received the same amount. In comparison with their respective groups the operated rat thrived best, the normal individual losing weight, but no more than a rat fed the ordinary amount. These results seemed to

indicate that the organism is capable of utilizing only a certain amount of the pituitary autacoids, and that an excess is eliminated without any deleterious effects.

The hypophyseal substance was given to the animal in a separate dish and from the outset the rats exhibited a marked predilection for the anterior lobes, eating with great avidity all that was administered. After two or three feedings the animals would snatch the glands out of the dishes in preference to any other food. In fact the glands were consumed with greater eagerness than the liver in the other groups.

The rats were weighed each week and their general condition noted. When an animal died a careful search was made for thyroid tissue. Any rats dying less than two weeks after the operation or from known accidental causes were discarded. The experiment proper commenced June 3, 1918, and terminated February 3, 1919. The remaining rats were killed and prepared for subsequent examination.

The entire group of rats, seventy in number, was subdivided into the four series. The subsequent history of each rat was recorded in tabular form, the data being secured from the record of weekly weighings and curves plotted from the same. Two distinct sets of tables were drawn up, the first of which, tables 1 to 4, commence the experiment with the initial weighing of each rat. The chief value of the results presented herein is to indicate the conditions of growth before operation. It was found that thyroidectomy caused but slight immediate effect; in some cases a loss of a few grams was noted at the time of the next weighing.

In the second set, tables 5 to 14, the same data are used but the comparisons begin with the date of operation. The results stated in this form are seen to emphasize the relations observed in the first set of protocols. Upon comparing the two sets the chief difference is to be found in the figures which are based on the weight changes, although the relative proportions of the different groups remain unchanged. Since the date of operation marks the true beginning of the experiment, the second set of tabulations will be more particularly considered.

In all of the tables, the sign X indicates that the animal died; the sign + shows that the animal is still living; the sign ϕ denotes death due to accident; 2/3 is the date of termination of the experiment.

The foregoing tables present the data in one comparative way, but as stated above certain facts are brought out more clearly by a different arrangement. In the following tables the comparison begins with the date of operation and a corresponding day for the unoperated animals.

TABLE 1

Control. Ordinary dietary with the addition of liver

GROUP NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
X A 25					
A 50	♀	6/4 to 1/20	201 to 246	+45	230
A 1	♀	6/3 to 2/3	184 to 180	−4	245+
A 46	♀	6/3 to 2/3	232 to 235	+3	245+
A 3	♀	6/4 to 2/3	211 to 233	+22	244+
A 28	♀	6/4 to 2/3	189 to 203	+14	244+
A 4	♀	6/4 to 2/3	250 to 174	−76	244+
A 20	♂	6/27 to 2/3	189 to 300	+111	221+
D 15	♀	8/26 to 2/3	76 to 158	+82	162+
E 34	♀	8/26 to 2/3	65.5 to 186	+120.5	162+
G 2	♂	7/8 to 2/3	20 to 173	+153	210+
G 71	♀	7/8 to 2/3	22 to 164	+142	210+
J 12	♀	6/27 to 2/3	35 to 103	+68	221+
J 90	♂	6/27 to 2/3	36 to 194	+158	221+

Total number of rats = 14. Average gain = 64.5 grams. Total number of rats dead (one was accidental) = 2. Average percentage of deaths = 7.6 per cent. Average duration of experimental life = 219.9 days.

TABLE 2

Control. Ordinary diet with the addition of anterior lobe

GROUP NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
A 11	♀	6/27 to 2/3	281 to 276	−5	221+
A 18	♀	6/27 to 2/3	288 to 336	+48	221+
A 19	♂	6/4 to 2/3	247 to 304	+57	244+
A 10	♂	6/4 to 2/3	263 to 288	+25	244+
A 82	♀	6/4 to 2/3	240 to 227	−13	244+
C 16	♀	6/27 to 2/3	48 to 255	+207	221+
C 57	♀	6/27 to 2/3	48 to 233	+175	221+
E 87	♂	8/26 to 2/3	49.5 to 154	+104.5	162+
E 16	♂	8/26 to 2/3	63.5 to 199	+135.5	162+
F 73	♀	8/7 to 2/3	36 to 206	+170	181+
G 13	♂	8/7 to 2/3	20 to 159	+139	181+
G 57	♀	8/7 to 2/3	21.9 to 128	+106.1	181+

Total number of rats = 12. Average gain in weight = 95.7 grams. Total number of rats dead = 0. Average duration of experimental life, 206.9 days. Percentage of deaths = 0 per cent.

Altogether there are ten distinct groups (A to J inclusive), these being arranged according to age, litters, etc. Group A contains thirty individuals, seventeen of which, after a month of study (in nearly every case, see tables 3 and 4) were operated the first week in July. The legends at the heads of the tables are self-explanatory, the X at the left

TABLE 3

Thyroidectomized rats fed upon the ordinary diet and liver

GROUP NUMBER	SEX	DURATION OF EXPERIMENT	DATE OF OPERATION	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
				grams	*grams*	*days*
ϕ A 9	♀	6/4 to 2/3	6/24			
ϕ A 8	♀	6/12 to 2/3	6/21	230 to 205	−25	235+
X A 6	♀	7/3 to 11 8	7/6	235 to 238	+3	128
X A 4	♀	7/8 to 10/15	7/8	157 to 127	−30	99
X A 91	♂	7/5 to 10/20	7/13	51 to 202	+51	107
X A 7	♀	6/4 to 10/7	6/20	216 to 125	−91	125
X A 17	♀	6/4 to 12/2	7/21	261 to 189	−72	181
X A 8	♀	6/27 to 8/28	6/28	105 to 84	−21	63
X A 22	♀	6/29 to 8/20	7/16	23 to 54.5	+31.5	52
X A 33	♂	7/13 to 9/26	9/13	17 to 51	+34	75
X A 29	♂	8/14 to 9/4	8/15	73 to 85	+12	21
X D 88	♀	8/26 to 12/2	9/12	46 to 120	+74	98
X D 14	♀	8/26 to 10/4	9/12	46 to 56	+10	39
ϕ E 6	♀	8/26 to 9/14	9/13	60.5 to 89	+28.5	19
X E 56	♀	8/26 to 11/18	9/12	45.5 to 76	+30.5	84
ϕ H 27	♂	8/6 to 1/20	8/9	99 to 316	+217	166
H 32	♂	8/7 to 2/3	8/9	79 to 315	+236	179+
X H 80	♂	8/8 to 8/30	8/9	67.5 to 90	+22.5	22
X I 43	♀	8/1 to 12/22	9/19	21 to 89	+68	143
I 45	♂	7/13 to 2/3	9/20	14 to 189	+175	204+
X G 5	♀	6/27 to 9/26	7/18	27 to 77	+50	91

Total number of rats = 21. Total number of rats dead = 18. (Three accidental deaths). Total number of rats alive = 3. Percentage of deaths =78.9 per cent. Average gain in weight = 40.8 grams. Average duration of experimental life = 111.1 days.

signifying a death presumably due to experimental conditions or to unknown causes in the controls. The ϕ connotes either an accidental death, doubtless due to shock, etc., as most of these deaths occurred the day following the operation, or to the escape of the animal. The date 2/3 indicates that the individual is still living.

Table 5 represents the histories of eight rats. The dates 6/28 and 7/3, were taken as the initial date to correspond with the operative dates. Of the eight individuals there were two deaths, one accidental. The average duration of life of the entire group is 207.8 days. The sign + in last column indicates that the animals are still living. The total

TABLE 4

Thyroidectomized rats fed upon the ordinary diet and anterior lobe

GROUP NUMBER	SEX	DURATION OF EXPERIMENT	DATE OF OPERATION	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
				grams	*grams*	*days*
A 35	♂	6/12 to 2/3	6/25	165 to 161	−4	235+
A 13	♀	6/3 to 2/3	7/3	98 to 130	+42	245+
A 58	♀	6/3 to 2/3	7/3	157 to 199	+42	245+
A 14	♂	6/4 to 2/3	6/18	87 to 250	+163	244+
X A 81	♀	7/6 to 12/22	7/6	208 to 293	+85	169
X A 12	♀	6/27 to 11/30	6/28	197 to 170	−27	156
X B 11	♀	6/4 to 10/7	6/25	71 to 133	+62	125
B 51	♀	6/4 to 2/3	6/20	67 to 229	+162	244+
B 15	♂	6/4 to 2/3	6/25	50 to 276	+226	244+
D 89	♀	8/25 to 2/3	9/13	56 to 104	+48	16+
ϕ D 30	♂	8/26 to 9/22	9/21	61 to 89	+28	
X E 18	♀	8/26 to 11/16	8/29	67.5 to 116	+48.5	82
X E 2	♀	8/26 to 10/25	9/13	56 to 104	+48	60
H 17	♀	8/7 to 2/3	8/9	64 to 196	+132	181+
H 78	♂	8/8 to 2/3	8/9	104 to 134	+30	180+
H 79	♂	8/8 to 2/3	8/9	97 to 327	+230	180+
X H 44	♂	8/7 to 10/16	8/7	70 to 85	+15	60
X I 3	♀	7/5 to 12/3	7/10	35 to 104	+69	151
I 24	♂	6/29 to 2/3	7/26	29 to 279	+250	219+
I 31	♀	7/5 to 2/3	7/10	49 to 234	+185	213+
ϕ J 70	♀	8/8 to 11/12	8/12 escaped			25

Total number of rats = 21. Average gain in weight = 105.3 grams. Total number of rats dead = 9; (two accidental). Average duration of experimental life = 179.5 days. Average percentage of deaths = 37.5 per cent. Total number of rats alive = 12.

gains in weight are 179 grams and the total losses are 185 grams, making the average 0.75 gram.

Table 6 shows the effect of the addition of the anterior lobe to the dietary of the rats. There were no deaths, the average duration of life was 218.6+ days. The average gain for the group is +27 grams.

TABLE 5

Group A. Control, fed upon normal diet and liver

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
X A 25	♀	6/28 to 12/1	276 to 183	−93	155
A 50	♀	7/3 to 1/20	218 to 246	+28	221+
A 1	♀	6/27 to 2/3	175 to 180	+5	221+
A 46	♀	6/27 to 2/3	220 to 235	+15	221+
A 3	♀	6/27 to 2/3	222 to 233	+11	221+
A 28	♀	7/3 to 2/3	187 to 203	+16	215+
A 4	♀	7/3 to 2/3	266 to 174	−92	215+
A 20	♂	7/3 to 2/3	196 to 300	+104	215+

TABLE 6

Group A_2. Control, fed upon usual diet and anterior lobe

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
A 11	♀	6/27 to 2/3	280 to 276	−4	221+
A 18	♀	6/27 to 2/3	288 to 336	+48	221+
A 19	♂	6/27 to 2/3	265 to 304	+39	221+
A 10	♂	7/3 to 2/3	255 to 288	+33	215+
A 82	♀	7/3 to 2/3	208 to 227	+19	215+

TABLE 7

Group A_3. Thyroidectomized rats fed upon normal diet and liver

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
ϕ A 9	♀	6/24 to 2/3	222 to 238	+16	
A 8	♀	6/21 to 2/3	207 to 205	−2	227+
X A 6	♀	7/6 to 11/8	232 to 238	+6	125
X A 4	♀	7/8 to 10/15	165 to 127	−38	99
X A 91	♂	7/13 to 10/20	81 to 102	+21	99
X A 7	♀	6/20 to 10/7	235 to 125	−110	109
X A 17	♀	7/2 to 12/2	265 to 189	−76	153
X A 8	♀	6/28 to 8/28	105 to 84	−21	61
X A 22	♀	7/16 to 8/20	48 to 54.5	+6.5	35
X A 33	♂	9/13 to 9/26	55 to 51	−4	13
X A 39	♂	8/15 to 9/4	73 to 85	+12	20

Group A_3, table 7, shows nine deaths out of ten operated animals, the average life after operation being 71.4 days, and of the group, 94 days. The sign + means that one rat is still living. Thus there is 90 per cent of deaths, an average gain of −20.5 (obtained by adding the gains and losses algebraically and dividing by the number of individuals). Of the rats which died, four showed myxedematous symptoms—rats A 6, A 4, A 7 and A 8.

On the other hand group A_4, table 8, exhibits 33.33 per cent of deaths and an average duration of life of 201.1 days for the group, that for the two dead being 162 days.

TABLE 8

Group A_4. Thyroidectomized rats fed upon normal diet and anterior lobe

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
A 35	♂	6/25 to 2/3	175 to 161	−14	223+
A 13	♀	7/3 to 2/3	128 to 130	+2	215+
A 58	♀	7/3 to 2/3	163 to 199	+36	215+
A 14	♂	6/18 to 2/3	87 to 250	+163	230+
X A 81	♀	7/6 to 12/22	208 to 293	+85	169
X A 12	♀	6/28 to 11/30	205 to 170	−35	155

TABLE 9

Group B. One litter. Thyroidectomized rats fed upon normal diet and anterior lobe

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
X B 11	♀	6/25 to 10/7	93 to 133	+40	104
B 55	♀	6/20 to 2/3	103 to 229	+126	228+
B 15	♂	6/26 to 2/3	75 to 276	+201	223+

Whereas from the previous group there is but one rat alive, here there are four. The average gain is 39.5 grams. A 81 seemed hyper-irritable, very nervous and maintained its head tilted to one side.

In B, table 9, another operated group to which the hypophysis was added, one death occurred, with an average duration of life for the group of 185 days and an average gain of 122.3 grams.

In C, table 10, there is an average life of 221+ days and gain of 191 grams.

In groups D and E, tables 11 and 12, 9/12 is taken as the start of the experiment for the normals to correspond with that of the operated animals.

The average life in D was 144 days for the normal; 51.5 days for the operated + liver; and 143+ days for the operated + pituitary; while the average gains were +95, +12.5 and +103; the per cent deaths, 0 per cent; 100 per cent and 0 per cent.

TABLE 10

Group C. One litter. Control fed upon normal diet and anterior lobe

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
			grams	*grams*	*days*
C 16	♀	6/27 to 2/3	48 to 255	+207	221+
C 57	♀	6/27 to 2/3	48 to 223	+175	221+

TABLE 11

Group D. One litter

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
Group D. Control, fed upon normal diet and liver					
			grams	*grams*	*days*
D 15	♀	9/12 to 2/3	84 to 179	+95	144+
Group D. Thyroidectomized, fed upon liver and normal diet					
X D 88	♀	9/12 to 12/2	80 to 120	+40	81
X D 14	♀	9/12 to 10/4	71 to 56	−15	22
Group D. Thyroidectomezid, fed upon anterior lobe and normal diet					
D 89	♀	9/13 to 2/3	56 to 159	+103	143+
ϕ D 30	♂	9/21 to 9/22			1

In the above litter the percentages of death were: 0.0 per cent; 0.0 per cent; 100 per cent and 100 per cent respectively; and the average life 144+ days; 144+ days; 67 days and 68 days, while the average gains were +78 grams; +82 grams; +9 grams and +76 grams. This lot presents the first and only instance where the life of a thyroidectomized and liver-fed animal after operation is nearly equal to that of the pituitary series. The condition of growth in these two series may

be compared by examining the group gain, which is +9 grams in the liver series and+76.5 grams in the pituitary individuals. In this as in the preceding families, the maximum duration of life is 144+ days in the controls, as the experiment commenced September 12.

The preceding set is largely composed of normal individuals. The average life is 180+ days in the pituitary-fed series and the corresponding ones in the other series. If there is any difference in the rate of growth, the figures favor the pituitary rats.

TABLE 12

Group E. One litter

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
1 Rat. Control, fed upon liver and normal diet					
			grams	*grams*	*days*
E 34	♀	9/12 to 2/3	108 to 186	+78	144+
Group E. Control, fed upon anterior lobe added to normal diet					
E 87	♂	9/12 to 2/3	92 to 154	+62	144+
E 16	♂	9/12 to 2/3	97 to 199	+102	144+
Group E. Thyroidectomized rats fed upon normal diet with liver					
ϕ E 6	♀	9/13 to 9/14			1
X E 56	♀	9/12 to 11/18	67 to 76	+9	67
Group E. Thyroidectomized rats fed upon normal diet with anterior lobe added					
X E 18	♀	8/29 to 11/16	67.5 to 116	+48.5	79
X E 2	♀	8/29 to 10/25	72.5 to 176	+103.5	57

The life of the normals in J is 200+ days as contrasted with 70 days in the control operated rodent, and 92 days (before its disappearance) in J 70, the pituitary-fed individual.

Upon making deductions from the above group, H 27 is regarded as living since the animal was killed near the termination of the experiment in attempt to ascertain how much thyroid material was present; no thyroid substance was found. There was one death after 20 days in the first group as opposed to one after 61 days in the pituitary animals. The average gain was 158.5 grams as compared with 101.8 grams although the average duration of life was 121+ days in comparison with 148.7+ days.

TABLE 13

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
Group F. Control, fed upon anterior lobe added to normal diet					
			grams	*grams*	*days*
F 73	♀	8/7 to 2/3	36 to 206	+170	180+
Group G_2. Control, fed upon liver added to normal diet					
G 2	♂	8/7 to 2/3	53 to 173	+120	180+
G 71	♀	8/7 to 2/3	65 to 174	+109	180+
Group G. Control, fed upon pituitary added to normal diet					
G 13	♂	8/7 to 2/3	20 to 159	+139	180+
G 57	♀	8/7 to 2/3	21.9 to 128	+106.1	180+
Group J (one litter). Control, liver added to usual diet					
J 12	♀	7/18 to 2/3	55.5 to 103	+47.5	200+
J 90	♂	7/18 to 2/3	59.5 to 194	+134.5	200+
Group J. Thyroidectomized rat fed with liver added to usual diet					
X J 5	♀	7/18 to 9/26	46 to 77	+31	70
Group J. Thyroidectomized rat fed with pituitary added to usual diet					
ø J 70	♀	7/14 to 10/14	(escaped)		92

TABLE 14

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
Group H. Thyroidectomized rats, fed with usual diet and liver					
			grams	*grams*	*days*
ø H 27	♂	8/9 to 1/20	99 to 316	+217	165
H 32	♂	8/9 to 2/3	79 to 315	+236	178+
X H 80	♂	8/9 to 8/29	67.5 to 90	+22.5	20
Thyroidectomized rats fed with usual diet with addition of anterior lobe					
H 17	♀	8/9 to 2/3	64 to 196	+132	178+
H 78	♂	8/9 to 2/3	104 to 134	+30	178+
H 79	♂	8/9 to 2/3	97 to 327	+230	178+
X H 44	♂	8/6 to 10/6	70 to 85	+15	61

Ignoring the two deaths in the first group, which were possibly due to tetany or some operative factor, there is one death 94 days after operation as compared with one after 146 days in the second series. The average duration of life of the groups is 113.5+ days and 182+ days. This despite the fact that the animals in the latter lot were operated two months prior to that of the first and at that time were younger and therefore less liable to live. The average gains were 51.3 grams and 161.3 grams. An individual analysis of each group therefore reveals the fact that the life is longer and the growth is greater in the pituitary fed operated rats.

TABLE 15

NUMBER	SEX	DURATION OF EXPERIMENT	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
Group I. Thyroidectomized rats fed upon usual diet to which liver was added					
			grams	*grams*	*days*
XI 43	♀	9/19 to 12/22	81 to 89	+8	94
I 45	♂	9/20 to 2/3	94.5 to 189	+94.5	133+
ø I 47		10/10 to 10/11			1
ø I 13		9/13 to 9/14			1
Group I. Thyroidectomized rats fed upon usual diet to which anterior lobe was added					
X I 3	♀	7/10 to 12/3	35 to 104	+69	146
I 24	♂	7/26 to 2/3	49.2 to 279	+229.8	192+
I 31	♀	7/10 to 2/3	49 to 234	+185	208+

A critical survey of the general welfare of the living rats at the termination of the experiment disclosed the following data: In respect to nutritive condition and the appearance of their coats the rats of the liver-fed controls were decidedly inferior to those of the pituitary-fed rodents. An individual scrutiny of the various groups revealed four emaciated rats with dirty, scrawny coats among twelve normal liver-fed individuals, as compared with one emaciated rat among three operated liver-fed animals, three emaciated rats among twelve operated pituitary-fed members, while the twelve normal pituitary-fed rats were in excellent condition.

It will, perhaps, be still more instructive to exhibit the results as expressed in the form of tabular summaries. First, for the sake of convenience the histories of all the rats are recorded in one table 16.

TABLE 16A

Normal rats fed upon normal diet with addition of liver

NUMBER	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
	grams	*grams*	*days*
X A 25	276 to 183	−93	155
ϕ A 50	218 to 246	+28	200
A 1	175 to 180	+5	221+
A 46	220 to 235	+15	221+
A 3	222 to 233	+11	221+
A 28	187 to 203	+16	215+
A 4	266 to 174	−92	215+
A 20	196 to 300	+104	215+
D 15	84 to 179	+95	144+
E 34	108 to 186	+78	144+
G 2	53 to 173	+120	180+
G 71	65 to 174	+109	180+
J 12	55.5 to 103	+47.5	200+
J 90	59.5 to 194	+134.5	200+

Total = 14. Dead = 2 (1 accidental)

TABLE 16B

Normal rats fed upon hypophysis added to normal diet

NUMBER	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
	grams	*grams*	*days*
A 11	280 to 276	−4	221+
A 18	288 to 236	+48	221+
A 19	265 to 304	+39	221+
A 10	255 to 288	+33	215+
A 82	208 to 227	+19	215+
C 16	48 to 255	+207	221+
C 57	48 to 223	+175	221+
E 87	92 to 154	+62	144+
E 16	97 to 199	+102	144+
F 73	36 to 206	+170	180+
G 13	20 to 159	+139	180+
G 57	21.9 to 128	+106.1	180+

Total = 12. None dead

Table 17 shows the percentage of deaths in each series. In tables 5 to 16, as in the following tables, the final results are so arranged that the groups may be compared with each other in the form of group averages. Here the total average is obtained by adding the group averages and dividing by the number of component groups. But since the constituent groups differ in number this total average figure, whether

TABLE 16C

Thyroidectomized rats fed upon normal diet with addition of liver

NUMBER	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
	grams	*grams*	*days*
♀ A 9	222 to 238	+16	227
A 8	207 to 205	−2	227+
X A 6	232 to 238	+6	125
X A 4	165 to 127	−38	99
X A 91	81 to 102	+21	99
X A 7	235 to 125	−110	109
X A 17	265 to 189	−76	153
X A 8	105 to 84	−21	61
X A 22	48 to 54.5	+6.5	35
X A 33	55 to 51	−4	13
X A 39	73 to 85	+12	20
X D 88	80 to 120	+40	81
X D 14	71 to 56	−15	22
♀ E 6			1
X E 56	67 to 76	+9	67
♀ H 27	99 to 316	+217	165
H 32	79 to 315	+236	178+
X H 80	67.5 to 90	+22.5	20
X I 43	81 to 89	+8	94
I 45	94.5 to 189	+94.5	133+
♀ I 77			1
♀ I 13			1
X J 5	46 to 77	+31	70

Total 23. Dead 20 (5 accidental)

representing percentages or gains in weight, is not as significant as it would be if the groups were strictly comparable in respect to size. To obviate this discrepancy in size another figure is calculated by considering all the rats in each series as members of one common family. To secure this relation each individual change is considered and the total sum divided by the number of members. The use of this result makes

the different series more directly comparable. This procedure intensifies instead of weakening the relations as found by group comparisons. Both results are recorded in the tables but the emphasis will be placed upon the figure in which all of the rats in a series are considered as belonging to one lot, prefixed in the tables by the sign +, whereas the sign ϕ precedes the other.

TABLE 16D

Thyroidectomized: fed upon normal diet with addition of anterior lobe

NUMBER	INITIAL AND TERMINAL WEIGHT	GAIN OR LOSS	DURATION OF EXPERIMENTAL LIFE
	grams	*grams*	*days*
A 35	175 to 161	−14	223+
A 13	128 to 130	+2	215+
A 58	163 to 199	+36	215+
A 14	87 to 250	+163	230+
X A 81	208 to 293	+85	169
X A 12	205 to 170	−35	155
X B 11	93 to 133	+40	104
B 55	103 to 229	+126	228+
B 15	75 to 276	+201	223+
D 89	56 to 159	+103	148+
ϕ D 30			1
X E 18	67.5 to 116	+48.5	79
X E 2	72.5 to 176	+103.5	57
H 17	64 to 196	+132	178+
H 78	104 to 134	+ 30	178+
H 79	97 to 327	+230	178+
X H 44	70 to 85	+15	61
X I 3	35 to 104	+69	146
I 24	49.2 to 279	+229.8	192
I 31	49 to 234	+185	208+
ϕ J 70			70

Total number of rats = 21; number dead = 9 (2 accidental)

Table 17 shows the average percentage of deaths to be 7.6 per cent; 0 per cent, 78.9 per cent and 36.8 per cent for the respective series.

It is interesting to note that although there were nineteen rats in each of the thyroidectomized groups at the time of operation, ignoring the accidental deaths, the present number is three as compared with twelve (see table 18).

An inspection of table 19 shows that the average life of the operated individuals from the date of operation to the termination of the experiment or the death of the animal is 93.5 days in the liver-fed series, and 174.8 days in the pituitary-fed.

TABLE 17

Percentage of deaths in different groups

GROUP	NORMAL	NORMAL PLUS PITUITARY	THYROIDECTOMIZED	THYROIDECTOMIZED PLUS PITUITARY
	per cent	*per cent*	*per cent*	*per cent*
A	14.2	0	90.0	33.0
B				33.3
C		0		
D	0		100.0	
E	0	0	100.0	100.0
F		0		
G	0	0		
H			33.3	25.0
I			50.0	33.3
J	0		100.0	
0 Average percentage of deaths	2.84	0	78.8	37.5
+ Average percentage of deaths	7.6	0	78.9	36.8

TABLE 18

Numerical distribution and fate of the rats

	NORMAL	NORMAL PLUS ANTERIOR LOBE	THYROIDECTOMIZED	THYROIDECTOMIZED PLUS ANTERIOR LOBE
Total number	14	12	23	21
Total number dead	2	0	20	9
Total number accidental deaths	1	0	5	2
Total number other deaths	1	0	15	7
Total number alive	12	12	3	12

While the above figures refer to the average duration of life after operation of all of the animals, whether living or dead, a consideration of tables 16C and 16D shows that of the thyroidectomized animals which died, those of the pituitary series lived the longest, 110 days as compared with 71 days.

In the above table the individuals in each series are regarded as members of one group. Since the figures obtained by considering the individual group averages are almost identical with those in the table, they are not given here. In comparing the figures representing the duration of life of the thyroidectomized animals although there is a marked difference in favor of the pituitary animals, it is of added importance to understand the connotation of the symbol +, table 19C. In the one group it indicates that the possibility of future life and maintenance is determined by the fat of three rats, (see table 18) while there are twelve members in the other group. Any speculation as to the probable duration of life of these animals will be in favor of the pitui-

TABLE 19

Duration of life (average); counting from the date of operation

NORMAL	NORMAL PLUS ANTERIOR LOBE	THYROIDECTOMIZED AND LIVER	THYROIDECTOMIZED AND ANTERIOR LOBE
A. Duration of life of all individuals from the time of operation to death or the termination of the experiment			
days	*days*	*days*	*days*
193.5	196.9	93.5	174.8
B. Duration of life of the animals which died during the experiment			
175.5		71	110
C. Duration of life of the individuals which lived throughout the entire experiment			
196.4	196.9+	179.3+	200.8+

tary-fed individuals, as shown from the previous histories of the two groups. If the figures, obtained by considering the rats as members of the same or different groups, be compared, but very little difference can be noticed and this is of slight significance.

An idea of some of the rates of growth may be gained from table 20 which is computed by a consideration of the gains and losses before the occurrence of death or the termination of the experiment. The figures representing the liver-fed rats, normal and operated, show the least gain, while those of the pituitary-fed animals are more than doubled, in both the normal and the operated series. The lowest gain is in the operated-liver series, and the highest is in the operated-pituitary group,

but this is practically equivalent to that observed in the normal pituitary series. The results obtained from a study of individual groups, lowest line of table, exhibit the same relative differences, although the weights are higher in each case since individual deviations assume a greater value.

In answer to the possible objection that these differences might well be due to corresponding differences in weight of the animals at the outset, it will be of value to examine the initial and final records of the animals. There is, also, the objection that the higher mortality in the operated-liver series might be due to the use of younger animals, in which the effects of operation would be the most severe. But inspection of the tables shows the difference between the operated animals, only 13 grams, to be in favor of the operated-liver animals, as they were heavier at the time of operation, 113 grams in their case and 100.0 in the other. But the difference is not enough to be significant either

TABLE 20

Average gain in weight in grams. The comparisons include the interval between the date of operation and the termination of the experiment

NORMAL	NORMAL PLUS ANTERIOR LOBE	THYROIDECTOMIZED	THYROIDECTOMIZED PLUS ANTERIOR LOBE
grams	*grams*	*grams*	*grams*
+41.2	91.0	33.7	92.1
0 75.4	118.4	40.3	106.4

way. Neither is the difference between any of the weights sufficient to account for the marked deviation in the final weights; 156.1 to 197.3 and 113.0 to 136.3 in the liver-fed series and 138.3 to 230.4 and 100 to 192.1 in those animals which were fed with pituitary.

In the normal animals the difference of initial weights, 17.8 grams in favor of the pituitary-fed animals and not the liver series. Although this difference of 17.8 grams is too insignificant to account for the great deviation between the terminal weights of the liver and pituitary series, tables 1 and 2 show the weights before the administration of any liver or hypophyseal substance. Before the administration of the hypophysis the initial weight of the liver group was 125.7 in contrast to 133.8 in the pituitary group. If the final conditions of growth are to be inferred from the initial weight the lighter group or the younger would have the choice as here the growth would be most rapid. But this small difference of 8.1 grams would in no case explain the large difference in weight at the termination of the experiment.

These results are intensified by a further differentiation in which the initial and terminal weights are calculated separately for the rats which died and those which are still living. Upon comparing the thyroidectomized animals which died it was found that the liver-fed individuals lost weight, 111.0 grams to 104.2 grams, while the pituitary-fed rats gained, 88.7 grams to 125.4.

Thus an inspection of the experimental data shows that the administration of anterior lobe to thyroidectomized rats has a pronounced beneficial action improving their general condition, accelerating their growth and prolonging their lives.

The cause of death in the operated animals is not certain. That it was due either to a thyroid or a parathyroid deficiency is unquestionable since there was no evidence of infection and but one death of the controls. That the effect is produced by lack of thyroid is strongly indicated. The longevity of many of the animals after operation would exclude the parathyroid-deficiency hypothesis. It might be added that the duration of the experiment is longer than has been usually reported in studies upon thyroidectomized rats. Also, the general condition of the animals up to the time of death suggests a thyroid deficiency. Four of the animals which were not fed the anterior lobe exhibited typical myxedematous symptoms. By these I refer to the general appearance and habits of the animals. In most of the cases which died there was a marked dullness, the rats being somnolent and exhibiting a disinclination to move spontaneously. Since several individuals had been very wild and difficult to handle before the operation, the change to a dull, lethargic condition was very significant. The general nutrition was very poor, as the animals without exception in the liver-fed series were extremely emaciated just before death. The skin exhibited an appearance of lowered resistance, being devoid of hair in places and in four cases was covered with scabby sores. In the pituitary-fed animals which died, some showed emaciation but in no case was the condition so extreme.

DISCUSSION OF EXPERIMENTAL RESULTS

I have been unable to find any previous record of definite experimental data upon the results of the administration of the anterior lobe in cases of thyroidectomy, although there is good reason for considering this portion of the hypophysis.

Schäfer (51) states that the pituitary cannot take the place of the thyroid in operated animals, and that the pituitary extract is not able to take the place of the thyroid extract in the treatment of goiter and myxedema. However, he presents no evidence for this statement.

The clinical evidence upon substitution experiments is contradictory. Pineles (42) reported two cases of acromegaly with concomitant myxedematous symptoms. These symptoms were ameliorated by the administration of thyroid gland but were not influenced by pituitary tablets. This result was to be expected since the primary cause of the myxedematous symptoms was a hyperactivity of the hypophysis and therefore the addition of still more of the pituitary autacoid could have no effect. On the other hand, Pal (38) stated that infundibular extract was of value as a therapeutic agent in the treatment of hyperthyroidism. In his use of pituitrin the most noteworthy effect was that the patients felt so much improved subjectively that they requested the resumption of the treatment whenever it was discontinued. The effect was noticed only in those cases where the internal secretion was disturbed. Still more striking clinical evidence in favor of the functional relationship of the two glands is furnished by Richter (46); he reported that four cases of ambulatory Graves' disease were materially benefitted by the administration of desiccated anterior lobe over a considerable period. All of the cases exhibited well-marked characteristic symptoms of the disease and all, because of their circumstances, were forced to forego the advantages of complete rest and favorable hygienic treatment. However, remarkable improvement was secured and such symptoms as nervousness, the exopthalmos and tachycardia were ameliorated.

An analysis of the data furnished by the protocols of the present experiment indicates clearly that the administration of the anterior lobe of the hypophysis exerted a beneficial effect upon thyroidectomized rats. The action of the pituitary autacoid or autacoids not only ameliorated the condition of the operated rats, in which a deficiency of the thyroid hormone had been created, but actually lengthened the life of the animals. A favorable influence of the anterior lobe was seen also in the case of the normal rats where the coats and condition of nourishment were improved.

The question now arises as to the significance of these results. It has been assumed that in the absence or hypofunction of the thyroid there has been a direct functional substitution of the pituitary autacoid. Another possibility is that the beneficial action is not so much due to a functional relationship between the thyroid and hypophysis as to an

influence exerted by the pituitary substance upon the organism as a whole. In order to ascertain which of the above assumptions is correct, extensive, carefully controlled experimentation is necessary.

If the adherents of the Rogowitsch hypothesis are correct in their assumption that the hypertrophy of the hypophysis following thyroidectomy is physiological, then the favorable influence of pituitary feeding in cases of thyroid hypofunction is an indication of a direct substitution.

There are two objections to this idea of a functional reciprocity. The one is patho-histological in nature and the other is chemical. Trautmann advances the conclusion that the changes observed in the hypertrophied pituitary indicate a degeneration. Therefore the enlargement would indicate a pathological condition instead of a physiological hyperfunction. But these changes might well be the result of increased work or that the cells are taxed beyond their strength in the compensatory effort to perform a double function. Careful histological examination is necessary to ascertain whether there are any cellular evidences of increased functioning in the hypertrophied pituitary. In addition it is essential to discover the true significance of the increased production of colloid. If the colloid substance is to be regarded as an excretory product then its increased output would indicate increased activity upon the part of the cells. Most workers have reported an increased formation following thyroidectomy. The other objection sometimes advanced is based on the relative importance of the iodine content. The failure to detect iodine in the pituitary after thyroidectomy is considered as evidence contra-indicating a functional relationship, on the assumption that the iodine is an essential constituent of the thyroid autacoid. It certainly forms a large percentage of Kendall's thyroxin, constituting as much as 60 per cent. But Kendall emphasizes the indol group as the most important functionally and this same group might be present in the hypophysis or in Robertson's tethelin. On the other hand several investigators have expressed the opinion that the iodine is not an essential component. Again Kendall (31) states,

> For reasons given hereinafter, it appeared desirable to emphasize the presence of the oxy-indol nucleus and it appeared equally desirable not to emphasize the presence of iodin.

He then proceeds to demonstrate that the physiological activity of thyroxin is due to the CO-NH groups.

In his explanation of some of the phenomena concomitant with thyroid disease and apparently anomalous exceptions, Kendall emphasizes

the necessity for the presence of some group similar in structure and function to that of the thyroid autacoid. Thus he states that patients with complete atrophy of the thyroid have a basal metabolism rate of 40 per cent below normal and that the administration of thyroxin alone can bring back and maintain the normal metabolism rate. Now he assumes the complete or nearly complete absence of thyroxin in complete atrophy. In answer to the question what maintains the energy output from 100 per cent below normal up to 40 per cent below normal (the point of basal metabolism in the absence of thyroxin), he assumes the presence of some chemical substance in the body with the *same groupings* as in thyroxin. According to his interpretation of the function of iodine, the halogen merely renders the active groups more reactive. Thus,

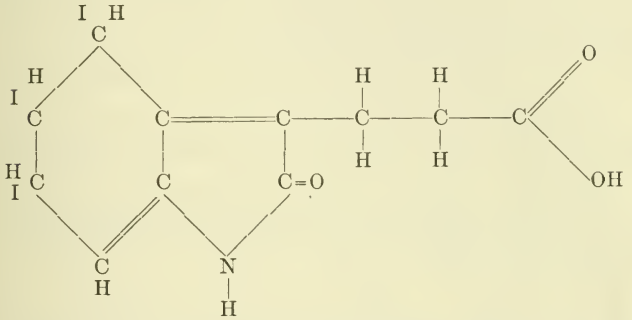
> in the absence of iodine it would take a greater working pressure to bring about its reaction. The substitution of iodine by hydrogen or chlorine or bromine would undoubtedly be followed by an alteration in the degree of reactivity of the substance, but its *gross chemical nature and properties* would not be altered thereby. That the iodine breaks off from the molecule and is used as iodine per se for any purpose, seems absolutely impossible, because Plummer (44), has shown that this substance functions for as long as from 15 to 21 days after being administered . . . .

If the iodine plays a minor rôle, how are the beneficial effects of iodine therapy to be explained? Kendall allocates the symptoms of hyperthyrosis following the administration of iodine to the excessive liberation of thyroxin caused by the stimulating action of iodine, since thyroxin in large amounts will produce hyperthyroid symptoms. Again, the beneficial effects of iodine in myxedema depend entirely upon the amount of active thyroid substance. If the ill effects are due to a partial failure of the gland to maintain a normal supply of thyroxin, the administration of iodine would enable the thyroid substance to make sufficient thyroxin. On the other hand in the condition of complete atrophy no amount of iodine would avail. Again, he considers the animals with the iodine-free thyroids to be analogous to myxedematous individuals in which there is a complete atrophy of the thyroid and a metabolic rate below normal. Here again some other group must be present.

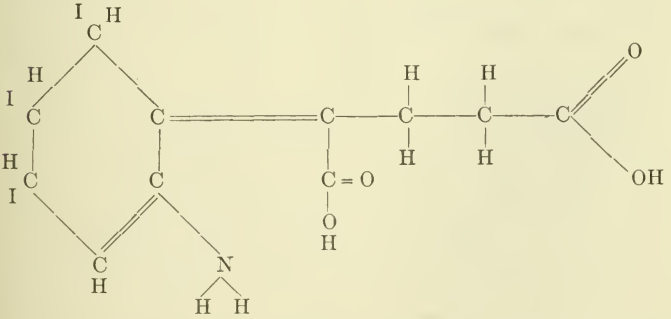
Shumway (55) clearly differentiates between the action of iodine and the thyroid active principle in his experiments with paramecia. He reported that thyroid substance produced an increase of growth of sixty-five per cent in the rate of division while iodothyrin and iodine failed to give this effect.

If the above reasoning is correct the objection advanced by Simpson and Hunter, based on the failure to detect iodine in the hypophysis of thyroidectomized animals, has slight significance, if any. Even if iodine were assumed to be vital to the thyroid substance there could still be a substance so nearly similar in structure and function in the hypophysis that upon a diminution of the former, the latter could be substituted with partial or complete success.

In this connection it is of interest to note the possible similarity in structure between the active principles of the two glands. According to Kendall, thyroxin has the following structure:



He further stated that within the body the indol ring opens up assuming the structure:

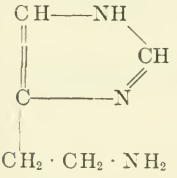


On the other hand two different substances have been isolated from the hypophysis. The researches of several investigators have empha-

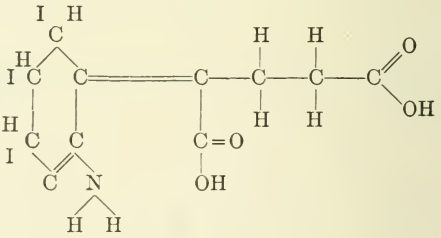
sized the similarity between the properties of the active principle of the posterior lobe and β-iminozolylethylamine. Some have gone still further and identified the two substances. For an excellent discussion of the active principle of the posterior lobe Barger's *The Simpler Natural Bases* (4), and the work of Schmidt and May (52), should be consulted. The only supposedly active principle which has yet been isolated from the anterior lobe is tethelin, considered by Robertson (47) as the active principle, par excellence. Since Robertson found β-iminozolyethylamine in tethelin the idea was advanced that the tethelin was the mother substance of the β-iminozolyethylamine of the posterior lobe. Accordingly, Schmidt and May (52) studied the physiological activities of the split product of tethelin. As a result of their investigation they concluded that the active substance of the posterior lobe was to a certain extent derived from the splitting of a substance present in the anterior lobe.

A consideration of the literature shows therefore an unanimous agreement that β-iminozolyethylamine as a very important principle in the hypophysis.

A comparison of the structures of β-iminozolyethylamine and thyroxin at once reveals the presence of a benzene ring in thyroxin but not in the pituitary principle.

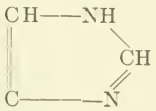


β-iminozolyethylamine



Thyroxin, open form.

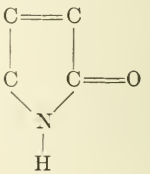
However, there is a closed ring in each.



(1)

Closed ring of β-iminozolyethylamine.

and



(2)

Closed ring of thyroxin.

If the closed form of thyroxin opens in the body it would not be unreasonable to assume that the grouping (1) could also open up at its most reactive point, the NH_2 radical, and function in place of the thyroid ring.

The structural analysis of the known autacoids of the two glands, therefore, reveals a sufficient similarity to indicate the possibility of substitution. In addition to this similarity in chemical structure, a study of the two glands in relation to metabolism brings forth further correlations. A removal of either gland causes disturbances in carbohydrate and fat metabolism, as well as in the sexual activities and general growth of the body. Therefore even if *no* iodine be present after thyroidectomy, in view of the similarities noted, it is reasonable to assume that the pituitary hormone can, in a time of thyroid deficiency, take the place of the latter in the metabolic processes.

Still further evidence in favor of the idea that the hypertrophy of the pituitary is physiological is furnished by the experiments of Livingston (35), who reported that thyroid feeding in thyroidectomized rats did prevent the hypertrophy. It would be interesting to note whether pituitary feeding would also prevent the hypertrophy.

Again, Hoskins interprets the hypertrophy of the pituitary in thyroidectomized amphibian larvae as being physiological. His assumption is based upon the subsequent gigantism noted in thyroidless larvae.

To recapitulate, the objections to the idea of a direct vicarious relationship are twofold: the failure to find iodine in the hypophysis after thyroidectomy and the possibility that the hypertrophy indicates pathological processes. On the other hand there is the possibility that the hypertrophy is physiological and that the iodine might not be a necessary factor. There are also similarities in chemical structure and functions.

Before a definite decision for or against the idea of a direct functional reciprocity can be reached, more evidence is needed. That such a reciprocal relation might be one-sided, is indicated by clinical substitution experiments. Although it may be assumed that the pituitary does, in time of a thyroid deficiency, function for the latter, the thyroid apparently cannot take the place of the hypophysis. Thus Climenko (11) reported a case of infantile hypopituitarism in which there was an absence of distinct myxedematous symptoms and no response to thyroid medication but a decided amelioration after the administration of pituitary extract, the whole dried gland. Again, Stephenson (58) applied thyroid therapy to a case of dyspituitrism without success.

Instead of a functional reciprocity it might be supposed that the beneficial effects obtained are due to an excess of pituitary hormone which affects the organism as a whole. That this would very well be the actual rôle of the hypophyseal substance is possible when the widespread disturbances are noted which result from pathological conditions. Thus to quote Falta (20), page 250:

> The regressive changes in the thyroid gland that so frequently become established in the later stages of acromegaly might well be regarded as a partial phenomenon of the degenerative alterations that in the later stages of acromegaly involve not only these organs that are the seat of the tendency to grow fostered by acromegaly, but also the entire body as well. Hence in the later stages of acromegaly we may often see myxoedematous symptoms, even in the absence of previous manifestations of hyperthrosis.

Again:

> The vascular system in the later stages of acromegaly almost always shows changes . . . . not rarely in addition to the enlargement of the heart there is found an enlargement of the liver, the spleen, the stomach and the intestines . . . . kidneys . . . . suprarenals . . . . In many cases the pancreas was found to be sclerosed . . . . and often of enormous size. A persistent thymus . . . . has been reported quite frequently . . . . The examination of the blood in acromegaly shows not rarely a reduction in the number of erythrocytes and in the hemoglobin contents. . . . In the majority of cases the differential count shows a mononucleosis and not rarely an increase in the number of eosinophiles.

Important abnormalities of metabolism are also associated with the disease. Some of the more important are disturbances in fat carbohydrate and salt metabolism; spells of polyphagia. Attention has been called repeatedly to changes in function of the genital glands, and condition of the vegetative nerves and often a wasting away of the body.

An enumeration of the above symptoms concomitant with hyperpituitarism or acromegaly is indicative of some of the ways in which the hypophysis might affect the entire organism. These conditions are supposedly due to an excess of the hypophyseal substance which resulted from an abnormal condition of the anterior lobe. Since there is such a remarkable correlation between the hypophysis and the other organs, it is but natural to infer that in case of a physiological or pathological dearth of the thyroid hormone the pituitary might tend to restore the normal equilibrium. Evidence that this does actually occur is found in a compensatory hypertrophy. On the other hand, an excess of hypophyseal substance administered would now cause not pathological alterations in the other organs but a restoration of the normal "hormone balance."

SUMMARY

1. The administration of the anterior lobe of the hypophysis has a very beneficial action upon the maintenance and growth of thyroidectomized rats. Aside from the amelorating effect upon the general condition of the animal, the life is definitely prolonged.

2. The beneficial effect might indicate a direct substitution in which the pituitary autacoid takes the place of the thyroid hormone in a compensatory effort to establish normal metabolism. Or the results obtained might be due to a stimulating effect upon the total metabolic processes A definite decision can only be obtained by extensive study of the various factors involved.

3. An attempt has been made to summarize the existing evidence for a possible direct substitution and to answer objections to this explanation of the results.

The writer wishes to express his appreciation of Prof. S. S. Maxwell's counsel and interest throughout the entire investigation. He is also indebted to Miss L. Burke for her personal assistance in carrying out the experiment.

BIBLIOGRAPHY

(1) Allen: Science, N. S., 1916, xliv, 755; Anat. Rec., 1918, xiv.
(2) Alquier: Cited by Delille, 113.
(3) Aschner: Cited by Leva, Med. Klin. (Berlin) 1915, xi, 1266; Abstract Journ. Endocrinology, i, 255.
(4) Barger: The simpler natural bases, New York, 1914, 110.
(5) Baumann: Zeitschr. f. physiol., Chem., 1895, xxi, 319.
(6) Bell: Quart. Journ. Exper. Physiol., xi, 77.
(7) Benda: Cited in Falta, 323.
(8) Bourneville and Bricon: Arch. d. Névrologie, xii.
(9) Boyce and Beadle: Journ. Path. and Bact., 1892, i; 1893, ii.
(10) Calderara: Arch. ital. de. Biol., 1908, l, 190.
(11) Climenko: Amer. Journ. Dis. Chil., 1916, xii, 596; Abstr. Journ. Endocrinology, 1917, i, 88.
(12) Cimoroni: Accademia medica di Roma, November 25, 1906; Arch. ital. Biol., 1907, xlviii, 387.
(13) Comte: Ziegler's Beitr. z. Path. Anat., 1898, xxiii, 90.
(14) Coulon: Virchow's Arch., 1897. cxlvii, 53.
(15) Cushing: The pituitary body and its disorders, Philadelphia, 1912.
(16) Degener: Quart. Journ. Exper. Physiol., vi, 111.
(17) Delille: L'hypophyse et la médication hypophysaire, Paris, 1909.
(18) Dolega: Ziegler's Beitr. z. Path. Anat., 1891, x.
(19) Eichorst: Deutsch. Arch. f. klin. Med., 1916, cxxiv, 207.

(20) Falta and Meyers: The ductless glandular diseases. 2nd Ed., Philadelphia, 1916.
(21) Gley: Arch. d. physiol. norm. et Path., 1892, 311.
(22) Gourfein-Welt: Arch. d'Ophthalmologie, Paris, xxvii, 1907.
(23) Halliburton, Candler and Sikes: Quart. Journ. Exper. Physiol., 1909, ii, 229.
(24) Halpenny and Thompson: Cited by Schäfer. The endocrine organs, 1916, 116.
(25) Herring: Quart. Journ. Exper. Physiol., 1908, i, 281.
(26) Hofmeister: Fortsch. der Medizin, 1892, iv.
(27) Hoskins and Hoskins: Proc. Soc. Exper. Biol. and Med., 1918, xv.
(28) Houssay: First Nat'l Congress of Medicine, Buenos Aires, 1916; Abstr. Journ. Endocrinology, ii, 497.
(29) Josefson: Anat. Rec., 1917, xi, 363. Cited in Falta, 323.
(30) Kamo: Kyota Igak. Zasshi, 1917, xiv, 1; Jap. Med. Lit., 1918, iii, 33.
(31) Kendall: Journ. Amer. Med. Assoc., 1918, lxxi, 871; Journ. Endocrinology, 1918, ii, 81; Mayo Clinic Collected Papers, 1917, ix, 209; Trans. Assoc. Amer. Phys., 1916, xxxi, 134.
(32) Klebs: Allg. Pathol., 1889, ii, 433.
(33) Kojimo: Quart. Journ. Exper. Physiol., xi, 319.
(34) Leonhardt: Virchow's Arch., 1897, cxlix, 341.
(35) Livingston: Proc. Soc. Exper. Biol. and Med., 1914, xi.
(36) Lucien and Parisot: Réunion biologique de Nancy, December 8, 1908.
(37) Nièpce: Goitre et Cretinisme, Paris, 1851.
(38) Pal: Deutsch. med. Wochenschr., 1915, xli, 1537; Abstr. Journ. Endocrinology, 1917, i, 262.
(39) Pardi: Lo Sperim., Journ. 1916, Abstr. Arch. Pediat., 1917, xxxiv; Abstr. Journ. Endocrinology, 1918, ii, 78.
(40) Parhon and Goldstein: Les Sécrétions internes, Paris, 1909.
(41) Pellegrini: Pensiero Medico (Milan) 1916, vi, 289, 301, 313, 325; Abstr. Journ. Endocrinology, 1918, ii, 506.
(42) Pineles: Cited in Falta, 259.
(43) Pisenti and Viola: Centralbl. f. d. med. Wissensch., 1890, 450 and 481.
(44) Plummer: The thyroid hormone and its relation to metabolism. Presented before the Assoc. Amer. Physicians, May 6, 1918, Atlantic City.
(45) Ponfick: Zeitschr. f. klin. Med., 1899, xxxviii, 1.
(46) Richter: Med. Herald, 1916, xxxv, 307; Abstr. Journ. Endocrinology, 1917, i, 114.
(47) Robertson: Journ. Biol. Chem., 1916, xxiv, 416.
(48) Rogers: Journ. Exper. Zoöl., 1918, xxiv.
(49) Rogowitsch: Ziegler's Beitr. z. Path. Anat., 1888, iv, 453.
(50) Rosenhaupt: Cited in Falta, 323.
(51) Schäfer: The endocrine organs, 1916, 116.
(52) Schmidt and May: Journ. Lab. and Clin. Med., 1917, ii.
(53) Schnitzler: Wiener med. Wöchenschr., 1896, 657.
(54) Schönemann: Virchow's Arch., 1892, cxxix, 310.
(55) Shumway: Journ. Exper. Zoöl., 1917, xxii; Abstr. Journ. Endocrinology, 1917, i, 350.

(56) Simpson and Hunter: Quart. Journ. Exper. Physiol., iii, 121; iv, 257.
(57) Smith: Science, N. S. 1916, xliv, 280; Anat. Rec., 1916, xi, 57.
(58) Stephenson: Brit. Journ. Chil. Dis., 1916.
(59) Stieda: Ziegler's Beitr. z. Path. Anat., 1890, vii, 537.
(60) Thaon: Cited by Delille, 113.
(61) Torri: Cited by Delille, 114.
(62) Traina: Cited by Delille, 113.
(63) Trautmann: Frank. Zeitsch. f. Path., lxxx, 173.
(64) Wells: Journ. Amer. Med. Assoc., 1897.

STUDIES IN SECONDARY TRAUMATIC SHOCK

I. The Circulation in Shock after Abdominal Injuries

JOSEPH ERLANGER, ROBERT GESELL and HERBERT S. GASSER

From the Physiological Laboratory of Washington University, St. Louis

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When the Physiological Committee of the National Research Council adopted as part of its war program a coöperative investigation of surgical shock, this laboratory decided to devote itself to the study of those phases of the problem to which its facilities seemed best adapted. Formal, though brief reports on the progress of the work have been made to the Committee from time to time, and a few brief preliminary notes covering certain phases of the problems have been published (1), (2), (3). Now, with the cessation of the urgent press of war work, time is taken to prepare more complete reports of our experiments.

It is well at the outset to set forth clearly the condition it was our intention to study. In this connection the distinction must be drawn sharply between so-called primary and secondary shock, a distinction more readily drawn in the case of man than of animals. According to Cowell (4) in *primary shock*

> the man suddenly becomes pale, clammy, and pulseless; and a low pressure may be found as soon as it is possible to make a reading, 15 to 20 minutes after the man has been hit.
>
> When, on the other hand . . . . a (wounded) man previously in good condition develops similar shock symptoms, a condition of *secondary wound shock* may be said to exist.

Whether or not there are grounds for believing that these are two wholly different conditions is a question that does not concern us here. But it is important to bear in mind that the conditions studied in the laboratory presumably resemble secondary more nearly than primary shock as defined by Cowell.

One of the problems with which all were at first concerned was how to recognize and how to produce "shock" in animals. In his first communication on the subject, the Chairman of the Physiology Committee

suggested that the criteria of shock enunciated by Mann (5) be taken as our standard. These are: *a*, "loss of sensibility, as shown by the lack of the necessity of administering an anesthetic when the eye reflex is present;" *b*, "pallor of the mucous membranes;" *c*, "a small, weak pulse;" *d*, an "irregular, rapid, shallow or gasping respiration;" and *e*, a blood pressure lowered to one-third or one-fourth its original level. These signs are not all equally easy to recognize. For this reason, and for others also, we were not always certain that we had succeeded in getting into this condition all the animals which we had good reason for believing were in "shock." In the dog, the eye reflex is a most uncertain index. An animal certainly not in "shock" may have no reflex whatever; and on the other hand, it may, of course, be absent in animals in "shock." Again, animals in "shock" with eye reflex either present or absent, may begin to move shortly after the anesthetic is removed; though to be sure, in every case of "shock," properly so-called, only the lightest anesthesia is necessary to keep the animal quiet. Furthermore, Mann's blood pressure criterion is usually an altogether impossible one. The arterial pressure at the beginning of our experiments most often lay between 100 and 125 mm. Hg., sometimes lower, sometimes higher. Taking Mann's larger fraction, namely, one-third, these animals, according to his criterion would not be in "shock" unless their pressures had fallen to 33 to 40 mm. Hg.; or, taking his smaller fraction, one-quarter, to 24 to 31 mm. Hg. But when, during the induction of shock, the pressure passes below 40 mm. Hg., the animal has passed beyond shock—it is dying. As a matter of fact, Mann himself does not adhere to this specification (6).

We do not believe that in the present state of our knowledge shock can be defined to the entire satisfaction of all. And yet, for any given investigation of the subject it is necessary, in order to be able to compare the results of different procedures, to have some standard state to compare. A fall in arterial pressure is certainly one of the manifestations of developed shock and there can be very little reason for doubting that the state of shock is present when, as a result of traumatization, the blood pressure has fallen slowly but consistently to the level of 50 mm. Hg. Indeed it is almost safe to state when the arterial pressure has thus fallen to this level that the circulatory failure has become irreversible; though to be sure by certain procedures, such as the administration of adrenalin, the arterial pressure can be raised temporarily. Clinical experience is in accord with this opinion. Thus, according to Archibald and McLean (7), cases in which the systolic and diastolic

pressures fall as low as 75 and 40 mm. Hg. respectively, rarely recover. The geometric mean of this pulsatile range of pressure cannot be very far from 50 mm. Hg. There are other reasons for selecting 50 mm. Hg. as the level for comparison. If an animal with a pressure of 50 mm. Hg. is not in shock it is only a question of time, if this pressure is maintained, before shock properly so-called will supervene. In other words any procedure that will carry the arterial pressure down to about 50 mm. Hg. and hold it there for a sufficient length of time will, if it does not lead too rapidly to dissolution, bring on the picture of shock. Furthermore, it frequently happens, when an animal is exposed to conditions that lead a shock-like failure of the circulation, that after the arterial pressure begins to fall the descent is relatively rapid until the pressure comes to lie somewhere between 60 and 40 mm. Hg., when it is apt to become less rapid, so that the pressure for hours may hover in the vicinity of 50 mm. Hg. before the more rapid fall begins that leads to death. We realize, as one of us has emphasized (8), that the circulation may be severely disturbed, as indicated by a reduced volume flow of blood, even before the blood pressure begins to fall. But unfortunately there is at present no satisfatory or convenient way of recognizing in man the imminence of shock through the use of this particular sign. For these reasons we adopt in this investigation a blood pressure criterion of shock and arbitrarily take the level of 50 mm. Hg., when certain other signs also are present, as surely indicative of the presence of shock.

The procedures we have employed for the purpose of bringing on shock can best be described in connection with the several sets of experiments we have performed. Their significance in relation to the mechanism of shock will be considered in the final discussion.

GENERAL METHODS

For the most part the animal employed in this investigation has been the dog. All were anesthetized with ether. At first a preliminary dose of morphine was administered but, gaining the impression—by no means, though, a conviction—that the morphine delayed the onset of shock, the use of morphine was soon discontinued. Throughout the experiment the anesthesia was as light as possible and usually quite uniform.

Arterial and venous pressures. The carotid pressure was followed by a mercury manometer throughout all of the experiments. In some

experiments the portal pressure also was followed either continuously or from time to time. In these cases the jugular pressure also was recorded from time to time. The former pressure was taken from the vena gastro-lienalis. This vein is easily exposed through an abdominal incision just large enough to permit of the delivery of the spleen, and without exposing or even touching the intestines.

The main difficulties to be overcome in recording the venous pressures through prolonged experiments, besides obviating the action of valves, consist in the recognition and the prevention of obstruction by coagulation, by kinking or by extraneous objects. These difficulties we have succeeded in minimizing through the use of the following method: An obliquely bored three-way stopcock is so connected with a pressure bottle containing salt solution, in which is dissolved 0.01 mgm. hirudin per cubic centimeter, with a manometer, and with the vein that by turning it into one position the manometer can be raised until it records a pressure slightly in excess of the anticipated venous pressure, whereupon, by turning the cock into the other position, the manometer is thrown into communication with the vein and sinks to the level of the venous pressure, a corresponding volume of hirudin solution, usually not more than 0.05 cc., running into the vein. This manipulation is repeated at intervals of about five minutes throughout the course of the experiment. The total volume of hirudin solution thus washed into the circulation need not exceed 10 cc. even in the longest experiment. Clotting has rarely occurred. The method quickly discloses any interference with the transmission of the pressure back from the portal vein, when the readings are taken from the splenic vein, or back from the right auricle, when the readings are taken from the jugular vein. The importance of such a means of control when the record, as is often the case, may be without any oscillation whose diminution or cessation would disclose an obstruction, cannot be over-emphasized. Methods resembling ours have been employed by other investigators.

Inflow method. The tone of the peripheral arteries has been followed by means of a modification of a method previously employed in this laboratory by Bartlett, also in a study of shock (9). From time to time determinations are made of the rate of flow of salt solution under a constant pressure, one so high as to nullify the effects of changing venous and collateral (arterial) pressures, through a part of the peripheral resistance from time to time momentarily isolated in such a way as not to injure the nerves or the nutrition of the part concerned. The method differed from Bartlett's only in that the injection period was

both controlled and recorded electro-magnetically; it was thus freed entirely of the errors inherent in reaction time.

For the most part, the injections ("inflows") were into the hind-leg ("femoral") area; though a few have been made into an intestinal area and into the liver. When the intestine was employed care was taken not to handle or even to remove from the peritoneum the part into which the injection was to be made. Examination at the close of the

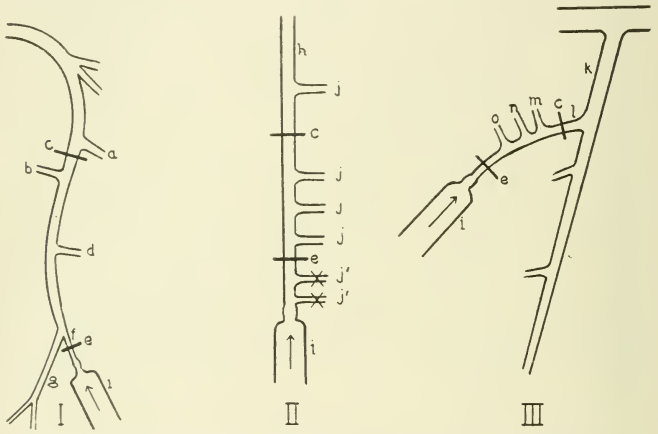


Fig. 1. Diagrams showing the arrangements for inflow experiments.

I. Femoral inflow. Main branches of the Arteria femoralis with the position of the inflow cannula, *i*, of the electromagnetic obturator, *e*, and of the clip *c*. *a*, A. profunda femoris; *b*, A. femoralis anterior; *d*, A. femoralis postica superior; *f*, A. saphena; *g*, A. articularis genu suprema.

II. Intestinal inflow. *j*, Aa. ileae (*j'*, ligated) of *h*, A. mesenteria superior.

III. Hepatic inflow. Main branches, *m*, Ramus hepaticus dexter; *n*, Ramus hepaticus sinister; and *o*, Ramus hepaticus medius of *l*, A. hepatica; *k*, A. coeliaca.

experiment showed that the perfused area usually included one or two loops of the lowermost part of the ileum.

The diagrams shown in figure 1 will serve to indicate how and where the injections were made. To determine the inflow rate, the clamp, *c*, is first adjusted on the artery. Then, by operating the electromagnetic obturator, *e*, with a chronographic marking key, salt solution under a high pressure is permitted to run into the artery, in the direction shown

by the arrow, for from three to five seconds, rarely longer; then the clamp, *c*, is removed. Under ordinary circumstances, the circulation of the part receiving the solution is stopped not over a half-minute for each estimation. The inflow period employed has been such as would allow about 2 to 6 cc. of solution to enter the circulation, the considerations being to allow in a sufficient volume to minimize the effect of distention of the arteries and of the changing viscosity, and yet not inject into the circulation a volume of salt solution so large as to materially affect the general trend of the state of the circulation. The total volume injected before the arterial pressure reached the level of 50 mm. Hg. exceeded 100 cc. in only three experiments, and usually it was considerably less; and the total injected before death, excepting cases in which injections were made for special purposes, in only two instances exceeded 200 cc. As the injection of this total quantity usually extended over a period of many hours, it may be regarded as relatively small in amount. If it had any other than the usual transitory effects on the circulation, we were unable to recognize them. A continuous perfusion method, such as that of Sollmann and Pilcher (10), would have been preferable; but in experiments lasting as long as have these, such a method is precluded by the tremendous edema that eventually develops. The method of Morison and Hooker (11) seemed objectionable on account of the possible consequences of the great change in vascular bed that is made in association with the period of observation.

Bartlett tested the inflow method and found that it revealed changes in peripheral resistance, and that other physiological changes in the circulation were without influence on it. We have completely confirmed Bartlett's tests. The accuracy of the method cannot be measured in absolute terms; but the experiment illustrated by figure 2 demonstrates, by the regularity of the form of the curve as the vasomotor tone dies away after the extreme constriction produced by cerebral anemia, that variations in peripheral resistance are correctly indicated.

Precautions must, of course, be taken to avoid adventitious plugging of the blood vessels. Our results show that if any plugging did occur, in at least the vast majority of cases it was not sufficiently extensive to mask the main effect. In very long experiments clots sometimes form in the main artery just in front of the cannula. To insure against vitiation of results by an unrecognized partial obstruction of this kind, the rate of inflow into the unclipped artery during a fraction of a second was noted from time to time, and inflow readings were accepted only when the rate of flow into the unclipped artery greatly exceeded the rate

of the flow into the clipped artery. If a clot did form and could not be forced on into the general circulation (not into the part perfused), determinations were continued on the femoral artery of the other side; or if the artery perfused happened to be unpaired, inflow readings were discontinued. When the transfer is made from one femoral to the other, the new *normal* inflow rate is unknown. This, however, is of but little consequence; for the method is relative only, and if the conditions

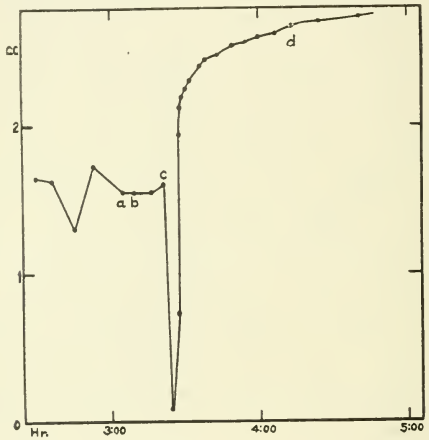


Fig. 2. Illustrating the capabilities of the inflow method (femoral). A reading was made at *a* simultaneously with a peripheral stimulus of the vagus that stopped the heart; it shows that a mere fall in arterial pressure, before time for reflex effects has elapsed, is without effect on the inflow rate. At *c* the ventricles were fibrillated; the subsequent readings show the effect of extreme asphyxial stimulation of the centers, followed by its death. At *d* the great veins were cut; the result (no change) shows that venous back pressure is without effect on inflow rate.

at the time of the transfer are fairly constant, the first of the new series of inflows may be taken as a new level from which to regard the trend that obtained while the last of the old series were made. And, in any event, the normal inflow times of the two femorals of any one animal are probably very nearly alike.

In each experiment the animal is first made entirely ready for the induction of shock; that is to say, all of the preliminary operations and

cannulations are made. Then from one to eight, usually three, inflow readings are made for the purpose of determining the normal vasomotor tone. These readings, however, are rarely if ever constant; indeed they usually fluctuate widely, as widely as the vasomotor tone is known to fluctuate normally. It was easy to demonstrate that at least some of these early fluctuations were vasomotor reflexes started by some definite manipulation, such, for example, as placing the clip upon the artery. It is interesting to note that as a rule the readings become much more regular as the experiment proceeds; for this observation indicates that the vasomotor reactions diminish with time. Although the wide fluctuations in inflow rate at the opening of the experiment made it difficult to determine the norm, yet they served the useful purpose of proving that in the preparations for the experiment the nerve supply to the peripheral resistance concerned had not been injured. It is possible that by the time the preliminary inflow readings are obtained a certain amount of peripheral constriction already has developed (8).

Charts of results. In order to simplify the analysis of the lengthy records the results have been plotted on a system of coördinates. We are reproducing a number of the curves thus constructed so that those who are working on shock may have the opportunity of seeing the data upon which our conclusions are based. In reading these charts it should be borne in mind that the venous and arterial pressures are plotted on different scales; to bring out sharply the small fluctuations in venous pressure, large ordinates are necessary. It should also be borne in mind that vasomotor tone is the reciprocal of the "inflow rate."

THE CIRCULATION IN SHOCK AFTER ABDOMINAL INJURIES

In this paper the results are given of our study, by the methods described above, of the mechanical changes in the circulation occurring during the development of shock by procedures directly affecting the abdominal viscera.

SHOCK BY EXPOSURE AND MANIPULATION OF THE INTESTINES

Ten experiments of this character have been performed. In table 1 it is seen (exps. 7, 8, 9, 10, 19, 21, 26, 58 and 59) that, disregarding the preliminary procedures, the experiments lasted from $5\frac{1}{2}$ to 11 hours each, and that the pressure level of 50 mm. Hg. was reached in from $3\frac{1}{2}$

to about $7\frac{1}{2}$ hours, most often in about 5 hours. In one case it had not fallen to that level even after 11 hours had elapsed. When calculated, however, from the time manipulation of the intestines was begun, and

TABLE 1

Shock after abdominal operations

EXPERIMENT NUMBER	DURATION OF EXPOSURE*	PRESSURE ABOVE 50 MM. HG.	PRESSURE 50 MM. HG. OR BELOW	MANIPULATION BEGUN AFTER EXPOSURE	BETWEEN MANIPULATION AND 50 MM. HG.	REMARKS
	hours	*hours*	*hours*	*hours*	*hours*	
7	6	$3\frac{1}{2}$–$4\frac{1}{2}$	$1\frac{1}{2}$–$2\frac{1}{2}$	3(?)	$\frac{1}{2}$–$1\frac{1}{2}$	Exposure and manipulation
8	8	$6\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$	4	Exposure and manipulation
9	$5\frac{1}{2}$	5	$\frac{1}{2}$	$\frac{1}{2}$(?)	$4\frac{1}{2}$	Exposure and manipulation
10	$6\frac{1}{2}$	$3\frac{1}{2}$	3	$\frac{1}{2}$	3	Exposure and manipulation
19	8	6	$1\frac{1}{2}$	$1\frac{1}{2}$	4	Exposure and manipulation. Long preliminary operation involving manipulation of intestines
21	$7\frac{1}{2}$	$5\frac{1}{2}$	$2\frac{1}{2}$	3+	2	Exposure and manipulation. Long preliminary operation involving manipulation of intestines
26	11			$\frac{1}{2}$		Exposure. Scarcely shocked after 11 hours
58	6	$5\frac{2}{3}$	$\frac{1}{3}$	$\frac{1}{2}$	5+	Exposure and manipulation. Position of inflow cannula prevents thorough manipulation
59	$9\frac{1}{2}$	$7\frac{1}{2}$	2	$\frac{1}{2}$	7	Exposure and manipulation. Position of inflow cannula prevents thorough manipulation
13	$6\frac{1}{2}$	3	$3\frac{1}{2}$	**	**	Evisceration
16	6	5	1	**	**	Evisceration
17	5	$2\frac{2}{3}$	$2\frac{1}{3}$	**	**	Evisceration
22	2+	0	2			Exposure in sympathetic-less animal
23	6	3−	$3\frac{1}{3}$	$\frac{2}{5}$	$2\frac{1}{2}$	Exposure and manipulation in sympathetic-less animal

* The time necessary to make the preliminary operation is not included.

** Evisceration was performed immediately and required about 15 minutes.

when this was thorough, this fall was accomplished most often in about 4 hours. It is also seen that the pressure was 50 mm. Hg. or lower for from 20 minutes to 3 hours, most often for about $1\frac{1}{2}$ hours.

Peripheral resistance

Femoral inflow. The early effect on the femoral inflow rate of exposing and manipulating the intestines has not been entirely constant; it is fair to say, though, that practically invariably the inflow rate is reduced, and usually below the initial range (see figs. 3, 4, 5, 8, 9). This constriction as a rule persists through the period of maintained arterial pressure and often for some time after the arterial pressure begins to fall (figs. 3, 4). It then gradually gives way to dilatation, so that by the time the arterial pressure has fallen to the level of 50 mm. Hg., the

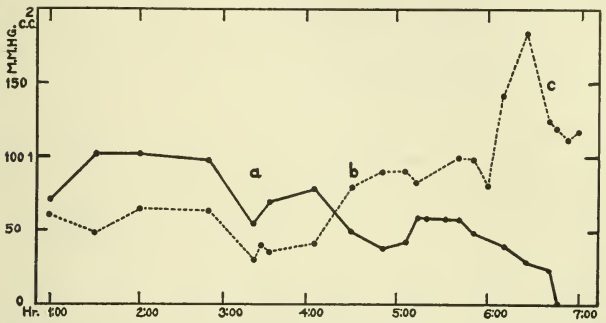


Fig. 3. Experiment 7. Shock by exposure and manipulation of the intestines. Arterial pressure, —•—•—; femoral inflow rate, ···•···•··· . *a*, some time after exposure and manipulation; *b*, three minutes after manipulation; *c*, asphyxiated to end.

inflow rate practically without exception has been as high as, or higher than, the highest initial inflow rate. As the end approaches, the inflow rate often accelerates (figs. 3, 8). Sometimes there is a retardation (asphyxial) at the moment the heart or respiration stops, followed by an acceleration (figs. 5, 8, 9). In some cases a mortal increase in the inflow rate has failed to occur; indeed the asphyxial decrease occurring at this time sometimes has failed to pass off entirely (figs. 3, 4, 9).

This occasional failure of the inflow rate to increase beyond the normal after death is worthy of a moment's consideration. It has been seen not alone after exposure of the intestines but as a matter of fact at the conclusion of long lasting experiments in general and also after adrenalin injections in experiments of even short duration. In part,

at least, it is due to constriction of the larger arteries, including the femoral itself; for the rate of flow through the unclipped artery at this time may be but little faster than the rate through the clipped artery. The first explanation of this phenomenon that suggests itself is that it is due to the formation of an obstruction (a clot) in the artery. But this conclusion is rendered untenable by the behavior of subsequent inflows; for these almost invariably slowly increase from trial to trial (see, for example, fig. 4). The only explanation of the phenomenon that seems at all to fit the facts is that as a result of prolonged asphyxia the arteries pass into a state resembling post-mortem contraction, such as has been described by MacWilliam (12). It is worthy of note that Sollmann and Pilcher (13) found that in post-mortem paralysis of the vasomotor center the outflow, followed by their method, also often did not quite recover its former rate.

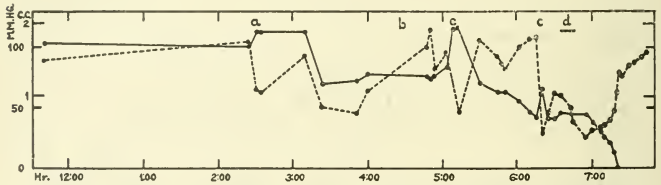


Fig. 4. Experiment 8. Shock by exposure and manipulation of the intestines. Arterial pressure, —•—•—; femoral inflow, ···• ·•··· *a*, intestines exposed; *b*, changed from left to right femoral artery; *c*, adrenalin, 1.0 cc. 1:20,000, intravenously; *d*, asphyxia.

Mesenteric and hepatic inflows. No lengthy explanation is needed to make it clear that mesenteric and hepatic inflows can be followed only with great difficulty in experiments in which the intestines must be exposed and manipulated in such a way as to protect and leave undisturbed the part into which the injection is being made. We have succeeded in making fairly satisfactory observations in three of the experiments. The results, upon the whole, have been the same as in the case of the femoral inflow.

In one of the experiments (fig. 5) both the femoral and hepatic (actually hepato-duodenal) inflows were followed. If allowance is made for the fact that the hepatic and femoral readings were not made simultaneously, the general parallelism of the two inflow curves, excepting the readings made after death, is striking. Some time after manipulation

of the intestine, and at a time when the arterial pressure reaches about 50 mm. Hg. (5:30), both of the inflow readings are high, the hepatoduodenal being well above the initial range, the femoral at about the upper limit of normal. The terminal (presumably asphyxial) slowing of the femoral inflow rate is followed by the usual post-mortem acceleration; that of the hepatic, however, is not, possibly because a clot was beginning to form.

In experiment 21 (fig. 6) the hepatic and intestinal inflows were followed. The former showed no consistent changes. It should be noted, however, that at *b* (see fig. 6) it became necessary to force a clot on into the hepatic circulation. The intestinal inflow was not redetermined until the arterial pressure had fallen to 50 mm. Hg. some 3 hours after exposure of the intestines. The inflow rate then was well above normal. Then, after a lapse of 40 minutes, the inflow rate was found to be markedly diminished and it remained so to the close of the experiment. This final decline of the inflow rate might have been due to the slow formation of an obstruction (clot).

In experiment 26 (fig. 7) the intestines were merely exposed (at *a*) and not manipulated. After 10 hours of exposure, the arterial pressure was still 80 mm. Hg., but finally it fell to 52 mm. Hg. After it had been at the latter level for 4 minutes a clot formed in the inflow artery. The experiment was then brought to a close by puncturing the heart. Exposure of the intestines in this experiment led to a very marked reduction in the intestinal inflow rate over a period of 8 hours. Then the inflow rate increased. It reached practically the low limit of normal and was still on the increase at the time the last reading was made.

Although these experiments cannot be regarded as entirely satisfactory, they nevertheless clearly confirm the results obtained in the study of the femoral inflow rate and indicate that in shock produced by exposure and manipulation of the intestines the hepatic and intestinal (splanchnic) areas, as well as the femoral (somatic) area constrict, or, at least, do not dilate, during the early stages of shock induction and dilate as the pressure falls toward the level of 50 mm. Hg. The differences in the behavior of the intestinal inflows in the different experiments possibly are attributable to differences in the amount of handling the intestines suffered, and also to the time relation obtaining between the actual handling of the intestines and determination of the inflow rate.

The behavior of the arterial pressure and of the inflow rate as we have described and pictured them applies to the conditions obtaining while the intestines are lying quietly out of the abdomen in the intervals

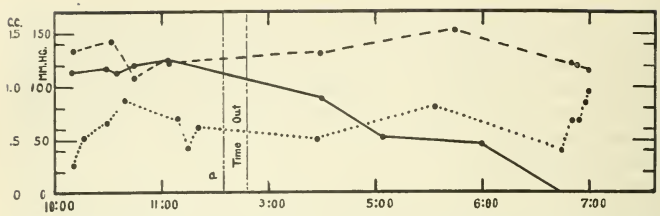


Fig. 5. Experiment 19. Shock by exposure and manipulation of the intestines. Arterial pressure, —•—•—; femoral inflow, ···•· •···; hepato-duodenal inflow, --•--•--. *a*, manipulation of intestines.

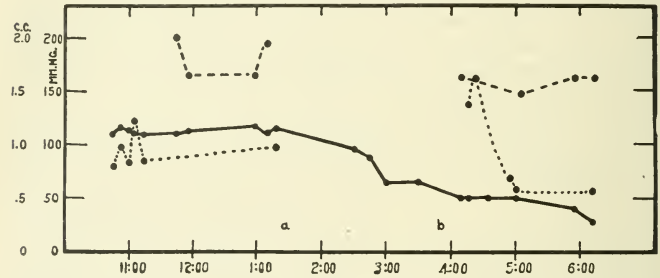


Fig. 6. Experiment 21. Exposure and manipulation of the intestines. Arterial pressure,—•—•—; intestinal inflow, ···•·••···; hepatic inflow, --•--•--. *a*, intestines exposed; *b*, clot in hepatic cannula forced on into capillaries (hepatic).

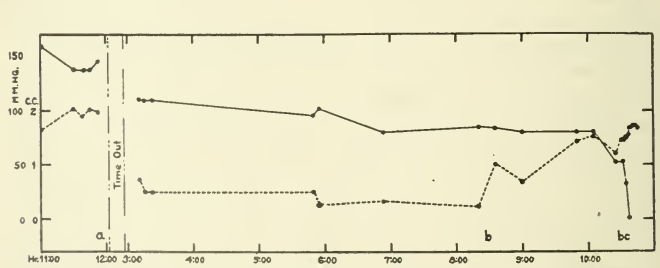


Fig. 7. Experiment 26. Exposure of the intestines. Arterial pressure, —•—•—; intestinal inflow, --•--•--. *a*, intestines exposed; *b*, clot removed from inflow cannula; *c*, animal killed by puncturing heart.

between manipulations. When observations are made during, or very shortly after, momentary, though it may be, rough, manipulation of the intestines the result, at least during the first hour or two of the period of shock induction, is quite different. The immediate effect of manipulation then (see fig. 3, *b*) is a sharp fall in arterial pressure and a momentary, and it may be marked, increase in (femoral) inflow rate. This unquestionably is a passing reflex dilatation. The relation this reflex bears to the basic change in peripheral resistance occurring during shock development has not been investigated.

Interpretation and discussion. Interpretating our results in terms of activity of the vasomotor center, it can be stated categorically that in the early stages of exposure of the intestines the tone of the center usually is increased; that by the time the arterial pressure has fallen to 50 mm. Hg. the tone of the center practically invariably is subnormal, but never is entirely lost. The latter finding confirms Bartlett (9). But even when the tone of the center is subnormal it still is capable of responding in the usual manner to asphyxia produced by suffocation or by sudden stoppage of the heart. The constrictions so induced, though, are very much smaller than those occurring in normal animals. In the last stages of the experiment, therefore, the condition of the center is below normal as regards both its tone and its reactivity.

The fact that vasoconstriction is the rule while the pressure is still high and that dilatation occurs as a rule only after the pressure has been low for some time, would seem to indicate that exposure of the intestines in some way tends to cause a fall in pressure, but that this tendency is combated by the vasoconstrictor center; and that this hyperactivity is maintained as long as the arterial pressure is high enough to maintain a sufficient supply of blood to the centers. It sometimes happens, however, that the inflow rates are not diminished even when the arterial pressure has begun to fall (fig. 8). In such instances, which have been very rare, it must be concluded that the reactivity of the center to lowered pressure is subnormal.

As is well known, there is a large literature, full of contradictions, on the state of the vasomotor center in shock. Inasmuch as practically all these views rest upon observations on, or inferences drawn from, shock as produced by exposure of the intestines or by some comparable procedure, a brief discussion of this subject is here in order.

One cause of the misunderstandings that have arisen in the discussion of this question seems to be a tendency on the part of those holding definite views to overstate the antagonistic views. Thus, those who

have maintained that the cause of shock is to be sought in fatigue, or depression, or exhaustion, or inhibition of the vasomotor center did not as a rule intend to imply, as some seem to believe, that the center is wholly without tone, or wholly unresponsive to its normal stimuli. While another cause of the discrepancies probably is revealed by the fact, brought to light by the present investigation, that the state of vasomotor tone is not the same in all stages of shock. Doubtless different investigators have been dealing with different stages of shock. An analysis of some of the more important contributions to this subject, with these sources of error in mind, will serve to make our contention clear.

It should be stated in the first place that the origin of the view that shock is due to diminished vasomotor activity rests upon inferences from clinical data and from indirect experimental data rather than upon direct methods of attack (14), (15), (16), (17), (18). Porter and collaborators (19), (20), (21), were the first to approach the problem with a view to testing the then prevailing hypothesis by more direct means than had previously been employed. They succeeded in showing that the fall in arterial pressure on stimulation of the depressor nerve, and that the rise in arterial pressure on stimulation of other afferent nerves are proportionally as great in shocked as in normal animals. They, therefore, maintain that the activity of the vasomotor center is not impaired in shock. Lyon and Seelig (22) using a similar method independently come to the same conclusion. Sollmann and Pilcher (23) rightly take exception to this method because of a fallacy in the underlying logic. They point out that a percentile rise in pressure, other things being equal, denotes some loss in activity on the part of the center; that an unimpaired center should be capable of producing an unimpaired absolute rise in blood pressure. Indeed, it is to us conceivable that a percentile rise might actually occur where the center is overactive. Thus a normal center that is stimulated to hyperactivity by a reduction in blood volume, for instance, and which cannot, even as a result of this hyperactivity, keep the arterial pressure up to the normal level, could effect only a relatively small additional, though, perhaps, still percentile, rise as a result of afferent stimulation.

Mann's methods of studing vasomotor tone were indirect (5). He goes further than his facts justify when he concludes that "the vasomotor center is not depressed or fatigued in shock;" his methods like those of Porter are capable of showing only that the vasomotor center possesses some degree of activity. Joseph and Seelig (24) also employed

an indirect method of following vasomotor tone. They conclude from observations on the rabbit that in shock the tone of the center is normal. But, as we interpret their results, they demonstrate merely that the center possesses some tone; they are not in a position to state what that degree of tone may be.

Muns (25) plethysmographed the leg of the dog and found that traumatizing the exposed intestines diminished the volume of the leg even when the arterial pressure remained constant. This observation is clearly indicative of a vasoconstriction in the early stage of shock production and therefore is in accord with our findings.

The methods employed by Sollmann and Pilcher (13) and by Morison and Hooker (11) are alike in that they determine the rate of flow of liquid under a constant pressure through blood vessels under the control of the vasomotor center; they, like the method of Bartlett, employed by us, are direct methods. The study of shock was only an incident in the work of Sollmann and Pilcher. In one experiment in which the arterial pressure had been reduced to 10(!) mm. Hg. by manipulation of the intestines the center failed to respond to asphyxia; it had completely lost its activity. Morison and Hooker performed six experiments upon the leg and two each upon the kidney and the intestine. It is impossible, though, to tell from their article in what stage of shock they determined the perfusion rate. They state in the text that in but one (leg) experiment the outflow was increased, and in their conclusions state that the rate of flow is decreased. One of us (8) has shown beyond peradventure that tissue abuse at once and definitively leads to a reduction in the flow of blood as measured in the salivary gland.

Finally, Wiggers (26) concludes (upon the basis of the configuration of the central arterial and intraventricular pressure curves) that from the moment the intestines are exposed and until the death of the animal the larger arteries are abnormally empty and the peripheral resistance low. Wiggers, apparently forgetful of his preliminary discussion, in which several possible explanations are narrowed down to one by a process of reasoning, by no means convincing, maintains that his evidence is a *direct* proof of the condition of the peripheral resistance, a conclusion with which we cannot agree. But, assuming for the moment that the method does indicate a diminution in peripheral resistance, it does not necessarily follow, as Wiggers himself admits, that the diminished peripheral resistance is the result of diminished central tone. The present methods have shown that early in shock production, ex-

cepting only the momentary response to manipulation of the intestines, the center, as a rule, maintains increased tone, both in the somatic area and in the parts of the splanchnic area that are not injured. We have not measured the peripheral resistance in the parts of the splanchnic area directly traumatized. According to Janeway and Ewing (27), manipulation of the intestines paralyzes the splanchnics locally, while in loops protected from exposure the vasomotor tone remains normal. This, presumably, is entirely a peripheral process. But inasmuch as the exposed loops are also the seat of an inflammatory process, it does not necessarily follow that the resistance to the flow of blood through them is diminished by the splanchnic paralysis. In the light of our direct determinations of the peripheral resistance in the early stages of intestinal exposure, it is obvious that only if the resistance were diminished in the traumatized regions would there be a way of accounting for the peculiarities of Wigger's central pressure curves on the basis of a reduction in resistance. As a matter of fact, the evidence available seems to support increased resistance to blood flow throughout the splanchnic area, rather than diminished resistance; for all investigators are agreed, as will be seen in a moment, that the portal pressure is lowered by intestinal exposure. A diminution in the resistance to blood flow in the intestinal area would have just the opposite effect.

Our experiments, as has been said, seem to indicate that in part, at least, the late loss in vasomotor tone is the result of the reduced blood flow that comes of the low pressure rather than the cause of the low arterial pressure. It is therefore conceivable that if shock could be induced more rapidly than we have succeeded in bringing it on, a low pressure might develop at a time when the tone of the center is still high. In such a case marked constriction might persist to the end.

Jugular pressure

The jugular pressure in the two experiments in which it has been followed (figs. 8, 9), varied at most 0.5 mm. Hg. Morison and Hooker (11) found that the caval pressure progressively falls; and Wiggers found that the so-called effective venous pressure falls. In shock produced by this method, therefore, the heart seems to be capable of moving on efficiently all the blood that is brought to it. This confirms the conclusion Mann (5) and others have come to on the basis of observations of another kind.

Here we may refer to a few casual observations made with the idea of testing the capabilities of the heart. In a few experiments we have from time to time determined the height to which the carotid pressure is raised when the aorta is temporarily occluded. This may be considerable even quite late in the course of the experiment, but it is not equal to that attained early in the experiment, nor is the raised pressure so well maintained. This failure of the heart in shock to equal the performance of the heart in the normal animal does not necessarily mean that the heart is at fault. For in shock the respiratory pump is apt to be feeble and the return of blood to the heart impaired.

Portal pressure

The portal pressure has been followed through one experiment and almost to the close of another. In the one complete experiment (fig. 8), upon exposure of the intestines the pressure fell from the unusually high level of 16 mm. to 7 mm. in the course of $2\frac{1}{2}$ hours. Owing to trouble caused by clotting, the data with regard to the peripheral resistance are of but little help here in arriving at an understanding of this behavior of the portal pressure. It might, though, have been due entirely to the fall in arterial pressure. Later (after 2:00) the portal pressure rose for about $1\frac{1}{2}$ hours; the fall in arterial pressure at this time was continuous. The inflow rate, here, is again confused by clotting; but later, when the shift was made to the other femoral artery, it was found that the peripheral resistance was diminishing. It is possible, therefore, that the rise in portal pressure which here occurs despite a falling arterial pressure, is to be explained by a giving way of the peripheral resistance. Later, the portal pressure begins to fall again and this fall is continuous to the end. Very little dilatation occurs during this last phase of the experiment. The falling arterial pressure may therefore be the cause of the fall in portal pressure.

In the other experiment (fig. 9) the behavior of the portal pressure was quite similar to that seen in the preceding case. After exposure of the intestines the pressure fell more or less steadily from 7.5 mm. Hg., reaching 5 mm. Hg. in the course of $2\frac{1}{2}$ hours. In the presence of a constant arterial pressure this fall may fairly be attributed to the peripheral constriction indicated by the inflow readings. During the next 3 hours the portal pressure was practically stationary. In the same period the arterial pressure for the most part was falling, while the inflow readings were quite variable; so much so that the data furnish no clear basis for the interpretation of the behavior of the portal pressure.

Our finding that exposure of the intestines, upon the whole, lowers the portal pressure, confirms Morison and Hooker (11), in most of whose experiments the animal was merely kept on the table under an anesthetic until the arterial pressure fell.

Fig. 8. Experiment 58. Shock by exposure and manipulation of the intestines. Arterial pressure, —•—•—; femoral inflow, ···• •···; portal pressure, —⊙—⊙—; jugular pressure, × ×. *a*, intestines exposed; *b*, partial clot in inflow cannula; *c*, clot removed; *d*, accidental injection of carbonate, respiration stopped but recovered in 8 minutes; *e*, changed to other femoral artery.

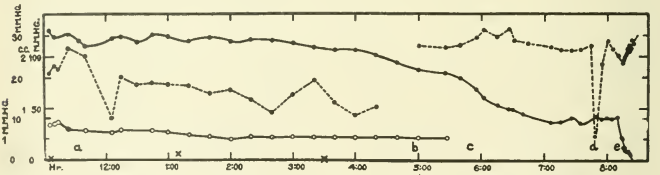


Fig. 9. Experiment 59. Exposure and manipulation of the intestines. Arterial pressure, —•—•—; femoral inflow, ···• •···; portal pressure, —⊙—⊙—; jugular pressure, × ×. *a*, abdomen opened; *b*, changed to other femoral artery; *c*, abdomen opened wider; *d*, injected strong carbonate; *e*, heart stabbed.

Inspection

In experiments of this kind one gains the impression that the larger veins of the splanchnic area do not contain more blood when the animal is in shock than when the intestines are first exposed. It is realized, however, that this method of estimating venous engorgement is too subjective to be of any great value, as is evidenced by the conflicting opinions of different observers.

When the intestines are first exposed, the peritoneum covering them is perfectly smooth and glistening. In time, however, fine beads being to form on it; these run together forming larger beads and eventually the fluid so formed streams off the bowels in considerable amounts. In several experiments, by placing the bowels on a tilted glass plate, the larger part of the fluid thus exuding was collected over definite periods. At times it dripped away at the rate of over 1 cc. per minute. The total amount formed probably never has exceeded 150 cc. This weeping of the peritoneal surface probably accounts in part for the concentration of the blood that occurs in this type of shock. The bowels also become boggy to the touch as though they were edematous. A certain amount of fluid must be abstracted from the circulation by this process also.

At post-mortem examination, petechial hemorrhages into the serosa are not uncommonly found. The mucosa of the intestines is deeply congested, of a deep, bluish-red color and the lumen of the bowel may contain some bloody material. On microscopical examination there is found relatively little hemorrhage into the mucosa. But the capillaries and the veins of the villi are tremendously distended with solid columns of corpuscles. It is undoubtedly this that gives the mucosa its deep red color, rather than the hemorrhage.

Summary

The immediate effect of exposure of the intestines usually is a vasoconstriction, often of a moderate grade (as compared with some other shock-producing procedures to be described later) affecting both the somatic and the splanchnic areas, with the possible exception of the parts directly traumatized; a fall or no change in the arterial pressure; a fall in portal pressure; and a slight, probably unimportant, change in the pressure in the right auricle. In view of the fact that the energy of the heart at this time is not reduced, this combination of effects seems to be accounted for best upon the basis of a reduction in effective blood volume; possibly also upon the basis of a dilatation in the traumatized region. The fact, however, that the portal pressure falls seems to exclude the latter, and to lend support to the view either that constriction is occurring in the traumatized area as well as elsewhere, or that exposure of the intestines mechanically interferes with the emptying of the veins of the splanchnic area, or that the distention of the splanchnic capillaries and venules with masses of corpuscles blocks

the entrance of blood into the portal area. Weeping from the serous surface of the injured viscera must tend toward reducing the blood volume.

The later consequences of exposure of the intestines are: continuation of the fall in arterial pressure; eventual diminution in vasomotor tone; and a slight rise, or a cessation of the fall, of the portal pressure. The absence of constriction, indeed, the development of an actual dilatation in the face of a low arterial pressure bespeaks a giving way of the vasomotor center. This giving way, presumably, is largely a reaction on the part of the center to the long-continued unfavorable conditions, among which must be included the low arterial pressure. The vasoconstrictor center, though, preserves a certain amount of tone and of reactivity to the end. The capillaries and veins of the villi of the intestines are greatly distended with blood.

SHOCK IN ANIMALS DEPRIVED OF SYMPATHETIC CONTROL[1]

Reflex inhibition of the tone of the vasoconstrictor center, affecting the splanchnic area in particular, may be regarded as one of the earliest of the hypotheses of shock founded upon experimental observation. If reflex inhibition of the constrictor center is the cause of the shock that results after exposure and manipulation of the intestines, it was felt that it should be more difficult to bring on shock in animals so prepared that there would remain but little opportunity for the play of such reflexes. It also has been maintained that shock is due to constriction of the portal radicles in the liver with consequent damming back of blood in the portal area.

A number of methods are available of putting these views to the test of experiment. One that we employed consisted in studying shock-producing procedures in animals after cutting the splanchnic nerves and removing the abdominal sympathetic chain. In this way the sympathetic connection of the abdominal viscera with the central nervous system is severed and the possibility of vasoconstrictor reflexes to them, to the parietal peritoneum, as well as to a large part of the soma, is excluded.

Two animals survived this rather difficult operation long enough for the immediate effects to wear off. Several weeks later the effects of traumatization were studied. The initial arterial pressures of these

[1] These experiments were done by one of us (H. S. G.) with the assistance of B. Landis Elliott.

animals were 80 and 100 mm. Hg.—not as low as was anticipated in the absence of the central vasoconstrictor control of practically all of the vessels below the level of the diaphragm.

Experiment 22. In this experiment, the data of which are collected in figure 10, the arterial pressure was falling when the registration of the pressure was begun, and by the time the abdomen was opened it had already practically reached the level of 50 mm. Hg. The fall in arterial pressure ("shock"), therefore, can scarcely be attributed in this case to the exposure of the intestines. Excepting possibly a slight increase early in the experiment and one aberrant reading, the inflow rate, followed in both the right and left femoral arteries, remained prac-

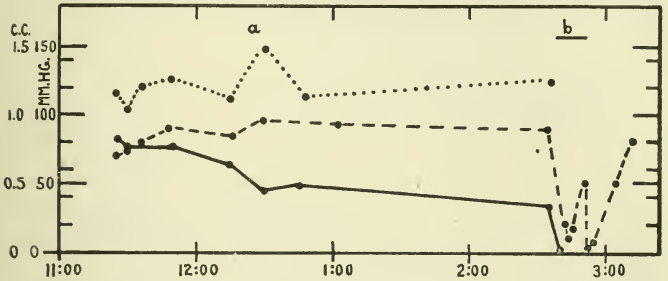


Fig. 10. Experiment 22. Shock after excision of abdominal sympathetic chain and section of the splanchnic nerves. Arterial pressure, —•—•—; right femoral inflow, ···•···•···; left femoral inflow, --•--•--. *a*, abdomen opened; *b*, adrenalin injected.

tically constant through shock and death. Evidently the preliminary operation had accomplished its end.

Experiment 26 (fig. 11). A pressure of 50 mm. Hg. was reached in something over 2½ hours after opening the abdomen, and the animal died about 3½ hours later. Early in the experiment each manipulation of the intestines (*b*, fig. 11) caused the arterial pressure to fall and the femoral vessels to dilate. This reaction presumably was mediated through the vasodilator mechanism; for the femoral area, the area in which the state of tone was followed, was isolated from the vasoconstrictor center by the preliminary operation. The afferent path of this reflex must, therefore, have been by way of the visceral fibers of the vagus nerve (28). Excepting these fluctuations, the inflow rate was

never, not even after death, more than 0.2 cc. per minute above or below a rate of 1.6 cc. per minute. Manipulation of the intestines in this case obviously hurried the animal into shock.

These two experiments prove that the preliminary operation had accomplished its object of freeing the lower parts of the body from vasoconstrictor influences. The level of 50 mm. Hg. was reached in one (exp. 22) about 1 hour after starting the record; in the other, in something less than 3 hours. Shock, therefore, developed faster in these animals than in any other of the animals in which it was induced by exposure of the intestines. The explanation of this fact may lie either in the rundown condition of the animals at the time they were experimented upon, or in the liberation of a large part of the body from vasoconstrictor control. If the latter is the correct explanation, it would

Fig. 11. Experiment 23. Shock by exposure and manipulation of the intestines after excision of the abdominal sympathetic chain and section of the splanchnic nerves. Arterial pressure,—•—•—; femoral inflow, ···•·····•·····•. *a*, abdomen opened; *b*, *b*, intestines manipulated.

constitute a reason for believing that the vasoconstriction usually present in the earlier stages of shock development in the case of undenervated animals is, as we have every reason for believing, an effort at compensation of something that is tending to lower the arterial pressure.

Furthermore, if the shock that is induced by exposure and manipulation of the intestines is due to the accumulation of blood in the splanchnic area, these experiments would serve to indicate that the accumulation is not attributable to the trapping of blood in the portal area through constriction (29), nor to a dilatation that comes about through loss of vasoconstrictor tone. The increased capacity of the peripheral vessels must develop either through the action of some *local* mechanism or through continuous central vasodilator stimulation. The latter alternative, to say the least, is highly improbable.

SHOCK IN EVISCERATED ANIMALS

Three animals have been followed through shock after removing the stomach, intestines and spleen. The operation was performed in such a way as to carry away with these organs a minimum of blood. The results in all three experiments were essentially alike (see fig. 12). The arterial pressure was well maintained for a while and then fell gradually, reaching the 50 mm. Hg. level in 3, 4 and 6 hours, respectively. Evisceration was always followed by a reduction in the femoral inflow rate lasting as long as 2 or more hours; in the case used here for purposes of illustration the constriction was the least marked and the most transient of all. At about the time the arterial pressure begins to fall rapidly, vasoconstrictor tone begins to give way and it becomes subnormal long before the animal dies. Death has occurred in from $4\frac{1}{2}$ to 7 hours.

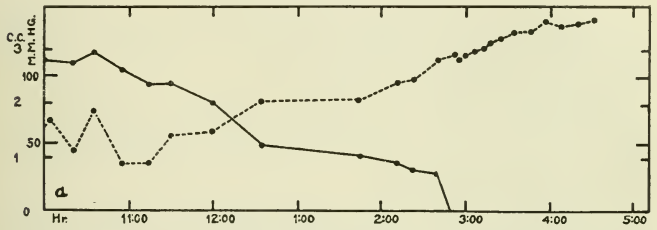


Fig. 12. Experiment 17. Failure of the circulation after evisceration. Arterial pressure, —•—•—; femoral inflow, ···•···•·· At *a*, the stomach, intestines and spleen were excised.

Discussion. These experiments were performed with the idea of testing the view, widely held, that the splanchnic area is primarily the location in which are enacted the events that lead to shock. This rôle has been assigned at various times to such processes as the accumulation of blood in the splanchnic and portal capillaries and veins due to splanchnic dilatation, the damming back of blood in the portal area as a result of an increase in hepatic resistance, loss of tone in the portal system, loss of blood "due to the reaction of the great delicate vascular splanchnic area to irritation—an acute inflammation of the peritoneum," (5), etc., etc. Some investigators have gone so far as to maintain that shock can be produced in animals solely by exposure and manipulation of the intestines.

The present experiments demonstrate not only that typical shock develops in animals in which none of these mechanisms has a chance to work, but in addition, that the vasomotor reactions and the duration of the several stages of shock development (see table 1) are essentially the same in the eviscerated as in the uneviscerated animal. Indeed, the vasoconstriction of the early stages of shock induction is more marked in the former even than in the latter. We do not wish to give the impression that exposure and manipulation of the viscera are not factors in the development of shock; the present experiments merely demonstrate that after the animal has been started on its way to shock, partly perhaps through the handling of the viscera that evisceration entails, none of the above-mentioned mechanisms is needed to hurry the animal along its downward course.

SUMMARY AND CONCLUSIONS

The changes in the circulation found in this investigation to occur during the development of shock brought on by exposure and manipulation of the intestines are as follows:

1. The arterial pressure at first may be lowered but little, if any. After some time, it may be after some hours, the pressure begins to fall, and this fall then continues more or less steadily, though often slowly, until the animal dies.

2. The changes occurring in the systemic venous pressure have been so small that they cannot be regarded as significant, excepting, perhaps, in demonstrating that cardiac failure has little, if anything, to do with the failure of the circulation.

3. The portal venous pressure falls continuously, though slowly, during the first 2 to 3 hours. It then ceases to fall or actually rises slightly until the arterial pressure has reached a comparatively low level, when the portal pressure again begins to decline.

4. The peripheral resistance, both somatic and splanchnic, at first practically invariably is increased. At about the time the arterial pressure begins to fall, or starts on its steady decline, and also at about the time the portal pressure starts to rise, the peripheral resistance begins to diminish and by the time the arterial pressure has reached the vicinity of 50 mm. Hg., the peripheral resistance practically invariably is below normal. But up to the time of death the vessels preserve some residual tone and the vasomotor center some, though slight, reactivity. In this respect the findings of Bartlett (9) are completely confirmed.

5. A considerable loss of fluid from the exposed bowel occurs as a result of transudation through the serous surface, and presumably into the tissues also. The capillaries and veins of the intestinal villi are greatly distended and tightly packed with red corpuscles.

6. No positive evidence has been obtained that the efficiency of the heart is impaired during the development of shock. Nevertheless, although the heart is capable of raising the arterial pressure as high as can the normal heart, we are inclined to believe that the heart in shock cannot maintain high pressures as long as can the normal heart.

7. The initial changes in the circulation can be explained best upon the assumption that the effective blood volume is reduced.

The loss of fluid into and through the tissues of the bowel and the sequestration of blood in the intestinal capillaries and venules suggest a mechanism through which a reduction in blood volume might occur.

But if this is the mechanism, the fact that after excision of the stomach, intestines and spleen the arterial pressure falls almost exactly in the same way as after exposure of and manipulation of the intestines, and the fact that the changes in peripheral resistance are also alike, necessitate assuming that blood is thus removed from circulation, not alone in the parts of the body directly traumatized, but elsewhere also.

It is impossible to determine, on the basis of the present experiments, what rôle, if any, the preliminary constriction plays in the subsequent failure of the circulation. But the experiments in which, by excision of the abdominal sympathetic chain and section of the splanchnic nerves, the entire posterior half of the body was removed from vasoconstrictor control, indicate, as might have been anticipated, that vasoconstriction is not essential to the development of a shock-like failure of the circulation.

Although at first the vasomotor center, as a rule, strives, by increasing the peripheral resistance, to compensate the processes that are tending to lower the arterial pressure, the reactivity of this center becomes subnormal long before the arterial pressure has fallen to the level of 50 mm. Hg.; this ultimate failure of the center to respond with a *strong* constriction to the stimulus of a low arterial pressure must be regarded as a secondary factor in the development of the low arterial pressure in this type of shock.

BIBLIOGRAPHY

(1) Erlanger, Gesell, Gasser and Elliott: Journ. Mo. State Med. Assoc., 1918, xv, 30; Proc. Amer. Physiol. Soc., This Journal, 1918, xlv, 546; Journ. Amer. Med. Assoc., 1917, lxix, 2089.
(2) Gasser, Erlanger and Meek: Journ. Mo. State Med. Assoc., 1918, xv, 232; Proc. Amer. Physiol. Soc., This Journal, 1918, xlv, 547.
(3) Erlanger and Gasser: Journ. Mo. State Med. Assoc., 1919, xvi, 98.
(4) Cowell: Journ. Amer. Med. Assoc., 1918, lxx, 607.
(5) Mann: Johns Hopkins Hosp. Bull., 1914, xxv, 205.
(6) Mann: Surg., Gynec. and Obst., 1915, xxi, 430.
(7) Archibald and McLean: Ann. Surg., 1917, lxvi, 280.
(8) Gesell: This Journal, 1919, xlvii, 468.
(9) Bartlett: Journ. Exper. Med., 1912, xv, 415.
(10) Sollmann and Pilcher: This Journal, 1910, xxvi, 233.
(11) Morison and Hooker: This Journal, 1915, xxxvii, 86.
(12) MacWilliam: Proc. Royal Soc., 1902, lxx, 109.
(13) Sollman and Pilcher: This Journal, xxix, 1911, 104.
(14) Keane, Mitchell and Morehouse: Circ. no. 6, Surgeon General's Office, 1864.
(15) Fischer: Samml. klin. Vorträge, 1870, x, 69.
(16) Meltzer: Arch. Int. Med., 1908, i, 571.
(17) Crile: Experimental inquiry into surgical shock, Philadelphia, 1899.
(18) Howell: Vaughan's Anniv. Vol., Ann Arbor, 1903, 51.
(19) Porter: This Journal, 1907, xx, 399.
(20) Porter and Storey: This Journal, 1907, xviii, 181.
(21) Porter and Quinby: This Journal, 1908, xx, 500.
(22) Lyon and Seelig: Journ. Amer. Med. Assoc., 1909, lii, 45.
(23) Sollmann and Pilcher: This Journal, 1910, xxvi, 233.
(24) Joseph and Seelig: Journ. Lab. and Clin. Med., 1915, i, 283.
(25) Muns: Proc. Soc. Exper. Biol. and Med., 1915, xii, 87.
(26) Wiggers: This Journal, 1918, xlv, 485.
(27) Janeway and Ewing: Ann. Surg., 1914, lix, 158.
(28) Fofanov and Tschalussov: Arch. f. d. gesammt. Physiol., 1913, cli, 543.
(29) Cannon: Boston Med. and Surg. Journ., 1917, lxx, 615.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

THIRTY-FIRST ANNUAL MEETING

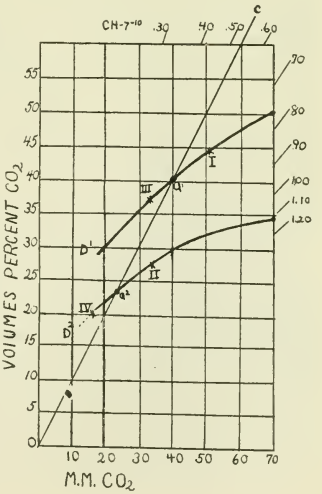
Johns Hopkins University, Baltimore, April, 24, 25, 26, 1919

IN MEMORIAM

Admont Halsey Clark
Ralph Edmond Sheldon
Frank Fairchild Wesbrook

The CO_2 dissociation curve as the complete expression of the acid base equilibrium of the blood. YANDELL HENDERSON and HOWARD W. HAGGARD.

In studying some problems of asphyxia and acidosis, we had occasion to plot a series of CO_2 dissociation curves. The data were from the blood of a dog which had received increasing amounts of HCl intravenously; and we made the following observation. With increasing dosage of acid and neutralization of the $NaHCO_3$ of the blood, the curves came to progressively lower levels. At the same time the points in the curves corresponding to the arterial blood, (the points *A1* to *A4* in the figure) fell further and further to the left. Furthermore when these arterial points were connected they were found all to lie on or close to a diagonal straight line, and this line when continued passed through the intersection of ordinate and abscissa.



Consideration of these graphic relations shows that such a line expresses the same C_H at every point throughout its length, for all points in it indicate the same ratio of dissolved CO_2 to combined CO_2. We term it the O-C line, or line of C

values. It expressed the inherent sensitiveness of the respiratory center to its chemical control. It demonstrates that the C_H and not the total CO_2 in the blood (i.e., not the concentration of HCO_3 ions) is the hormone of respiration. It enables us to modify the Haldane-Priestley law of respiration thus: The volume of the pulmonary ventilation varies inversely as the amount of alkali which in the condition of the blood at the time will afford the normal C_H. In other words, normal respiration adjusts the tension and content of CO_2, i.e., alkaline reserve, in the arterial blood to values corresponding to the point of intersection of the dissociation curve, whether high or low, with the O-C line.

Study of the CO_2 diagrams of many types of experiment shows that instead of merely two possible abnormal conditions, acidosis and alkalosis, there are theoretically four: *a*, high $NaHCO_3$ and high C_H; *b*, low $NaHCO_3$ and high C_H; *c*, high $NaHCO_3$ and low C_H; and *d*, low $NaHCO_3$ and low C_H. Regarding these four conditions, *a* probably occurs only in morphin poisoning and related conditions; *b* is the condition now termed uncompensated acidosis; *c* is perhaps what is now termed alkalosis; *d* is a condition which has not heretofore been distinguished from *b*. In its nature and proper mode of treatment *d* is however profoundly different from *b*. (The C_H scale given in the figure is merely illustrative).

Studies on the responses of the circulation to low oxygen tension. I. Types of variation in blood pressure and heart rate. CHAS. W. GREENE.

Schneider[1] has established the general type of compensation made by men undergoing the Air Service test to low oxygen tension in which the percentage of oxygen is gradually decreased during rebreathing. The heart rate is increased. The acceleration is slight at first beginning at from 16 to 12.2 per cent oxygen and growing more and more pronounced to the end of human endurance. The total increase may reach forty beats or more in an adequate reactor. In the ideal type systolic pressure holds till late in the test, till the oxygen is lowered to 14 to 9 per cent, then gives a gradual rise. The diastolic holds or rises slightly in the early stage and shows a fall at the low oxygen level often very marked at the end of the test. These are Schneider's types and he has published data including type charts in the Manual of the Medical Research Laboratory of the Air Service, War Department, 1918.

A number of marked variations from the types are selected and presented here for discussion, both from the cardiac group and from the vasomotor group. These types indicate that vascular compensation may be accomplished by a number of variations from the type. The following are listed: *a*, A sharp increase both in heart rate and systolic pressure at the beginning of the test, which hold at the new level and show the typical heart rate and blood pressure compensations to the end of the test. *b*, A high initial blood pressure response that holds on the new level with more than the average increase in the late stage of the test, but with slight compensation in heart rate. *c*, The converse of

[1] Journ. Amer. Med. Assoc., 1918, lxxi, pp. 1382-1400.

type *b*, a great increase in heart rate beginning early in the test and progressing to the end but coupled with little or no systolic compensation and often with early diastolic fall. *d*, Unusually high systolic pressures that fail in compensation and tend to collapse. The possible significance of these types is discussed.

Variations in alveolar air at low pressures. Brenton R. Lutz.

A preliminary report of twenty-four cases in which the alveolar air tensions were determined under conditions of rapid reduction of atmospheric pressure is presented. Reduced barometric pressures were produced in one of the low pressure chambers at the United States Army Medical Research Laboratory, Mineola, L. I., N. Y., at a rate simulating an air-plane ascent, that is, 352 mm. Hg. or 20,000 feet were attained in twenty minutes. Alveolar air samples were taken in Henderson alveolar air sampling tubes after the expiratory method of Haldane and Priestley. These were analyzed for oxygen and carbon dioxide with a Henderson-Orsat gas analyzer. Samples were obtained at sea level, 4,000 feet, 8,000 feet, 12,000 feet, 16,000 feet and 20,000 feet.

The average curve for oxygen and carbon dioxide shows a fall present at the fourth minute and continuing until the ascent was completed. The average sea level values were 103 mm. Hg. for oxygen and 39.6 mm. Hg. for carbon dioxide. The average values at 4,000 feet were 83.7 mm. for oxygen and 37.0 mm. for carbon dioxide; for 8000 feet, 66 mm. and 36 mm. respectively; for 12,000 feet, 53.2 mm. and 33.5 mm.; for 16,000 feet, 42.6 mm. and 31.3 mm.; for 20,000 feet, 34.8 mm. and 30 mm. These twenty-four cases indicate that in an ascent at the rate of 1000 feet a minute both the alveolar oxygen tension and the alveolar carbon dioxide tension begin to fall very early, reaching at 20,000 feet, 34.8 mm. Hg. for oxygen and 30 mm. Hg. for carbon dioxide.

Variations in respiration volume and oxygen consumption during the reduction of the oxygen tension and during short exposures at the reduced oxygen level. Max M. Ellis.

The changes in respiration volume and oxygen consumption of men at sea level, during the reduction of oxygen and during the first ten minutes at the new level of reduced oxygen, were obtained from a rebreather containing 54 litres of air. The carbon dioxid produced by the subject was removed by a potassium hydroxid cartridge.

The general response in volume was an increase in the per minute volume, as compared with the sea level volume, during the reduction of oxygen, followed by a decrease in the volume moved while at the new level of reduced oxygen. The per minute volume at the reduced oxygen level remained consistently higher than the sea level volume. The increase in volume moved was initiated in most cases before 17.5 per cent oxygen was attained.

The general response in oxygen consumption was the same as that in volume, a rise in the per minute oxygen consumption above the sea level

value during the reduction of oxygen, followed by a fall in the per minute consumption at the new level of reduced oxygen. The value at the reduced oxygen level was higher in the majority of cases than the sea level value.

The responses were more profound in subjects carried to equivalent altitudes above 10,000 feet.

Phenomena following indirect concussion of the skull. T. S. Githens and S. J. Meltzer.

Concussion was produced in completely etherized dogs by a weight falling on a board 4 cm. thick laid on the head in front of the occiput. Complete unconsciousness continued till the end of the experiment.

We wish to report briefly the following phenomena which were observed.

Brain. The lid and corneal reflexes were never lost. The eyes showed nystagmus for an hour or so after the concussion and afterward were moved in an apparently normal manner. Stimulation of exposed sensory nerves (e.g., supra-orbital) caused no sign of pain and no influence on respiration or other reflex effect even after four hours. A new nose-licking reflex was noted. If the nasal septum were pinched by forceps, no response occurred during the pressure, but on releasing it the tongue was protruded and licked the nose. This was seen in all the dogs in which it was systematically looked for.

Medulla. The medullary centers were surprisingly little affected. The blood pressure was usually very high soon after the concussion, and the respiration was noisy and irregular, soon becoming normal. Section of the vagus nerves interfered with respiration as in decerebrate animals. After section of one vagus, stimulation of the central end caused active expiration or expiratory stoppage.

Cord. For the first hour or so there was complete paralysis with loss of all reflexes and responses. Later, circulatory, respiratory and spinal reflexes returned and often became exaggerated. Many of the dogs showed a generalized tremor beginning within an hour and lasting until the animal was killed several hours or even a day later. In three dogs there was some indication of returning pain sense after two or three hours. Stimulation of the sciatic nerve after return of reflexes caused stiffness of one or more legs, often associated with emprosthotonos of the abdominal region and opisthotonos of the cervical; the pelvis being drawn forward and the head backward. Similar phenomena were obtained from stimulation of the brachial plexus. The muscles of the head itself were never involved. Stimulation of the cervical nerves never caused any response except active expiration.

Lesions. Only gross study of hemorrhage was made. The only characteristic lesion was a hemorrhage into the upper part of the cord extending from the calamus to the second or third cervical nerves. This was associated with laceration of the gray matter extending from the central canal into the dorsal horns. There was almost no hemorrhage within the skull.

Physiological effects of air concussion. D. R. Hooker.

Through the assistance of the National Research Council and the coöperation of the Ordnance Department of the Army, facilities were obtained for the study of the physiological effects of air concussion in animals. For the most part, anesthetized dogs were used. They were exposed at varying distances in front of large calibre rifles and if the concussion pressure amounted to approximately twenty atmospheres, a shock-like result was obtained, i.e., the arterial pressure fell immediately (five minutes) from a control value of 100–150 mm. Hg. to 40–60 mm. Hg.

After this condition was established, the animals remained alive for one to ten hours and efforts were directed to a study of some of the concomitant phenomena. Both the pulse and respiration were accelerated presumably as the result of the lowered arterial pressure. In a single case the blood catalase was not reduced. The alkaline reserve was normal immediately after exposure but fell markedly in the course of an hour. No air bubbles could be found in the vascular system nor was there evidence of intravascular fat sufficient to account for the results. The medullary reflexes, so far as tested, were all normal. That is to say, respiratory, cardiac and vasomotor responses were readily elicited upon sensory nerve stimulation. The eye-lid reflex was active.

The most interesting result was found in the venous side of the circulation. The venous pressure invariably showed a fall. This pressure was down immediately after exposure and continued subnormal until death, an observation which suggests, in view of the efficiency of the vasomotor mechanism, an inadequate venous filling as a causative phenomenon of the condition. This suggestion is strengthened by the appearance of the abdominal veins. If examined late in the condition, the veins were small, dark and relatively inconspicuous. If examined shortly after exposure, they were obviously very much enlarged. The fact that this enlargement of the abdominal veins accompanied a low venous pressure may be interpreted as indicating a failure of the venopressor mechanism. The late appearance of the veins, according with clinical observation, is doubtless due to transudation of blood plasma.

The sole pathological lesion found was a scattered hemorrhagic condition of the lungs, as has been noted by Crile. It is conceivable that this tissue injury released a toxic substance adequate to establish shock. But the extent of injury did not run parallel with the symptoms; in fact, many animals with an extreme grade of pulmonary involvement failed to show circulatory collapse.

On the general expression of the law of cicatrization of wounds. P. Lecomte du Noüy.

The cicatrization of surface wounds is a phenomenon which is likely to involve quite a great number of elementary phenomena. But as all the latter are related to each other by definite, although unknown, laws, it seemed that the resulting phenomenon, cicatrization, could be studied in the same way as the ordinary physico-chemical phenomena, in order

to see whether a general law could be assumed. To comply with this purpose, a technique had to be developed for measuring accurately the area of wounds. Sterilized cellophane was applied to the wound, and the edge was outlined with a wax pencil. This drawing was transferred in ink to an ordinary sheet of paper, and the area was measured by means of a planimeter. A curve was obtained by carrying the area, in square centimeters, in ordinates, and the time, in days, in abscissae. A great number of experiments on animals and on human beings during the war, brought forward a great number of interesting facts, which we shall try to review rapidly.

At first it was shown that the cicatrization of a wound was due to two different factors: contraction and epithelization, the first one being the most important.[1] When the wound is kept aseptic, the curve representing the cicatrization plotted as above said is perfectly geometric, and can be expressed by a mathematical formula. In this formula enters a certain coefficient, which we called "i," or index of cicatrization, which is inversely proportional to the age of the patient or of the animal. This means that by making one single measure of the area of the wound, and provided the age of the patient is known, one can draw the curve representing the normal healing of a wound.[2] Beside its scientific interest, this has many practical advantages. First: It allows the physician to compute the total time which is necessary for the wound to heal. Second: It gives indications as to the "physiological age of the patient" which, indicated by his own curve, may differ from his real age. Third: When it has been proved that a wound heals normally, comparison between the calculated and the observed curve will indicate the real value of the substance which is used on the dressing.[3]

This latter technique has been extensively used during the war to ascertain the action of antiseptics. It was found that most of them were irritating and interfered more or less with the normal process of cicatrization. Having a daily witness, a standard curve, to which the observed curve was always compared, it was a very easy and rapid work to determine the value of the so-called "cicatrizing substances." We found that no such thing exists so far. The ideal condition of perfect and most rapid healing is realized when the wound is kept practically sterile, or deprived of pathogenic microbes such as cocci, diplococci, streptococci and so forth. These conditions were realized with the Carrel method.

A device for injecting fluids intravenously. P. Lecomte du Noüy.

This device does away with piston, gears and valves. The only part which has to be sterilized is the piece of rubber tubing which is used with the needle. It aspirates and compresses, up to 30 or 35 cm. of mercury. It is controlled either by hand or by motor, and the flow may be regulated from 2 or 3 cc. a minute, or less, up to 50 cc. and more.

[1] Carrel and A. Hartmann: Journ. Exper. Med., November 1, 1916.
[2] du Noüy: Journ. Exper. Med., November, 1916; May, 1916; April, 1919.
[3] Carrel, du Noüy, Carrel: Journ. Exper. Med., August, 1917.
See also: Tuffier and Desmarres: Journ. Exper. Med., February, 1918.

A new form of the Mohr's specific gravity balance for small quantities of liquids. P. Lecomte du Noüy.

This balance gives the $\frac{1}{10}$ of a milligram by the means of a rider. The plunger being about 1 cc., the numbers read on the weights indicate the density of the liquid tested, if the apparatus has first been standardized with water.

An apparatus for measuring rapidly and accurately the surface tension of all liquids. P. Lecomte du Noüy.

It is based upon the well-known adhesion principle, of a disc or ring or square, brought into contact with the liquid. The tension is measured by the torsion of a wire, which torsion is simply read on a dial.

The behavior of the centriole and the centrosphere in the degenerating fibroblasts of tissue cultures. Warren H. Lewis.

In the normal healthy fibroblast the centriole lies close to one side or one end of the nucleus. The mitochondria do not appear to have any definite relation to it since they are arranged more or less parallel to the long axis of the cell. A few scattered granules are usually present. As degeneration progresses the granules increase in number and tend to accumulate about the centriole. As the process goes on the accumulation of granules about the centriole increases and each granule usually becomes surrounded by a vacuole.

Coincident with this accumulation of granules, the mitochondrial threads and rods become more or less radially arranged about the centriole. As the number of vacuoles and granules increases there develops about the centriole a clear area, the centrosphere, which gradually increases in size and may become as large as the nucleus. The centrosphere is usually quite free from granules, vacuoles and mitochondria. Protoplasmic strands, in which the mitochondria lie, extend from the centrosphere toward the periphery and often give a radial arrangement as the vacuoles. As degeneration proceeds the mitochondrial threads often break up into short rods and granules which may change into vesicles.

The small degeneration vacuoles and granules often exhibit quite active movements different from and more extended than the ordinary mitochondrial movements. The direction is from the region of the centriole or centrosphere to the periphery of the cell or *vice versa*. Are such movements due to cytoplasmic currents or to the metabolic interchanges between vacuoles or granules and the cytoplasm?

The orientation of the various structures about the centriole and centrosphere in degenerating fibroblasts suggests that the centriole and not the nucleus is the center of metabolic activity of the cell, the dynamic center of the cell according to Boveri.

This increase in the size of the centrosphere is probably a compensating reaction on the part of the cell induced by an upset in the normal metabolic balance between centriole and periphery through the accumulation of granules and vacuoles.

A comparison of the influence of secretine and the antineuritic vitamine on pancreatic secretion and bile flow. CARL VOEGTLIN and C. N. MYERS.

An alcoholic extract of dried brewers' yeast, after being submitted to the same purification as described for the preparation of Funk's vitamine fraction, causes, when injected intravenously, a marked increase in pancreatic secretion and bile flow. Extracts of the mucosa of the duodenum prepared in the same manner still possess their characteristic action on pancreatic secretion and also relieve the paralytic symptoms of polyneuritic pigeons.

Later experiments by Voegtlin have furthermore shown that liver, heart muscle and milk also contain a substance which stimulates pancreatic secretion and increases the flow of bile. The presence of the antineuritic vitamine in such extracts had been previously demonstrated. Hence it is concluded that secretine is not only located in the intestinal mucosa, but also in at least two other organs of the body.

The failure of previous workers to extract secretine from these organs is probably due to the use of improper chemical methods.

From these and other observations it is concluded that secretine and the antineuritic vitamine are very closely related, if not identical.

Electric conductivity in relation to shock and exhaustion. GEORGE W. CRILE and HELEN R. HOSMER.

In previous researches histologic and clinical findings have indicated that in exhaustion and shock there exists an apparent synthetic relationship between certain organs—notably the central nervous system and the liver. The work of Lillie, Osterhaut and Galleotti and other biophysical chemists suggests that further evidence of the rôle of these organs in exhaustion and shock might be secured by measurements of their electric conductivity.

The research of which this paper presents a preliminary report was therefore undertaken, the first measurements being made by Capt. G. B. Obear of the Case School of Applied Science, and the work continued by Miss Helen R. Hosmer.

Electric conductivity measurements of 107 rabbits have been made. This number includes 24 normal rabbits and groups of at least 6 rabbits, each of which were subjected to exhaustion from each of the following causes: prolonged insomnia, surgical shock, fright, infection, adrenalin injection, thyroid feeding, hydrochloric acid injection. We have studied also the effect of sleep and rest for different periods, of morphin alone and in the presence of infection, of prolonged ether anesthesia, of prolonged nitrous oxid-oxygen anesthesia.

These preliminary studies, *a*, have shown consistent antithetic changes in the brain and the liver; i.e., in every type of exhaustion the electric conductivity of the brain was decreased below the normal and the conductivity of the liver was increased above the normal; *b*, have indicated a general range of electric conductivity of the various organs and tissues.

In general, like changes under the same conditions were observed in the cerebrum and the cerebellum—these changes being most marked in the cerebellum. This finding is consistent with our observations of the comparative histologic changes in the cerebrum and cerebellum produced by exhaustion from the same causes.

The dependence of respiratory activity upon conditions in the central mechanism. F. H. Pike, Helen C. Coombs and A. Baird Hastings.

While respiratory activity may be automatic under certain conditions,[1] it is almost certainly not wholly automatic under the ordinary conditions of life. Afferent nerve impulses affect not only the frequency of the respiratory movements, but also their form. In this latter function the vagus and the afferent roots of the spinal nerves enter into the problem. We strongly support Baglioni's[2] contention on this point. The functional relation of the dorsal roots of the thoracic nerves to the efferent roots to the intercostal muscles may be regarded as merely a special phase of the wider functional relations of afferent to efferent roots as set forth by Sherrington. We have shown that strychnine convulsions of the respiratory muscles fail after section of the dorsal roots of the thoracic nerves, just as they fail in the skeletal muscles generally after section of the afferent spinal root.[3]

Other factors, generally considered to be related to the concentration of the hydrogen ions in the circulating blood, enter into the problem. The regulation of the respiration becomes at bottom a problem of the conditions and manner of stimulation of the nervous elements of the central respiratory mechanism. It has been shown[4] that restriction of the volume of blood flowing through the medulla oblongata increases the minute volume of the respiration. We have shown that there is also a reduction in the total carbon dioxide of the blood plasma. Hyperpnoea, or even dyspnoea, follows partial or complete occlusion of the four cerebral arteries in the cat, and the concentration of the carbon dioxide in the plasma falls rapidly. Restoration of the cerebral circulation is followed by a prompt cessation of the hyperpnoea and a rise in the carbon dioxide of the plasma.[5]

The changes in respiration on partial or complete occlusion of the cerebral arteries are opposite in direction to what one would expect if the change in the concentration of carbon dioxide and the related change in the concentration of the hydrogen ions were the only factors concerned. We can see no obvious source of large quantities of acid in these experiments, and we are not convinced that a low concentration of carbon dioxide in the blood is an unfailing indication of acidosis.

[1] MacDonald and Ried: Journ. Physiology, 1898, xxiii, 100; Stewart and Pike: This Journal, 1907, xix, 328; Winterstein: Arch. f. d. gesammt. Physiol., cxxxviii, 159.

[2] Baglioni: Zur Analyse der Reflexfunction, Wiesbaden, 1907.

[3] Moore and Oertel: This Journal, 1899, iii, 45.

[4] Geppert and Zuntz: Arch. f. d. gesammt. Physiol., 1888, xlii, 218.

[5] Proc. Soc. Exper. Biol. and Med., 1919, xvi, 49.

A consideration of our experimental data leads us to the conclusion that it is not merely the condition in the circulating blood, which Haldane emphasized, but also the set of conditions in the medulla oblongata itself, dependent upon at least four different factors, which determines the degree of respiratory activity. It is our present view that respiratory activity is regulated in such a way as to maintain the usual or normal set of conditions as nearly constant as possible. The activity of the respiratory mechanism during the restriction of blood flow through the medulla is independent of any known antecedent change in the group of afferent nerve impulses and, to this extent, is automatic. But in the normal action of the central mechanism of respiration, there is a summation of all the various forms of stimulation, chemical and nervous, by the nerve cells present therein.

Observations on decerebrate cats. Lois Fraser, R. S. Lang and J. J. R. Macleod.

It has been shown by one of us that the respiratory volume remains fairly uniform for three or four hours in most decerebrate cats after the effects of the initial etherization have passed off. Hyperpnoea usually develops later and this is accompanied by a depression in the alveolar CO_2 percentage and a lowering of the arterial blood carbonate. In the present investigation it was intended to study the effect produced by causing the animal to breathe through valves into a closed system of wide-bore tubing provided with soda-lime absorption bottles and a Gad-Krogh spirometer. The object was to find the exact degree of oxygen deficiency at which increased pulmonary ventilation would supervene, and to seek for evidence as to whether this hyperpnoea is associated with changes in the alveolar air (R.Q.) and blood (arterial-blood CO_2 + PH) that could be attributed to the appearance in the organism of unoxidized acid.

Although a sufficient number of data has not as yet been collected to answer these questions, the interesting observation has been made that a decided hyperpnoea develops a few minutes after connecting the animal with the respiration tube and spirometer. The evidence of this hyperpnoea is obtained partly by observing the tracing produced by the spirometer, or by a tambour connected with the tracheal tube before the valves, and partly by analysis of the alveolar air. The results with the latter have invariably shown a decrease in the percentage of CO_2 and a decided rise in the respiratory quotient, while the oxygen in the inspired air is still well above 15 per cent.

Similar results were obtained in two experiments in which an excess of oxygen was added to the system before causing the animal to respire into it. There can be no doubt that the slight resistance offered to expiration has served to increase the excitability of the respiratory center. This may be due to afferent stimuli set up either by the more distended condition of the alveoli (acting on the center through the vagi) or by the distended condition of the thoracic walls (acting through the muscle nerves). The following figures will serve to illustrate the results.

NUMBER OF EXPERIMENT	RESPIRATORY VOLUME IN CUBIC CENTIMETERS PER MINUTE		RESPIRATORY QUOTIENT		O_2 PERCENTAGE IN ALVEOLAR AIR DURING OBSERVATIONS
	Before	During	Before	During	
XXIV	1080	1845	0.82	1.63	14.4
XXVIII	600	1200	0.69	1.58	15.34
XXVI	905	2000	0.85	1.02	15.25
XXIX	1120	2250	0.85	1.27	17.7

Changes in hydrogen ion concentration in the sweat and urine following exercise and heat. GEORGE A. TALBERT.

Data were presented in support of the following conclusions:

1. That intense exercise of only twenty minutes duration will increase the acidity of the urine.
2. That heat produced in a sweat cabinet will in a large majority of cases increase the acidity of the urine of the subject.
3. That the sweat of man whether produced by exercise or heat is most invariably acid.
4. That heat sweat is always more acid than work sweat.

The effect of shattered hemoprotein on the colorless blood corpuscles. CLYDE BROOKS.

A protein substance has been isolated from the blood fibrin of the ox which though there is no toxic action produces an artificial leucocytosis. This substance is prepared by the methods previously outlined.[1] The main purpose has been to separate the protein from the blood without destroying the labile molecules which are apparently active in producing the leucotonic action. These labile molecules may be enzymes, vitamines or various adsorbed bodies which might cling to the fibrin as it is first coagulated.

When this protein is injected intravenously or intramuscularly it induces an artificial leucocytosis. There is no apparent preliminary sinking of the number of polymorphonuclear leucocytes immediately following the injection, but a steady upward trend. After several hours new or young leucocytes, and also atypical leucocytes appear in the blood stream, very much as after the injection of typhoid vaccine.[2] There is no reaction (chill followed by elevation of temperature, etc.) following the injection of the protein.

On the relation between the velocity of Beta rays from radium and their physiological effect. ALFRED C. REDFIELD.

The physiological effects upon the eggs of nereis of homogeneous groups of Beta rays of different velocities are proportional to their ability to ionize air. Beta rays of low velocity produce a greater amount of physiological change than the same number of rays of high velocity.

[1] Science, February 23, 1919; N. Y. Med. Journ., March 15, 1919.
[2] Cowie: Arch. Int. Med., 1919; Journ. Amer. Med. Assoc., April, 1919.

These conclusions are consistent with, but do not prove the view that the physiological effects of radiations from radium and x-rays are due to the production by them of an ionization of protoplasm.

The concentration of alcohol in the tissues of hens after inhalation. T. M. Carpenter and E. B. Babcock.

Hens weighing about 2 kilos were placed in a 216 liter air-tight chamber saturated with alcohol vapor over periods varying from two to twenty-nine hours. The carbon dioxide was absorbed by partially slaked lime and oxygen was supplied from a well counterpoised spirometer connected to the chamber. The activity of the individual hens was registered by the method devised by Benedict. The animals were killed immediately after exposure and the alcohol distilled from the various tissues and determined by the Nicloux method. The amounts of alcohol per gram of the whole body and length of exposure are given in the following table:

Alcohol in hens after inhalation of its vapor (Milligrams of alcohol per gram of whole body)

HEN NUMBER	HOURS EXPOSED	ALCOHOL PER GRAM	HEN NUMBER	HOURS EXPOSED	ALCOHOL PER GRAM	HEN NUMBER	HOURS EXPOSED	ALCOHOL PER GRAM
42	16	0.03	10	27½	0.64	11	15½	2.19
37	2	0.06	29	16	0.81	13	15½	2.24
38	2	0.18	46	16	1.13	3	15½	2.26
39	2	0.20	33	8	1.38	44	16	2.35
25	3	0.25	24	8	1.51	35	4	2.43
21	4	0.27	40	16	1.68	9	29	2.51
5	16½	0.28	34	4	1.73	45	16½	2.77
43	16½	0.33	48	16	1.98	8	29	3.27
41	16	0.34	47	16	2.06	6	29	5.67
36	4	0.38	15	8	2.13			

Hens 5 and 10 were very active practically the entire time during their stay in the chamber.

Hens 40 to 48 were stimulated to activity intermittently with varying degrees of success by means of leads from an induction coil. All hens containing more than 1.5 mgm. of alcohol per gram of body substance showed signs of intoxication.

The relative concentration of alcohol of different tissues of 12 hens was on the average as follows:

Blood	100
Heart and lungs	86
Brain	84
Kidneys	79
Alimentary tract	78
Muscle	78
Spleen	75
Liver	72
Skin	56
Fat	11
Whole body	70

Three dead hens placed in the chamber for sixteen hours gave 1.15 to 1.22 and 2.09 mgm. of alcohol per gram. Diffusion through the skin played a rôle in the experiments with live hens as well as inhalation.

The metabolism of bile acids. M. G. Foster, C. W. Hooper and G. H. Whipple.

Very little work has been reported recently dealing with the bile salts, for the methods of analysis available are not only inexact but very laborious. Many substances present in bile are known to interfere seriously with the various published methods for bile acid determination. It is generally accepted that dog's bile contains only taurocholic and taurocholeic acids with, at the most, only a trace of glycocholic acid. The presence of 10 per cent glycocholic acid in the bile acid fraction will not modify the figures computed as taurocholic acid by our method.

A new method has been devised which consists in simple hydrolysis of mucin-free bile with sodium hydrate at the temperature of boiling water. Taurocholic acid is completely split into taurine and cholic acid. The taurine is estimated by the Van Slyke amino nitrogen technique, as we have found that taurine gives up all of its amino nitrogen in three minutes. By this method taurocholic acid, added in known amounts to bile, can be quantitatively recovered. Other substances known to be present in bile do not interfere with this method, which has an error not exceeding 6 to 8 per cent.

All observations were made upon bile fistula dogs which were in a normal condition of activity, weight and appetite. These dogs were kept on a careful routine of diet, exercise and bile collection. Six-hour collections were made daily with a thirty-minute period of preliminary drainage of the bile fistula. Like many other workers, we observed great variations in the excretion of bile acids in these bile fistula animals, but we found it possible to reduce these fluctuations in large measure by *diet control*. During fasting periods, these dogs approach a uniform level of bile acid elimination, but even in such periods there are fluctuations which we cannot explain. The elimination from hour to hour during the day is fairly uniform for any given dog, but the daily output may vary a good deal. As a rule the bile acid excretion is a little higher in the forenoon than in the afternoon.

Diets rich in meat proteins raise the level of bile acid output to a maximum, and periods of fasting will depress the bile acid output, not to zero, but to about 25 to 35 per cent of this maximum. Sugar feeding will depress the bile acids below the fasting level of excretion. It is evident that there is an endogenous as well as an exogenous source of bile acids. After periods of fasting, a diet rich in meat proteins will not lift the bile acid output to the usual maximum level, and may raise it scarcely at all for several days. This may indicate a deviation of certain food elements from bile acid formation to tissue protein construction. This is only one of many suggestive points which seem to indicate an important relationship between the *body protein* metabolism and the *bile acid* metabolism.

Small amounts of bile given by mouth show a prompt excretion of the contained bile acids within three to four hours. If not over 2 grams of taurocholic acid are given by mouth, we may expect about 90 per cent elimination in the bile within four hours. Large amounts of bile acids given by mouth may prolong this elimination over many hours or even days and the cholagogue action may last even longer than the increased bile acid excretion. By giving sugar with bile acids by mouth, the cholagogue action can be inhibited and a maximum concentration of bile acids in bile (7 to 9 per cent) may be attained in this way. Bile given at night in moderate amounts has no influence upon the bile acid output of the following day. Bile exclusion by means of a night abdominal binder to prevent any licking of the fistula does not modify the curve of bile acid elimination in our experiments. This holds for all diet experiments as well as for periods of fasting.

Taurine intravenously or by mouth does not influence bile acid elimination. Taurine plus cholic acid by mouth reacts exactly as does taurocholic acid and causes a cholagogue action with great increase in output of bile acids. Cholic acid alone gives a cholagogue action and with a rich nitrogen diet causes a great rise in bile acid output. This is not true during fasting periods and we must assume that this increase in bile acid production is limited by the amount of available taurine either from food or tissue protein. Cystine injection alone does not influence bile acid excretion but combined with cholic acid feeding reacts exactly as does taurine. This confirms other work to show that taurine is derived from the amino acid cystine.

Cholic acid is of unknown origin in the body. It gives certain chemical reactions like cholesterol, turpentine and camphor. Cholesterol feeding and the feeding of large amounts of red blood cells did not influence the output of bile acids in bile. Intravenous injection of large amounts of laked red blood cells was also negative. Feeding terpene hydrate and camphor alone or combined with taurine gave no positive results. Casein, caseinamino acids, beef extract (commercial) and gelatin feeding have no influence upon the bile acid excretion. Our experiments seem to indicate the derivation of cholic acid either from certain meat proteins in the food, or from the tissue proteins, or both. Cholic acid is normally the limiting factor which determines the level of bile acid excretion in bile fistula dogs.

On the compensation of ocular and equilibrium disturbances which follow unilateral removal of the otic labyrinth. Demonstration.[1] ALEXANDER L. PRINCE.

The ocular and equilibrium disturbances which follow unilateral destruction of the otic labyrinth in higher vertebrates are of short duration and are ascribed to the unbalanced activity of the intact labyrinth.[2] In the cat, these disturbances consist of deviation of the

[1] A report of these experiments appeared in the Proc. for Exper. Biol. and Med., 1917, xiv, 133.

[2] Wilson and Pike: Phil. Trans. Royal Soc., London, 1912, Series B, cciii, 127.

eyes toward the side of the lesion, accompanied by nystagmus, and of a tendency to roll, fall and execute circus movements, also in the direction of the injured side. These symptoms gradually diminish in intensity and have disappeared within forty-eight to seventy-two hours after operation.

The question arises as to the mechanism concerned in the disappearance of the disturbances occasioned by the removal of the labyrinth.

In the animals demonstrated, decerebration was performed, several weeks after complete recovery from the effects of the labyrinthine operation, by section of the brain stem just anterior to the corpora quadrigemina. This procedure is immediately followed by deviation of the eyes to the side of the lesion and recurrence of the symptoms of disturbed equilibration.

In view of these observations, the disappearance of the ocular and equilibrium disturbances following unilateral removal of the labyrinth is attributed to the activity of a compensatory mechanism. The nervous paths concerned in the process of compensation may be roughly localized in the cerebrum above the level of the corpora quadrigemina.

A new type of rebreather. Demonstration. Carl N. Larson.

To obviate the immobility of the Henderson-Pierce rebreather the capacity of which is controlled by intake and outlet of water (requiring water and sewer connections) a new type of rebreather has been devised and installed in the Medical Research Laboratory of the Air Service, War Department. The new form is light and portable, it is free from water system connections, it can be graduated so that the oxygen percentage of the inclosed air is known at every moment and checking gas analyses can be made at any moment during the progress of a test. The rate and volume of the respirations and of the oxygen consumption can be taken directly by the automatic recorder devised by the author and from the table of constants of the instrument.

A brief description of the new features of the machine is as follows: The air chamber is of two pieces suitably framed and supported. The lower piece is a double cylinder with common base and open at the top. Each is 15 inches high and the inner cylinder 14 inches in diameter. A space of 1 inch filled with water serves as a water seal. The upper part of the cylinder is single, open at the bottom to engage with the lower cylinder in the water seal. It is 15 inches tall by 15 inches in diameter. The movable lower portion is raised into position by its crank shaft and ratchet. The capacity of the instrument can be varied between 40 and 80 liters. In the center of the top section a water sealed spirometer of 6 liters capacity is countersunk. It opens directly and widely into the air chamber insuring even mixture of the contained air during the rebreathing. The top of the tank carries openings for inspiratory and expiratory tubes, controlled as in the Henderson-Pierce rebreather type. Two tubes controlled by valves open directly into the air chamber, one for introducing nitrogen, oxygen or other gases and the other for withdrawing samples for analysis. The whole apparatus by modify-

ing the size lends itself for use in respiration experiments of either physiological or pharmacological import on man or mammals.

The work of the United States Public Health Service in industrial physiology. FREDERIC S. LEE.

Since June, 1917, the Public Health Service has been carrying on an investigation of some of the problems of industrial physiology. The work is still continuing. Two representative factories have been studied, an eight-hour factory employing 36,000 workers with three shifts, and a ten-hour factory employing a maximum of 13,000 workers with two shifts. The subjects studied include the following: Diurnal course of output; lost time; stereotyped output; day versus night work; rest periods; accidents; labor turnover; muscular strength of workers; fatigue of workers; rhythm in industrial operation; some of the chemical phenomena of fatigue. The results will be presented in detail in a general report, which is soon to appear, and in special reports dealing with particular topics. Some of the broader features of the results are as follows:

1. Maintenance of output. In the eight-hour plant there is a more steady maintenance of production throughout the day; in the ten-hour plant, there is a marked decline of production at the end of each shift.

2. Lost time. In the eight-hour plant, work with almost full power begins and ends approximately on schedule and lost time is reduced to a minimum; in the ten-hour plant, work ceases before the end of the spell and lost time is abundant.

3. Stereotyped output. By this is meant the voluntary restriction of production by the worker. In the eight-hour plant, a stereotyped output is comparatively rare and production approximates physiological capacity; in the ten-hour plant, stereotyped output is very prevalent.

4. Night work. In the ten-hour plant, a twelve-hour night shift prevails. The output is characterized by a progressive decrease and an abrupt and marked fall during the last two hours. This suggests the advisability of shortening the duration of the night work in the interest of greater production.

5. Rest periods. Rest periods of ten minutes' duration during each of the two working spells were introduced in several departments of the two factories. In the eight-hour plant, the result was indeterminate; in the ten-hour plant, while there was great individual variation among the workers, in eleven of the twelve operations studied, the total output of the day was increased.

Rhythm in industry. A. H. RYAN and P. S. FLORENCE.[1]

Rhythmical movements, such as those occurring in machine operations, are characterized externally by an approximately uniform repetition. The time interval between operations or various steps in operations can be measured, and such measurements may be used as an index

[1] From the U. S. Public Health Service.

of the rhythm. A method was devised of fitting up the machine to be studied with electrical contacts so that each step in the operation could be recorded automatically. Chronometer records were obtained simultaneously, and by means of a signal magnet records were also made of any variations in the work or working conditions.

We have chosen the median speed of a series of operations as that more nearly representing the typical speed. From this the average deviation was obtained, and this divided by the median gives the percentage deviation from the median, or the coefficient of dispersion. The smaller this coefficient, the more rhythmical the operation or operator.

In a simple movement, such as tapping, the coefficient of dispersion on two subjects was 2.40 per cent and 2.54 per cent. In the facing and scoring fuse ring (handscrew-machine type) the coefficient in one operator was as low as 2.74 per cent. In the inexperienced worker the coefficient was as high as 8.34 per cent. In five operators on this machine the more experienced and faster operators gave the lowest coefficients. In 45 series of observations on the same worker, covering 2648 operations, the coefficient was 4.22 per cent. In beveling (also a handscrew-machine operation) the coefficient was likewise low. In the foot-press type of operation the coefficient was considerably higher, indicating less rhythm.

The fatigue of the day did not seem to lessen the rhythm of the operation. Distraction, such as counting the ringing of a bell, did not seem to interfere with the rhythm. A curve expressing the correlation of speed and rhythm indicates that there is an optimum speed for rhythm and that the rhythm is worse at either a slower or faster speed.

The possible importance of rhythm in industry and occupational fatigue may be briefly summarized as follows:

1. In relieving attention and its consequent fatigue.
2. In rendering more uniform the metabolism and recovery involved in the operation by evenly distributing the work.
3. In masking fatigue effects. Here the output curve may be maintained in spite of fatigue.
4. In increasing or decreasing accident hazard according to the type of accident causation.

Muscular tonus in relation to fatigue. A. H. Ryan and Sara Jordan, with the collaboration of A. B. Yates.[1]

Tonus was investigated in the hope of finding further methods for the detection of the more pronounced degrees of fatigue resulting from the day's work at different occupations and from the working day of different lengths, also for cumulative fatigue. This is a preliminary report of the results.

Among the methods devised by us was that of determining at different times the amount of tension required to produce a given amount of extension of a group of muscles. Present results are based on observa-

[1] From the U. S. Public Health Service.

tions made upon the pectorales and the soleus-gastrocnemius group. For the pectoral measurement the subject was placed in a standing position in an apparatus with his back resting against a support; the arms were elevated to the horizontal position and supported in metal slings suspended from the ceiling. One arm was fixed to an immovable standard while the other was pulled backward a constant distance by means of a cord and pulley. A delicate spring balance was inserted between the cord and the arm sling. A rigid graduated arc below the arm made it possible to achieve a constant extension at the different observations. In testing the soleus-gastrocnemius group the subject either sat or reclined on a table. The apparatus consisted of an upright board hinged to a horizontal board which was clamped to the table. The foot was fastened to the upright board and the axis of rotation about the hinge passed through the ankle. A handle with spring and scale attached made it possible to apply pressure in such a manner that the soleus-gastrocnemius group was extended, and to observe the degree of extension and pressure. The amount of tension required to produce a given degree of extension at the various times of observation was taken as the index of muscle tonus. A history was kept of sleep, work, rest, etc.

In subjects doing relatively strenuous work during the day, or where long hours were being spent in work, there was usually a decrease in the tonus in the evening as compared with the morning condition. This was more pronounced when the subject was losing rest (sleep). After lost rest the morning tonus was lower and the average tonus for the day was less than on days following a good night's sleep. Evidence was thus obtained of cumulative fatigue effects. Sleep at night or during the day was usually followed by a considerable increase in tonus. Strenuous work of short duration was usually followed by an immediate decrease in tonus. Psychic influences (excitement) seemed occasionally to produce an increase in tonus, although fatigue-producing conditions were recorded in the history. In subjects doing relatively light work and obtaining plenty of sleep the tonus varied during the day, the evening tonus being frequently greater than that observed in the morning.

The effect of fatigue on the bicarbonate content of blood plasma. A. Baird Hastings.

The following is a preliminary report of studies on fatigue conducted in the laboratory and in the field as a part of the program of the United States Public Health Service.

Evidence of the production and accumulation of fatigue substances in the organism was sought by studying the bicarbonate content of the blood plasma of normal animals. The Van Slyke apparatus for the determination of carbon dioxide and the McClendon electrode for the electrometric titration of plasma were employed to measure the bicarbonate content. The Clark hydrogen electrode was used to determine the reaction of the plasma. Fatigue was induced by causing the animals to run on a motor-driven treadmill whose speed could be varied up to ten miles per hour.

Fifty-five experiments on fifteen dogs, several rabbits and one man have yielded results of which the following example is typical. The bicarbonate content of the plasma of dog A, running continuously at an average speed of 6.7 miles per hour for 43.7 miles dropped from 57.5 to 47.9 volumes per cent of CO_2. The corresponding P_H values were 7.72 and 7.71. No significant differences of hydrogen ion concentration were found in plasma collected before and after fatigue even though the bicarbonate content had fallen 31 per cent. The rate and the maximum amount of fall of the plasma bicarbonate during the same degree of exercise varied from subject to subject. It may be stated, however, that in any one organism the rate of diminution of the bicarbonate content, was, within certain limits, a function of the rate of fatigue. Further, when the rate of exercise was below that required to exact from the subject his maximum muscular performance, the fall in the bicarbonate seemed to vary almost directly with the distance traveled. The bicarbonate of small dogs whose capacity for muscular activity could be quickly reached by the available speed of the treadmill, fell rapidly to a constant level, thereafter necessitating a gradual reduction in speed to permit them to keep in motion. With regard to the rate at which the reserve alkali returned to its normal concentration, it was found that in an animal which had been fatigued by running at a rapid rate for a comparatively short distance, the recovery was rapid; but when he had run a relatively long distance, the recovery was much slower. This would point to an accumulation of fatigue substances in the organism.

Results of P_H determinations of the urine of men exhibited no significant changes of the average hydrogen ion concentration in resting men, a slight increase in factory workers, and large increases in the case of men engaged in such strenuous exercise as participating in a Marathon or six-day bicycle race.

Effects of external temperature upon the toxicity of thyroid. O. O. Stoland and May Kinney.

The toxic dose of desiccated thyroid reported by different investigators varies greatly and since no satisfactory explanation for such variation has been offered, we have attempted to find the cause for such variation.

Three series of albino rats, fed on a diet consisting of crackers and milk, were kept at different temperatures, 32, 25 and 18°C. All except a few controls in each series were fed 0.2 gram of desiccated thyroid per day. The series kept at 32° lived an average of 7.3 days; that at 25°, twenty-two days and that at 18° more than thirty-two days. The toxicity, therefore, varies with the temperature, being greater with the higher temperatures.

The histological changes of the thyroid observed were as follows: All the animals fed on 0.2 gram thryoid per day developed the normal resting type of gland with low cuboidal epithelium. The controls kept at 32 and 25°C. also showed the resting type of gland, but the controls kept at 18°C. developed the active type of gland with small amount of colloid and columnar epithelium.

We would conclude: *a*, that external temperature is an important factor in determining the toxicity of thyroid gland fed to susceptible animals; and *b*, that in conditions which produce hyperplasia of the gland, hyperthyroidism produced by feeding of desiccated thyroid will bring about a change to the resting type of gland.

Physiological action of the thyroid hormone. E. C. KENDALL.

Thyroxin when injected into the animal organism does not produce any immediate physiological effect and if but a single administration is given a demonstrable action may not be produced. This applies to relatively enormous doses of the substance and it shows that the compound itself is not toxic. The toxic condition which is noted in thyroid disturbances and which can be readily produced by thyroxin results only after a long-continued administration of the substance. Three or four successive daily administrations are necessary in order to bring about a hyper-thyroidism. Investigation showed that thyroxin is rapidly eliminated from the body by means of the bile. In one case, over 60 per cent was thus excreted. Eight per cent of the iodine in the administered thyroxin was found in the urine and the remaining 32 per cent had doubtless been taken up by the thyroid of the animal. This explains why the single administration does not produce a physiological response. It is only the continued presence of the compound within the body which results in the increase in metabolic activity.

A method for the determination of minute amounts of iodine has been devised and it has been found that the average content in calves' blood is approximately 0.015 mgm. per 100 cc. of blood. The amount in the tissues is slightly greater, about 0.03 mgm. per 100 grams of tissue. The amount in the liver is still slightly greater, about 0.04 mgm. per 100 grams of liver. These figures show the very small amount of thyroxin functioning within the tissues, but they agree very well with the amount that must be there in order to satisfy the quantitative relation which thyroxin bears to basal metabolic rate. This relation has been found to be: 1 mgm. of thyroxin increases the basal metabolic rate 2 per cent in an adult weighing approximately 150 pounds.

The administration of thyroxin to the animal organism has been shown to produce an effect upon the metabolic rate which bears a quantitative relation to the amount administered. An injection of a derivative of thyroxin in which the hydrogen of the imino group is replaced has no effect upon the metabolic rate. This emphasizes the importance of the imino group in thyroxin and minimizes the importance of the iodine in the molecule. Other investigators have recently shown that in the metamorphosis of the tadpole the administration of iodine produced a great increase in the rate of metamorphosis. If the metamorphosis depends only upon the increase in the basal metabolic rate of the tadpole then thyroxin should increase the rate of the metamorphosis, but the derivative involving the imino group should not. If, however, iodine alone were concerned in accelerating the metamorphosis, then both thyroxin and the derivative should affect the metamorphosis. It was

found that both thyroxin and the derivative would cause a rapid metamorphosis of the tadpole of the bullfrog.

Thyroxin, therefore, appears to have two separate and distinct functions: The effect upon the metabolic rate which is brought about by the CO-NH groups within the molecule; and the physiological changes involved in the metamorphosis of the tadpole brought about by the iodine contained in the molecule. This action of the iodine is not specific to thyroxin, but can be obtained by a large number of other iodine compounds and by elemental iodine itself and appears to be inherent within the iodine atom. But the unique effect of thyroxin on the metabolic rate is due to the specific chemical structure of the molecule and this is not shared with any other substance so far known.

The pharmacology of acacia. Theo. K. Kruse.

1. A 25 per cent solution of acacia causes hemolysis, agglutination and darkening of blood. Such a solution is about one-third isotonic with blood. If such a solution is made up in saline, hemolysis is largely prevented.

2. Agglutination occurs in man, dog, cat and rabbit and does not occur in ox, frog and turtle. This phenomenon is not associated with viscosity, acidity, alkalinity nor the calcium content of acacia. Hypertonic solutions delay but do not prevent agglutination.

3. Rhythmicity of isolated tissues such as smooth muscle and cardiac strips is diminished, even in concentration of 0.01 to 0.1 per cent acacia in saline.

4. Urine secretion is decreased and sometimes nearly suppressed after acacia injection. This is a factor in the maintenance of blood volume by acacia.

5. Blood volume is well maintained partly at the expense of urine excretion.

6. Gum acacia does not mix readily with blood.

7. Acacia is tolerated in normal animals. In animals reduced by hemorrhage acacia is not tolerated, showing irregularities of heart and no improvement in blood pressure until blood, saline, dextrose or sucrose are subsequently injected. A 6 per cent acacia in 2 per cent sodium bicarbonate is less toxic. This is probably brought about by its increase in osmotic tension and by the augmenting effect of sodium bicarbonate upon the heart.

8. Pharmacological evidence does not support the use of acacia in man.

The treatment of the condition of low blood pressure which follows the exposure of the abdominal viscera. F. C. Mann.

The purpose of the investigation is to review experimentally all the more important methods of treating a condition which exhibits the clinical signs of surgical shock. The method of experimentation consists in exposing the abdominal viscera of a dog under a constant ether tension until blood pressure is decreased to the desired level, therapeutic procedures are then instituted.

The methods of treatment are divided into four divisions: (1) general measures; (2) special measures; (3) the use of drugs; and (4) attempts to restore fluid volume. The last method was investigated very extensively. It was found that under the experimental conditions blood or blood serum produced by far the best results. None of the artificial solutions were as beneficial as blood. Of the artificial solutions, normal salt solution was the least valuable and did not seem to produce as good results as distilled water. A hypertonic salt solution produced better results than a normal physiologic solution. Sodium carbonate and sodium bicarbonate produced some beneficial action as did also sodium sulphate. None of the saline solutions alone would restore and maintain blood pressure. Glucose was found to be of distinct benefit as were also cane sugar solutions. The so-called colloidal solutions produced the best results of all the artificial media. Gelatin solutions were found to be fully as efficacious as acacia solutions. Neither of these solutions appears to be without danger. Solutions of dextrin produced fair results. Various combinations of these different solutions were tried but none of them were found to be of as great value as blood.

The nutritive value of yeast protein. Thomas B. Osborne and Lafayette B. Mendel.

A graphic record was presented of the growth of rats beginning at ages of thirty-five to forty-five days for a year or more on a diet in which the protein and water-soluble vitamine were furnished by brewers' yeast alone. The foods consisted of:

	per cent
Yeast	30–40
Salt mixture	4
Starch	43–33
Butter fat	9
Lard	14

It is evident that the protein of yeast is "adequate" in the ordinary sense for nutrition. Details of this study will be published in the *Journal of Biological Chemistry.*

Further studies on the chemical composition of the brain of normal and ataxic (?) pigeons. Mathilde L. Koch and Oscar Riddle.

Previous observations of functional derangement in pigeons, i.e., lack of control of the voluntary movements—provisionally called ataxia—led to the conclusion that the seat of the disturbance was probably in the brain. Previous chemical analysis of the brain of these ataxic pigeons supplied evidence that the functional disorder is associated with deviations from the normal composition of the brain. These were interpreted as indicating chemical under-differentiation or immaturity of the brain of the affected individual. In other words, the brains of affected individuals of a given age appeared chemically more like the brains of normal individuals of a younger age. To confirm this, ten additional analyses were made of brains of pigeons of still other ages than those previously used; in order to obtain more complete data on the chemical

changes of the brain during development. Also, in order to determine whether this functional disorder was localized, most of the analyses were upon samples representing separate portions of the brain—the cerebrum having been analyzed apart from the rest of the brain (cerebellum and medulla).

The principal chemical changes noted in the growth series (from 45 to 2021 days) are: a general decrease in the per cent of water (82.6 to 78.4 per cent), of proteins (51.9 to 47.4 per cent) and of extractives (14.4 to 13.2 per cent) and an increase of lipoids (33.7 to 39.4 per cent). The younger affected pigeon brains suggested greater chemical under-differentiation or immaturity than older affected brains. There appeared no or slight localization of changes in composition in the two parts of the brain analyzed, i.e., changes are a little more pronounced in the cerebellum. That the brain is the seat of the disturbance in these ataxic pigeons has been partially confirmed by chemical analysis, the younger "most ataxic" pigeons showing the greater chemical under-differentiation.

Effect of pulse rate on the length of the systoles and diastoles of the normal human heart in the standing position. Warren P. Lombard and Otis M. Cope.

Continuing the work reported at the last meeting, the writers have now material consisting of 620 tests on 250 men and 15 women, and have estimated, in thousandths of a second, the length of the systoles and diastoles of more than 10,000 heart cycles. The carotid pulse and the respiration were recorded by tambours, and the time was written by a fork having fifty v. d. The records were taken on loops of smoked paper 170 cm. long. All readings were made under a glass, verticals being drawn from the point where the primary rise of the pulse first turned sharply up, and from the point where the bottom of the dicrotic notch was first reached. At least 15 consecutive cycles of each test were read. Diastoles are always affected by respiratory and usually by vasomotor influences; either one may show the more prominently in the plotted curves. Systoles always show the effects of respirations and sometimes vasomotor influences can be detected. As diastoles are longer and more variable than systoles, they have greater effect in determining the length of the pulse. The pulse rate, estimated from the length of individual cycles, may change 20 beats in a minute. If curves of the length of successive systoles and diastoles be plotted one above the other, and the time of the respirations is also indicated, the diastoles are seen to shorten promptly in inspiration and lengthen promptly in expiration. The systoles may or may not do this. The change in systole length may be delayed (just like the change in arterial blood pressure) so that the systoles may lengthen in inspiration.

If the average lengths of the systoles and diastoles of 250 men in the standing position are compared with their average pulse rates, and are plotted in a chart, in which the ordinates give the length of the systoles and diastoles in thousandths of a second and the abscissae the pulse

rates from 50 to 110, one sees that both the systoles and diastoles shorten as the pulse quickens, and that the diastoles shorten so much more rapidly than the systoles that the curves would have crossed had they been continued.

Accepting this chart as a standard, we found that the systoles of 84.8 per cent of the men differed not more than 0.015 second from the standard, 94 per cent not more than 0.020 second, and 6 per cent varied 0.021 to 0.025 second. The results obtained from the same man in two tests made perhaps weeks apart, generally agree better than might be expected, considering the probable difference in circulatory conditions. We found in the case of 68 men who were examined twice, 69.5 per cent gave systoles either longer or shorter than the standard each time. Also that in 75 per cent, the length of the systoles differed in the two tests less than 0.010 second.

One interesting fact observed was that of 15 women all gave systoles longer than the average length of systoles of men at the corresponding pulse rates in the standing position.

Effect of posture on the length of the systole of the human heart. Warren P. Lombard and Otis M. Cope.

The frequency of the beats of the left ventricle and, consequently, the pulse rate is determined by the rate of discharge of excitations from the sino-auricular node. One excitation starts the systole and the next excitation brings the diastole to an end. It is not known what influence stops the systole and starts the diastole. As the pulse rate quickens the length of systole lessens slowly and the diastole very rapidly. The shorter systoles might be associated with the lessened amount of venous blood reaching the heart during the short diastoles. Is there evidence that the amount of blood supplied to the large veins influences the length of the systoles of the left ventricle? Posture of body, by effects of gravity, would alter the rate of the return of blood to the large veins, and we have found that by the same heart rate the systoles of the left ventricle are 10 per cent longer in the sitting position, and 14 per cent longer in the lying-down position, than in the standing posture. This can be readily seen in a chart in which the average length of the systoles is plotted with respect to the average pulse rates by standing, sitting and lying-down postures. The change in the length of the systoles by different positions is not due to the changes in the rate of the pulse alone, because the systoles change out of proportion to the pulse. The curve of the average length of systoles has been found to correspond to the curve given by the formula $S = \frac{60}{K\sqrt{R}}$, in which S = systole, K = a constant and R = pulse rate. For standing postures a suitable constant appears to be 28.5, for sitting 26 and for lying down 25. It has also been found that the systole length in the standing posture just after exercise is slightly longer than before exercise, and the constant has to be changed from 28.5 to 27.5. Probably blood is returning to the heart more rapidly just after exercise. Height, and time of year,

have not been found to affect the length of systole. The effects of age of muscular exercise and of arterial blood pressure are being studied. As was stated in our first paper, the length of the systoles of the 15 women studied was longer than the average of men with corresponding pulse rates in the standing position. All but one of the women had longer systoles than men in all positions. Four women gave systoles not more than 0.008 second longer than men in the standing position. Three of these women reported having had considerable physical training, and two of the three gave systoles shorter than the average length given by men in the standing position just after exercise. One, who had 5 years gymnasium experience, gave systoles longer than men not only after exercise but in the sitting and lying-down positions. One cannot help wondering whether the longer systoles shown by women are due to the fact that most women take little vigorous exercise.

A comparative study of the relation of the cerebral cortex to labyrinthine nystagmus. A. C. Ivy.

During a series of cerebral ablations, it was suggested by Dr. F. T. Rogers that observations be made upon the nystagmus reaction. Wilson and Pike[1] and later Pike[2] have reported that the quick component of nystagmus was dependent upon the integrity of a cerebral reflex arc, i.e., the "removal of one cerebral hemisphere abolishes the quick movement when the slow movement of the eyes is directed to that side," or the removal of "the temporal and basal portion of the cerebral hemisphere" on both sides would completely abolish the quick component of nystagmus. Bauer and Leidler[3] report that the quick component is not dependent upon the cerebral cortex and thalamus and that with even extensive destruction of the mid-brain and probably with inclusion of the third nucleus does not abolish vestibular nystagmus.

This work has been done upon frogs, turtles, pigeons, rabbits, kittens, cats, pups and dogs. All operations upon mammals were done aseptically. When animals were comatose and when pressure symptoms were manifest, notation of such was made. Many of the animals lived indefinitely, or until some more radical operation was done. Observations have been made only upon rotatory and post-rotatory nystagmus, the speed and number of rotations being carefully controlled.

The brains of all operated animals have been examined and preserved.

A quick movement of the eyes upon rotation is present in the frog. It is irregular and slight in degree, for the deviation of the eye in the frog on rotation is not marked. It is influenced by such factors as temperature, handling and amount of rotation. Decerebration does not abolish the quick movement, but often increases the reaction.

A true eye, as well as head and neck, nystagmus is present in the turtle provided the temperature of the turtle is between 10°C. and 39°C. Following decerebration there follows a marked increase in the post-rota-

[1] Wilson and Pike: Arch. Int. Med., 1915, xv, 31.
[2] Pike: Proc. Soc. Exper. Biol. and Med., 1917, xiv, 76.
[3] Bauer and Leidler: Monatsch. f. Ohrenheilkunde, 1911, xlv.

tory response. Destruction of the cortex of the optic lobe does not abolish the quick component, but injury to the basal portion of the optic lobe does.

Complete decerebration with extensive injury of the thalamus in the pigeon does not abolish the quick component provided the temperature of the bird is kept normal by keeping the bird in an incubator. Rogers[1] has shown that in such animals all reflexes are diminished, or disappear, if the temperature of the bird is not normal.

In dogs hemi-decerebration and extensive injury to the thalamus does not abolish the quick component of nystagmus, but causes a temporary (3 to 4 months) increase in the post-rotatory nystagmus when the animal is rotated opposite to the side of the lesion. The rotatory nystagmus is increased when rotation is to the side of the lesion. The increase consists chiefly in a greater number of movements rather than an increase in the period of duration, which, however, does occur. If the remaining motor area is removed in a hemi-decerebrate animal, an increase in the post-rotatory nystagmus occurs when the animal is rotated opposite to the side of the fresh lesion. If the animal is comatose, manifests marked pressure symptoms, or if there is an injury to the third nerve, the quick component of nystagmus is absent, although deviation is present.

Rabbits and cats manifest the same results. Kittens and pups likewise show the same phenomena but the increase is more temporary, i.e., does not last longer than from four to six weeks.

Hence the conclusion is warranted that the quick component of eye nystagmus is not due to the integrity of a cerebral reflex arc, but is a lower type of reflex over which the cerebrum exercises its well recognized inhibitory influence. It is not meant by this that the cerebrum has the power to voluntarily reduce the number of nystagmic reactions following rotation.

Studies on the secretion of the pyloric end of the stomach. A. C. Ivy.

Heidenhain[2] demonstrated that the pyloric mucous membrane produced a thick, clear, alkaline secretion, which digested fibrin and coagulated milk.

In a series of animals prepared like Heidenhain's dog and in another series prepared with pyloric pouch with nerves intact, observations upon the character of the pyloric secretion have been made.

The rate of secretion of the entire pyloric mucous membrane is 2 cc. (average) per hour in animals with an isolated pouch. In animals with nerve supply to the pouch intact the rate of secretion is 5 cc. (average). The secretion is more or less continuous, no significant increase occurring after meals or water. The secretion is not increased in amount by subcutaneous injections of either gastrin or secretin. It shows no variations in amount with changes in the character of the food.

[1] Rogers: Journ. Nervous and Mental Diseases, 1919, xlix, no. 1.
[2] Heidenhain: Arch. f. d. gesammt. Physiol., 1878, xviii.

The secretion is thick and viscid—very similar to egg white. It is clear and very slightly alkaline in reaction.

The pepsin content of the secretion from the isolated pouch is lower than that from the pouch with the nerves intact, the former amounting to 0.5 to 1 mm. (Schiff's modification of Mett's method), the latter amounting to 2 to 3 mm. No significant change occurs following a meal. There is a slight rise in peptic activity during the third and fourth hours after a meal. Subcutaneous injection of gastrin caused a slight increase in peptic activity.

Its specific gravity varies from 1.009 to 1.013.

Chemical analyses made by Mr. B. Raymond have given up to date the following results per 100 cc. of pyloric secretion: Total solids, from 1.331 grams to 1.795 grams; total ash, from 0.558 to 0.903 gram; total nitrogen, from 0.095 to 0.144 gram; total chlorides, from 0.458 to 0.519 gram.

Studies on experimental gastric and duodenal ulcer. A. C. Ivy.

A study has been made *a*, of the occurrence of ulcer and other pathological lesions in the stomach and the duodenum of the dog; *b*, of the experimental production of chronic gastric ulcer in the dog; *c*, of the relation of the location of the ulcer to changes in gastric motility; *d*, and of the nervous mechanism involved in hypermotility of the stomach in experimental duodenal ulcer.

As judged from the results of eight hundred and fifty autopsies upon the stomach and duodenum in healthy and diseased dogs, it is apparent that chronic ulcer of the stomach and duodenum is very rare. In healthy dogs a chronic ulcer has never been found. In diseased experimental animals two typically chronic ulcers have been found, one in a thyroid-parathyroidectomized animal complicated by distemper, which was present prior to the operation, and a second in a ligated-pancreatic-duct animal, which was complicated by an acute stomatitis and cachexia. On the other hand petechial hemorrhages and hemorrhagic erosions are found not infrequently in the gastric and duodenal mucosa of the healthy dog. These lesions, however, occur more much frequently in the experimental and diseased and cachectic animals.

Typical chronic ulcers have been produced experimentally by making lesions of the pyloric and duodenal mucous membrane and then feeding cultures of virulent streptococci. A series of five healthy animals gave negative results, the lacerations of the mucous membrane healing in from six to ten days. A series of five cachectic animals showed indurated ulcers when they died or were killed four to six weeks following the lesion of the gastric mucous membrane. As a control, lacerations were made in two cachectic animals which were not fed streptococci. These lacerations healed in from twelve to fifteen days. The interpretation given to these results as related to the etiology of chronic gastric ulcer is as follows: given a petechial hemorrhage or an hemorrhagic erosion, along with a general lowered resistance accompanied by a hypo-acidity, bacteria swallowed are implanted in the abrasion, cause an inflammation

which is followed by an induration, which results in poor blood supply to the edges of the ulcerated area and an indefinite delay in the growth of mucous membrane to cover the abrasion. This work is being continued by a method more subject to experimental control.

In studying motility in ulcer, ulcers were made by the submucosal injection of 2 cc. of a 5 per cent silver nitrate solution. An ulcer located in the fundic portion of the stomach has no effect upon the motility and emptying time of the stomach. Ulcer in the pyloric portion of the stomach caused in three out of five dogs an increase in the motility of the empty stomach. In one of the five the emptying of the stomach was delayed two hours. Autopsy revealed an extensive scar just proximal to the pyloric sphincter. Ulcer of the duodenum caused an increase in the motility of the empty stomach in all of the six dogs studied. The emptying of the stomach was delayed more than two hours in every case. These results show that the clinical symptoms of ulcer with reference to disturbed motility and emptying time of the stomach and even loss of weight can practically be duplicated experimentally.

Animals with both vagi and both splanchnics cut along with excision of the coeliac plexus in which duodenal ulcers were made show an increase in motility of the empty stomach and a delayed emptying time.

In other words the mechanism of increased motility and delayed emptying of the stomach in duodenal ulcer is intrinsic. Whether it is due to increased irritability of the intrinsic nervous reflex or altered metabolic rate is yet to be determined.

An experimental study of a possible mechanism for the excitation of infections of the pharynx and tonsils.[1] STUART MUDD and SAMUEL B. GRANT.

The present experiments were undertaken in the hope of throwing light upon the mechanism by which chilling of the body surface may excite infection of the mucous membranes by their indigenous bacteria. The authors devised simple wire "applicators" for holding in apposition with the skin or exposed mucous surfaces the terminals of thermopiles in circuit with a D'Arsonval galvanometer. From the thermometer and galvanometer readings the temperatures of the surfaces beneath the thermopiles may be computed and temperature changes accurately followed.

The cutaneous chilling in our experiments caused only inconsequential changes in blood temperature and pressure. Rate and depth of respiration were kept constant. Superficial temperature varied directly with rate of blood supply, and was an index of local vasomotor tone. This thermogalvanometric method, checked by observations of color change, showed that chilling of the body surface causes reflex vasoconstriction and ischemia—not, as hitherto assumed, congestion—in the mucous membranes of the palate, faucial tonsils, oropharynx and nasopharynx.

Inhalation of amyl nitrite causes a sharp rise in mucous membrane

[1] The detailed paper will appear in the Journ. Med. Research, xl, no. 1.

temperature parallel to the skin flush. The temperature of an oropharynx, chronically inflamed for almost two years, failed to fall with cutaneous chilling, but normal vasodilatation followed amyl nitrite inhalation. The reflex arc to the mucous membrane vessels had thus presumably been interrupted in its peripheral motor elements. A throat with history of inflammation extending back only a week showed no blanching with chilling.

Scar tissue showed reflex vasoconstriction parallel to that of the neighboring skin. The earliest scar tested and proved to have vasomotor fibers was at the site of an operation performed a month before for removal of a keloid.

In four instances exposure was followed by a "cold" or sore throat.

It does not seem improbable that the ischemia of the mucous membranes incident upon cutaneous chilling might so disturb the equilibrium between the host and the bacteria in the tonsillar crypts and folds of the pharyngeal mucosa as to excite infection.

The antiscorbutic properties of green malt. J. F. McClendon and Wyman C. C. Cole.

Owing to the scarcity of antiscorbutics in winter an investigation of their production indoors seemed worth while. Sprouted seeds and their extracts were shown by our collaborator, Paul F. Sharp, to have a greater hydrogen ion concentration than pure water and hence might be subjected to a certain amount of heat or storage in the moist state without entire loss of antiscorbutic properties. Cereal grains and legumes were chiefly used but the former were found to be more resistant to mould. Sprouted wheat, heated to 70°, is quite tender and palatable, and the same may be said of rye. Corn moulds easily but is sometimes utilizable. Barley has an adherent husk that makes it unsuitable for similar use but more suitable for malt extracts. A special mill for grinding green malt was devised. If the mash is brought momentarily to 70° the oxidases are partially destroyed and the wort may be evaporated in vacuo to a syrup that needs no sterilization or preservative. Since the wort is acid and never boiled and the malt is not dried the antiscorbutic properties are largely retained. Thirty-five guinea pigs have been used in testing the antiscorbutic properties of green malt and extract. Although we have not finished our quantitative estimations we are glad to report that a young guinea pig may live about two months and probably indefinitely on a diet of sprouted barley and dry oats without symptoms of scurvy whereas controls died in three weeks. Since this diet is probably deficient in salts and proteins and fat soluble A, we are repeating the experiments with the addition of condensed milk. Malt extract is often recommended for babies and nursing mothers, but the malt is kiln-dried and the wort is boiled and hence the extract is deficient in antiscorbutics. It seems evident that the only source of antiscorbutics in mother's milk is in the mother's diet. The use of malt extract prepared by our method might avoid the necessity of feeding orange juice to infants when oranges are very difficult to obtain.

Owing to the discrepancy between the results of Weill and Fuerst on the age of sprouting barley at which the antiscorbutic property appears, it is important to note that these authors did not record the temperature and by raising the temperature the barley may be made to sprout four times as fast as it does in most breweries. We found that the malt had little antiscorbutic power before the acrospire projected beyond the grain but had marked antiscorbutic power when the acrospire projected to a distance equal the length of the grain.

The electric conductivity and polarization of skeletal muscle. J. F. McClendon and Ferdinand A. Collatz.

Our object was to make more accurate determinations of the electric conductivity of muscle than have been recorded. A Vreeland oscillator giving a pure sine wave of 1000 oscillations per second was used as the source of current, and this was cut down by means of a rheostat so as not to stimulate curarized muscle. Frog's muscles were packed in a glass tube having at each end a chamber containing a platinized gold electrode in Ringer's fluid, separated from the muscle by a gold grid. A Wheatstone bridge was made, having zero inductance. The capacity of the conductivity cell and muscle fibers was balanced by condensers made of pure gold plates immersed in water. A tuned telephone was used as zero-instrument. Since a tone-silence cannot be obtained unless the capacities are balanced, the method enabled us to estimate the capacities of the conductivity cell and muscle, and by subtracting the capacity of the cell, the capacity of the muscle was obtained. Since the muscle has considerable capacity and does not contain metallic plates, it must contain plates or membranes of non-conducting or poorly conducting substance. These membranes are evidently the seat of polarization in the muscle.

An attempt was made to determine the difference in conductivity of the stimulated and unstimulated muscle. By cutting out resistance at the oscillator the current could be caused to stimulate the muscle, but it was difficult to tell when stimulation occurred with the muscles packed in the tube. Curarized turtle's muscle was placed between two bright platinum discs that were rigidly fixed and which slightly squeezed the muscle. On stimulating the muscle its electric conductivity increased but the increase was less as the muscle became fatigued and might be 200 per cent in a fresh muscle and 10 per cent in a muscle that had been stimulated several minutes. The experiments were done in a constant temperature room but the temperature of the interior of the muscle could not be exactly controlled. The backs and edges of the platinum plates were paraffined. The contraction of the muscle changed the shape of the projecting edges of muscle. Notwithstanding the sources of error, we cannot explain the fact that the stimulated muscle is always at least 10 per cent more conducting than the unstimulated without assuming that the polarization within the muscle decreases on stimulation.

Intra-cellular acidity in Valonia. W. J. Crozier.

The cells of the marine green alga Valonia are sufficiently large to permit the extraction of 3 cc., or in some cases more, fluid from the central vacuole of a single cell. In normal cells the reaction of this intracellular fluid, containing CO_2 under considerable tension is pH = 5.9, and is maintained at this point regardless of the external reaction (between pH = 6.6 to 9.5), so long as the cell is healthy. The death process is accompanied by an increased alkalinity, approaching that of the sea water (pH = 8.1), and by the penetration of SO_4'' into the cell-sap.

The control of the response to shading in the gill-plumes of Chromodoris. W. J. Crozier.

Between 15° and 32° and in sunlight not too intense, the gill-plumes of Chromodoris zebra respond by contraction when they are shaded. This contraction, due to the activation of receptors locally contained, leads to the reflex retraction of the whole gill crown within its collared pocket. The degree of extension of the gill crown as a whole is a function of the light intensity, but is controlled through a separate set of receptors. The nudibranch is photokinetic.

The sensitivity of the plumes to shading is abolished when the alkalinity of the sea water is reduced to pH = 7.9 ±, and the retraction of the gill crown is furthermore completely inhibited at an alkalinity slightly lower. At pH = 8.4 the gills remain totally concealed. The "protective" reaction to shading is thus a response superimposed upon the simple system of fundamental activities (protrusion, retraction) which is concerned with regulating the gaseous exchange of the nudibranch.

Postural activity of the empty stomach. T. L. Patterson.

The experiments in this report were undertaken with the view of studying the postural activity of the empty stomach of the bullfrog. The balloon method was used and just enough air was introduced with a syringe to produce a constant pressure of 2 cm. in the manometer. The necessary pressure for animals measuring from twelve to thirteen inches was obtained with 10 cc. of air and this was used as the constant throughout the experiments.

After stomostomy and the obtaining of gastric hunger records, the animals were divided into three groups, namely: (1) Animals having both vagi sectioned with the splanchnics intact; (2) animals having the splanchnics cut with the vagi intact; and (3) animals with both the vagi and the splanchnics cut.

Section of both vagi or the vago-sympathetics in the neck of the frog with the splanchnics intact leads to an increase in the volume capacity of the stomach, since to obtain the constant pressure of 2 cm. in the manometer 15 cc. of air must now be used instead of 10, but this condition is only temporary. There is a complete reëstablishment of the postural activity of the stomach which takes place rather suddenly on the tenth day following the cutting of the nerves. Exactly the same

phenomenon occurs after section of the splanchnic nerves with the vagi intact, but in this case there is a decrease in the volume capacity of the stomach, so that only 4 cc. of air are required to produce the constant pressure of 2 cm., but here again there is a complete reëstablishment as above on the tenth day following the operation.

When both sets of extrinsic nerves are sectioned, thus isolating completely the stomach from the central nervous system, there is again an increased volume capacity of the stomach from the normal or 10 cc. level to 15 cc. from which there is only a partial recovery. Usually on the thirteenth day following the operation a drop from 15 to 13 cm. of air occurs but it never returns to the old level. Therefore, since the local reflex mechanism of the gastric wall is incapable of bringing about a complete physiological readjustment of the posturally acting stomach (empty) when completely isolated from the central nervous system, but is capable when partially isolated, it shows that both the extrinsic and intrinsic nervous mechanisms take part in the maintenance of the postural activity, and that the stomach when completely isolated develops in time, within itself, a new level of postural activity which as determined by volume capacity is greater than the normal.

On the effect of antipyretics on the hearing. D. I. Macht, J. P. Greenberg and S. Isaacs.

The effect of a large number of antipyretic drugs and their combinations was studied on the hearing of normal human subjects. The following drugs were utilized: acetanilid, acetphenetidin, pyramidon, antipyrin, lactophenin, melubrin, salol, aspirin, sodium salicylate and quinin. The following combinations were also studied: acetanilid plus sodium bicarbonate, acetanilid plus salol, acetphenetidin plus salol, antipyrin plus aspirin, antipyrin plus salol, acetanilid plus acetphenetidin, aspirin plus salol, and some others. It was found that some antipyretics decrease the acuity of hearing, while others increase it, and that certain combinations produce unexpected synergistic effects. Among the most interesting findings are the following:

Acetanilid, aspirin and salol each markedly decrease the acuity of hearing. Antipyrin, pyramidon and acetphenetidin all tend to render the hearing more acute. A combination of acetanilid with salol instead of decreasing the hearing actually renders it more acute. A very interesting observation is the one concerning the combination of acetanilid with sodium bicarbonate. It was found that while acetanilid definitely decreases the hearing, when given alone, and while sodium bicarbonate produces no change in the acuity of hearing when administered by itself, a combination of acetanilid with sodium bicarbonate actually increases the distance limit of hearing in the same subjects. Some experiments have been made with the object of explaining the latter phenomenon. The complete data will be published in the *Journal of Psychobiology.*

Action of corpus luteum extracts on the movements of isolated genito-urinary organs. D. I. Macht and S. Matsumoto.

The present authors have been engaged for some time in the study of the physiological action of various glandular extracts, and more particularly of their influence on the genito-urinary organs.

The authors have studied extracts of fresh corpora lutea of the sow, and also extracts of various commercial preparations of the desiccated corpus luteum substance in respect to their action on the vasa deferentia of the dog, cat, rabbit, guinea pig and the rat, and have found the most suitable and most sensitive preparation for testing the corpus luteum extracts to be a freshly excised vas deferens of the rat in Tyrode's solution. Such preparations, when treated with some corpus luteum extracts, may react by contractions in solutions corresponding to concentration of 1:2500 of the fresh gland, and they almost always react to concentrations of 1: 1000 of the fresh gland. It was interesting to note that the vas deferens, though very sensitive to the effects of the corpus luteum, does not react to extracts of ovarian substance proper. As far as the authors have been able to gather other data, both experimental and clinical, it seems that the activity of corpus luteum extracts, as indicated by the vas deferens preparations, runs parallel to the activity of those preparations as indicated by the other data. This organ, therefore, seems to furnish a convenient method of comparing the physiological activity of various corpus luteum preparations and some criterion for the testing of various chemical principles derived therefrom. Complete data of the present investigation will appear in due time in the *Journal of Urology*.

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STUDIES IN SECONDARY TRAUMATIC SHOCK

II. Shock due to Mechanical Limitation of Blood Flow

JOSEPH ERLANGER and HERBERT S. GASSER

From the Physiological Laboratory of Washington University, St. Louis

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INTRODUCTORY

The first phase of our study of the shock problem consisted in an examination of the mechanical changes in the circulation occurring in shock as induced by procedures directly involving the abdominal viscera, that is to say, in the type of shock most commonly investigated (1). Another method of inducing shock that has received some attention is the so-called mechanical shock of Janeway and Jackson (2). These investigators have studied the effect of temporary, partial occlusion of the inferior vena cava, and on the basis of their data have drawn certain conclusions with regard to the mechanism of the circulatory disturbance resulting therefrom, and with regard to its bearing on the problem of shock in general.

We have produced, and have studied by our methods, this type of shock. In the effort to interpret the results obtained, we have also included in our program a study of the mechanical changes in the circulation induced by partial, temporary obstruction of the aorta. Our experiments on the effects of temporary anemia induced in these two ways form the subject of the present paper.

SHOCK BY TEMPORARY PARTIAL OCCLUSION OF THE INFERIOR VENA CAVA

The method employed by Janeway and Jackson consisted in placing a thread around the cava in the thorax and then reducing the arterial pres-

sure to 30 to 40 mm. Hg. for 2 hours by means of graded tension exerted upon the vein through the thread. They found that after deocclusion, shock developed within 18 hours, the arterial pressure in the meanwhile sometimes recovering even the normal level.

The method of obstructing the vena cava finally adopted by us differed somewhat from that of Janeway and Jackson in that, instead of opening the chest and laying a ligature around the cava, a clamp was devised by which graded compression could be exerted upon the cava between the liver and the diaphragm through a small abdominal incision. Our method of following the tone of the peripheral arteries (1) gives the best results when shock develops within 5 or 6 hours. In many instances a period of occlusion lasting 2 hours sufficed to bring on shock within this limit. But not infrequently it became obvious, soon after deocclusion, that the failure of the circulation was not going to run its course within the optimum time limit of the method. In such instances, in order to hurry the process, the clamp was again applied to the cava for periods which were varied in duration to suit the needs of the case. In other instances, the onset of circulatory failure was hastened by holding the arterial pressure lower than 30 to 40 mm. Hg. It will be seen as the subject is developed that, in so far as the experiments overlap, they essentially confirm Janeway and Jackson. The methods employed in following the arterial, the systemic and portal venous pressures, and the peripheral resistance have been described in the preceding paper (1).

Experimental data

The results we have obtained have not been uniform enough to lend themselves to illustration by a single type experiment. Instead, it will be necessary to describe and discuss individually the results of several of the experiments before making an attempt to generalize.

Experiment 4 (*fig. 1*). By means of a thread passed around the cava through an opening in the thorax, the arterial pressure was held down at first to 30 mm. Hg., and later to 18 mm. Hg. During this period the inflow rate (femoral) at first was slowed somewhat (asphyxial constriction) but soon it began to increase and this increase continued steadily up to the reading made immediately after deocclusion (loss of constrictor tone). After deocclusion the arterial pressure rose momentarily as high as 55 mm. Hg., but otherwise was very low until the reading made at 3:17. Throughout this period the inflow rate remained high. Then, for a period of about 50 minutes, the arterial pressure remained above 50 mm. Hg., for a time reaching 64 mm. Hg. The inflow rate here steadily diminished and by

4:25 had practically reached the normal level (partial recovery of vasomotor tone). But now, coincidently with a rapid fall in arterial pressure, the respiration gave evidence of failing and it became necessary at *e* to give artificial respiration. At this time the inflow rate rapidly increased.

In this experiment the variations in constrictor tone can be regarded as secondary to the effects on the center of the changes in arterial pressure. It is obvious that the diminished peripheral tone is not the cause, or at least not the sole cause, of the low arterial pressure; for if it were, the arterial pressure at 4:15, *e*, when the inflow rate was nearly the same as at the opening of the experiment, should have been up to the initial level. The fact that it was not presumably finds its explanation either in a diminution in the effective blood supply or in cardiac weakness.

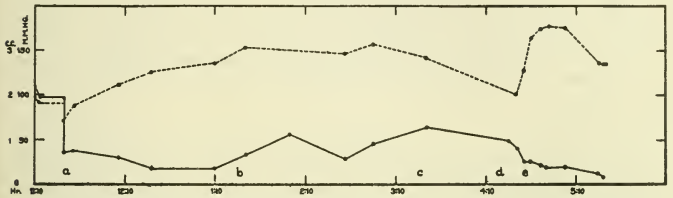

Fig. 1. Experiment 4. Temporary partial occlusion of the inferior vena cava in the thorax. Arterial pressure, —●—●—●—; femoral inflow, -●-●-●-●-. *a*, cava occluded; *b*, cava opened; *c*, thorax closed, artifical respiration off; *d*, respiration bad; *e*, artificial respiration.

In *experiment 20* (*fig. 2*), intestinal and hepatic inflow determinations were made. As the latter were unsatisfactory, no further reference need to be made to them. The vena cava at first was so occluded, at *a*, as to lower the arterial pressure to 82 mm. Hg., and then for 2 hours, *b*, to 34 mm. Hg. With deocclusion, the arterial pressure rose in the course of about 20 minutes to 100 mm. Hg., but it then fell and reached the level of 50 mm. Hg. about 15 to 20 minutes later. Later the heart stopped suddenly, as though the ventricles had fibrillated. Within a few minutes after beginning the more complete occlusion, it was found, *c*, that the ether could be dispensed with, and it was not necessary to administer it again.

After determining the normal inflow rate, inflow readings were not again made until the vena cava was deoccluded. The first reading after the period of occlusion was at the low limit of the initial range; but three subsequent readings, the last of which was made while the pressure was at 50 mm., Hg., were at the upper limit of the initial readings. With death there was some further increase in the inflow rate, followed by a steady decrease (constriction of the larger arteries?).

The intestinal inflow readings in this case show that after deocclusion the center has lost much of its activity; for in the presence of a very low arterial pressure, which ordinarily would have constituted a very strong stimulus, the center is not able to maintain more than the normal grade of arterial constriction.

They also show that the center possesses some residual tone up to the moment of death.

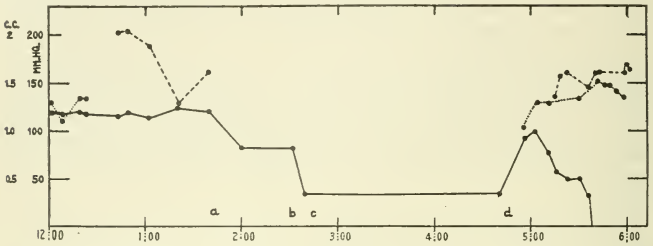

Fig. 2. Experiment 20. Temporary partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; hepatic inflow, – –●– –●– –; intestinal inflow, . .●. .●. .●. . . *a*, cava clamped; *b*, cava clamped more; *c*, ether discontinued; *d*, unclamped.

Experiment 50 (*fig. 3*). In this experiment the occlusion was of such a grade as to reduce the arterial pressure from 120 mm. Hg., to, for the most part, 40 to 60 mm. Hg., and was continued for 2 hours 8 minutes, *a* to *b*. The arterial pressure obtaining during occlusion in this experiment was much higher, therefore, than in experiment 4 (fig. 1) and somewhat higher than in experiment 20 (fig. 2). During the period of compression the femoral inflow rate decreased during about 40 minutes, eventually falling below the lowest of the initial readings; it then steadily increased so that at the time of deocclusion it was decidedly above normal. In this stage of the experiment we again see, first, the effort of the vasoconstrictor center to compensate the low pressure, and then a loss in the tone of the center. The latter effect may be due either to fatigue, or to functional incapacity of the center from the diminished blood supply, or to functional incapacity of the peripheral mechanism, also resulting from the reduced blood flow. Reasons for discarding the last explanation will be presented later. But whatever its cause, the loss of function at this time is not complete, as is demonstrated by the further increase in inflow rate that occurs at the close of the experiment.

With occlusion of the cava, the portal pressure rose at once from 8 mm. Hg. to 12 mm. Hg., but then fell promptly to 10 mm. Hg. This level was then maintained, possibly falling a bit toward the close of the period, as the arterial pressure declined. It is interesting to note how constant the portal pressure remains, varying not more than 1 mm. Hg., while the arterial pressure falls from 73 to 40 mm. Hg., and while the vasomotor tone (splanchnic, presumably, as well as somatic) swings from well above normal (11:30 to 11:40) to well below normal (1:00).

There can be no doubt but that the arterial pressure falls when the cava is occluded because of the accumulation of the blood in the peripheral veins and capillaries. This experiment shows that a degree of occlusion of the cava that causes a rise of only 2 mm. Hg. in the portal pressure (a corresponding, and prob-

ably greater rise in pressure occurs also in the systemic veins peripheral to the obstruction (2)) is all that is necessary to cause an accumulation of blood in the veins of sufficient magnitude to lower the arterial pressure to 60 mm. Hg.

The jugular pressure (one reading only) also was increased slightly (1.2 mm. Hg.) by the occlusion. In view of the fact, just commented upon, that back pressure on a central vein produces very marked effects even when the rise in venous pressure caused thereby is slight, this rise may mean that the heart here is suffering from the effects of a deficient coronary circulation.

Upon deocclusion, the arterial pressure rose at once from 34 to 65 mm. Hg., then more slowly (in about one-half hour) to 80 mm. Hg., after which it began slowly to sink (until 2:30). The femoral inflow during this phase of the experiment, continued to increase for about 10 minutes and then progressively decreased until 2:30. The portal pressure actually rose momentarily upon deocclusion and then sank progressively, until by 2:30 it was only 6 mm. Hg.,—that is, below the normal of 8 mm. Hg. The jugular pressure shows a further slight, and possibly, insignificant rise in this phase of the experiment.

Fig. 3. Experiment 50. Temporary partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; portal pressure, —○—○—○—; jugular pressure, —x—x—x—; femoral inflow, – –●– –●– –. *a*, cava clamped; *b*, clamp removed; *c*, slow asphyxiation; *d*, fresh air restored; *e*, temporarily clamped cava; *f*, clamped trachea.

The increase in vasomotor tone, the rise in arterial pressure and the fall in the portal pressure seen in the phase beginning some 10 minutes after deocclusion, is a combination that is easily accounted for; it is merely necessary to assume that the volume of blood liberated by deocclusion permits the heart to raise the arterial pressure. This soon improves the condition of the vasomotor center, and the peripheral resistance consequently increases. The fall in portal pressure is then explained by this increase in the peripheral resistance. Reduced vasomotor tone is not the whole trouble, however; for despite a peripheral resistance that is only a bit below the initial range, the arterial pressure is far below normal.

In the last phase of this period (1:45 to 2:30), despite the continued increase in the peripheral resistance and a further decrease in portal pressure, the arterial pressure falls. Failure of the heart or a diminution in the effective volume of blood alone could bring about this combination of events. The same inference must be drawn from the fact that, although at the end of this phase vasomotor tone lies within the normal range (though at the upper limit, to be sure), the portal and arterial pressures are well below normal. Inasmuch as the jugular

pressure continues to increase, even though slightly, during this phase of the experiment, the possibility must be entertained that failure of the heart is partly the cause of the fall in arterial pressure.

At this point, *c*, the animal was made to rebreathe the air contained in a 20 liter tank. The vasomotor tone, for some unknown reason, at first decreased, but it then increased, presumably through the action of the asphyxia that eventually developed. While the vasomotor tone was decreasing, the arterial pressure fell and the portal pressure rose. This is exactly the complex a vasodilatation should produce. But although, during the subsequent increase in vasomotor tone, the portal pressure fell, the arterial pressure, instead of rising, fell still further. This behavior of the arterial pressure possibly is to be attributed to the action of the asphyxia upon the heart.

With the cessation of rebreathing, *d*, the peripheral arteries dilated markedly. The arterial pressure at the same time fell, while there was but little change in the portal pressure. By this fall the arterial pressure was carried down to the level of 50 mm. Hg. and, excepting a slight momentary recovery, it did not again get above that level. At this time, vasomotor tone and portal pressure both were below normal.

Now, *e*, the effect was determined of momentarily clamping the inferior vena cava. When the clamp finally became tight the vasomotor tone diminished sharply, the arterial pressure fell sharply, and the portal pressure rose sharply, though slightly. At the same time the respiratory center failed and the clamp was removed and recourse had to artificial respiration. Within a minute or two the respiratory center became spontaneously active again and artificial respiration was discontinued, the vasomotor tone improved, the arterial pressure rose, and the portal pressure sank. This procedure was tried a second time with the same results, at least, in so far as the arterial pressure and the respiratory center are concerned (venous pressure and inflow readings were not made).

It will be noted that the effect of clamping here is very different from what it was at the beginning of the experiment; instead of causing arterial constriction it causes dilatation. This result presumably is to be explained by the nearness of the medullary centers to the limit of their viability; interference with the circulation, instead of causing them to respond as they do usually, with increased activity, lowers their activity. Throughout this stage of the experiment the general trend of the vasomotor tone was downward, while the arterial pressure and the portal pressure, essentially, were stationary. The animal was then killed by clamping the trachea, *f*. The arterial and portal pressures fell promptly, while the inflow rate was further accelerated. The jugular pressure (5 estimations) increased slightly throughout the experiment. Toward the end of the experiment, only the lightest anesthesia was needed to keep the animal from moving.

Experiment 45 (*fig. 4*) is described because it presents some new points of interest and also throws light on some of the events of the preceding experiment. It should be stated at the outset that this animal had very much enlarged thyroids and the enlargement of the carotid arteries often associated with that condition. It is possible that some of the unusual findings are referable to this fact. A general survey of the results of the experiment may be given before taking them up for discussion in detail.

Occlusion of the vena cava (at *a*, fig. 4) lowers the arterial pressure from 88 to 42, but eventually to about 30 mm. Hg. After 18 minutes, at *b*, respiration and heart fail. Through cardiac massage coupled with artificial respiration, the heart recovers and the circulatory changes then progress without any apparent break in continuity; but the respiratory center does not recover. Even before this failure of the respiration and the heart occurred it was realized that the caval occlusion, though carrying the arterial pressure no lower than it has in other experiments, was going to cause too profound a change in the circulation; therefore, as is indicated in the figure at *c*, the clamp was opened a bit. The arterial pressure rose slightly and then returned to its previous level. The effect of this partial release of the cava, though seemingly ephemeral, probably was long lasting; for it checked somewhat the rate of fall of the arterial pressure. At *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l* and *m* the clamp was partially opened until the jaws were wide apart, whereupon the clamp was removed. Inasmuch as after *h* the arterial pressure failed to show even the temporary, slight elevation up to this time in evidence

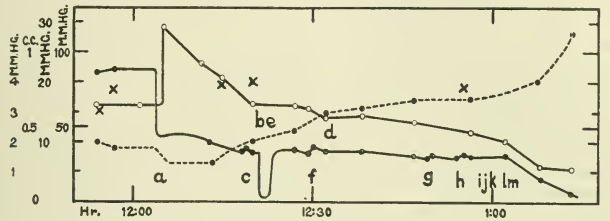

Fig. 4. Experiment 45. Temporary partial occlusion of the inferior vena cava (hypertrophy of thyroid). Arterial pressure, —●—●—●—; jugular pressure, x x x ; portal pressure, —○—○—○—; femoral inflow, – – ●– – ● – – ●– –. *a*, cava clamped; *c*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l* and *m*, partial unclampings; *b*, heart and respiration stop; recovery by cardiac massage and artificial respiration.

with each partial decompression, it may be assumed that the clamp was then wide open. The fall in arterial pressure at the end, to all appearances, was due to failure of the heart. The jugular pressure, which was high at the outset, did not change materially during the course of the experiment.

The portal venous pressure, also, was high at the start (16 mm. Hg.) and was markedly increased by compression of the cava, reaching the astonishing value of 29 mm. Hg.; but it then gradually fell and in 17 minutes was back practically to its original level. It then fell more gradually until failure of the heart caused the arterial pressure to fall rapidly, when the portal pressure likewise fell more rapidly.

The femoral inflow rate, as is always the case, at first diminished upon obstructing the cava. It is quite likely that, had a reading been made immediately after applying the clamp, a still slower inflow rate would have been found. The inflow rate then rapidly increased, after a time, however, at a diminishing rate. Eventually, when the heart finally failed, the inflow rate again accelerated.

We may now attempt an interpretation of these results. The damming back of blood by occlusion of the cava lowers the arterial pressure. The vasomotor center at first is stimulated by the anemia and constriction results. Despite this constriction, however, the arterial pressure is not raised and the anemic center begins to lose in activity.

An interesting stage of the experiment is where the vasomotor tone, while diminishing, crosses the normal level (at about *c*); for it so happens that at the same moment the portal pressure and the jugular pressure likewise are at their initial levels; the arterial pressure, though, is subnormal. For estimating the efficiency of the heart at this time the behavior of the jugular pressure, as has been said, is of but little assistance. While it is true that the heart stopped at *b*, it is to be presumed that this stoppage was secondary to failure of the respiration; for after resuscitation of the animal by cardiac massage and artificial respiration, the heart continued to beat, whereas the artifical respiration had to be continued during the rest of the experiment. The lowness of the arterial pressure must therefore be attributed either to accumulation of blood in the veins and capillaries or to extravasation. The conditions here were unusually favorable for the development of both of these processes. The high portal pressure would favor distension and filtration, while the slight pressure gradient in the splanchnic capillaries (difference between arterial and portal pressures), amounting for the most part to from 13 to 18 mm. Hg., only, would favor the transudation that comes with stagnation of the circulation. The brevity of the period, as will be made clear in another connection, does not invalidate the latter explanation.

The heart, started by massage and supplied with blood now aerated by artificial respiration, is able to continue its work about 40 minutes longer, when, owing to the continued low and falling arterial pressure, it again fails. At the time the respiratory center fails, *b*, the tone of the vasomotor center, it will be noted, is only a little below normal. In order to make sure here (at *d*) that the respiratory center had actually failed and was not merely in a state of apnoea, artificial respiration was discontinued for a period of 80 seconds: the animal made not the slightest effort to breathe. This observation confirms the well-known fact that the vasomotor center is one of the most resistant of the bulbar centers to cerebral anemia (3). The vasomotor center continues to lose in tone, though before the heart finally fails, the tone tends to strike a level. During this period, the portal pressure falls more rapidly than the arterial pressure, while the jugular pressure shows no material change. This combination of circumstances can scarcely be ascribed alone to increasing incapacity of the heart, nor can it be attributed to the diminishing vasomotor tone, for it is inconceivable that this could cause the portal pressure to fall more rapidly than the arterial pressure. Diminution in the effective volume of blood, therefore, again seems to be the sole factor accounting satisfactorily for the loss, in large part at least, in the efficiency of the circulation in this stage of the experiment. This is all the more remarkable because it is in this stage that the removal of the caval clamp ought to have made possible the return to the general circulation of some of the blood which had been trapped in the veins. The question arises, why does not the blood thus released become effective? Has it disappeared from the blood vessels, or has an increase in the capacity of the arterioles and capillaries through loss in vasomotor tone, and also of the veins, caused it to disappear? These are questions that must

be left unanswered. But whatever may be the explanation of this interesting circulatory state, it is to be noted that despite the early complete failure of the respiratory center, the vasomotor center possesses, until the heart fails, the capacity of holding the inflow rate well below that permitted into the unbridled arterioles.

Finally, we wish to call attention to the fact that but for the failure of the respiratory center, the condition to which this animal was reduced resembled typical shock; ether was no longer necessary, and the arterial pressure was below 50 mm. Hg. The grade of vasomotor depression that obtained before the heart finally began to fail, probably would not of itself have brought on shock; presumably it required this, plus the mechanical disturbance in the circulation that was caused by occlusion of the vena cava. However this may be, it is obvious that this animal was more susceptible to the effects of caval occlusion than any of the other animals thus far considered; and that the grade of circulatory inefficiency which, in other animals treated in the same way, does not supervene until some time after deocclusion, in this case develops while the cava is being occluded. We will have occasion to refer to this subject again in connection with the next experiment.

Experiment 47 (*fig. 5*). We describe still another of this series of experiments because of its interest in several respects. For some unknown reason this animal, like the preceding one, had unusually high initial jugular (3.0 to 3.5 mm. Hg.) and portal (14 to 15 mm. Hg.) pressures; and, as in the preceding case, it was necessary to open the clamp early in order to keep the animal alive. When the cava was so clamped (at *a*) as to carry the arterial pressure from the initial value of about 140 mm. Hg., down to 46 to 40 mm. Hg., the jugular pressure was scarcely affected, whereas the portal pressure, exactly as in the preceding experiment, rose immediately to 27 mm. Hg. and then slowly fell. The rate of femoral inflow decreased immediately and then slowly increased. At *b*, just 20 minutes after applying the clamp, the heart became irregular and, lest it might stop, the clamp was opened somewhat. A tremendous slowing in heart rate and increase in pulse amplitude resulted; the arterial pressure rose and the portal pressure fell. Despite this improvement in the circulation, and despite the fact that the occlusion in this experiment had not been any more severe than usual and had not lasted nearly as long as usual, the animal stopped breathing (at *c*). Artificial respiration was then started, without ether; within a few minutes the heart-beat improved and the clamp was then tightened a bit. The animal now began to breathe spontaneously and artificial respiration was discontinued (at *d*); but 5 or 6 minutes later (at *e*) it became necessary to resume artificial respiration. The arterial pressure soon fell below 40 mm. Hg.; so the clamp was gradually opened, and though by 12: 18 (at *f*) it was wide open, the circulation did not improve.

The inflow rate gives the clue to at least one of the causes of the circulatory failure. The estimation made a few minutes after the first failure of the respiration, *c*, showed that the peripheral resistance was well below the lower limit of the initial estimations; and subsequently a very marked further dilatation developed. Evidently both centers, respiratory and vasomotor, had become inefficient.

Even though the cava was now wide open, the arterial and portal pressures continued to fall, and as death seemed imminent a large dose of adrenalin (1 cc. of 1:1,000) was given intravenously (at *g*). The arterial pressure rose at once to 234 mm. Hg., indicating that the heart was still capable of as much exertion as in a normal animal. The inflow determinations show that the rise of pressure was due to a peripheral constriction which practically amounted to complete closure of the arteries. But within 18 minutes (12:45) the arterial pressure had fallen to 45 mm. Hg., despite a peripheral constriction that was still almost complete. The jugular pressure, which had mounted about 0.5 mm. Hg. while the circulation was failing (possibly an effect of the artificial respiration), after the administration of the adrenalin fell 1.5 mm. Hg., possibly as a result of some action of the adrenalin upon the heart, or of the removal of the *vis a tergo* through closure of the arterioles. The portal pressure, which had fallen to 5 mm. Hg., was raised by the adrenalin injection to 15 mm. Hg., but fell again and by 12:45 was at 7.5 mm. Hg., a usual value in normal animals. At this particular moment, therefore, despite normal jugular and portal pressures, despite a heart that is capable of elevating arterial pressure quite as high as can a normal heart, and despite a high peripheral

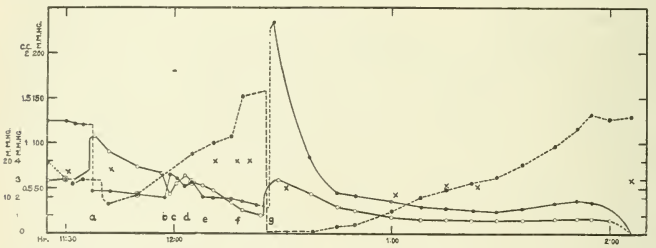

Fig. 5. Experiment 47. Temporary, partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; jugular pressure, x x x ; portal pressure, —○—○—; femoral inflow, – – ● – – ● – –. *a*, cava clamped; *b*, partial unclamping; *c*, respiration stops, artificial respiration started; *d*, artificial respiration discontinued; *e*, artificial respiration resumed; *f*, clamp being gradually opened, at *f*, is wide open; *g*, 1 cc. 1:1000 adrenalin intravenously.

resistance, the arterial pressure is insufficient to maintain life. At this time the respiratory center was still inactive and it may be surmised that the vasoconstrictor center also was inactive or, at least, depressed. It is conceivable, therefore, that while the adrenalin was constricting the arterioles in certain parts of the body, in others the vessels were still maximally dilated. But experiments to be described in another connection, show that the vessels of both the somatic and splanchnic areas are constricted by these large doses of adrenalin. It does not seem possible, therefore, that in the areas not acted upon by adrenalin the resistance would be low enough to permit of as large a fall in arterial pressure as is here seen. Consequently there is left but one other factor upon the basis of which it is possible to account for the failure of the circulation, namely, an insufficient effective volume of blood.

Summary and discussion

The experiments that have been cited as illustrative of the results obtained after partially obstructing the cava can be divided into two groups upon the basis of the rate of onset of circulatory failure. In one group the circulatory failure comes on some hours after deocclusion; in the other, much earlier, it may be while the cava is still occluded.

The results obtained in the *first group* may be generalized and interpreted as follows:

While the cava is occluded the rise in venous pressure causes blood to accumulate in the veins, both systemic and portal, and possibly in the capillaries also, and the arterial pressure consequently falls. The vasomotor center, stimulated by the anemia thus produced, immediately calls forth a peripheral constriction; but later the anemia begins to tell on the center and its tone begins to give way. The moment at which this change takes place can often be recognized: while the tone is increasing the clamp on the cava must be tightened more and more in order to hold the arterial pressure down, but while the tone is diminishing it becomes necessary to gradually loosen the clamp in order to prevent the pressure from falling too low. The rise in portal pressure (the pressure in the systemic veins peripheral of the clamp has not been measured) caused by the occlusion which leads to the sequestration of blood may be surprisingly small and may be taken to indicate how small is the resistance that is needed in order to cause a marked diminution in the effective volume of blood. The effective blood pressure, that is, the difference between the arterial and venous pressure, with respect to that obtaining in the second group of cases, is relatively large; the flow of blood, therefore, is relatively free.

When the cava is unclamped in those instances in which the fall in pressure begins fairly promptly (within a few hours), the arterial pressure rises abruptly at once and then more and more slowly, it may be, to attain a height of 80 or 90 mm. Hg. or more; but sooner or later, usually in less than an hour or two, the pressure starts on the decline that leads to death in the course of some hours. When the onset of the shock-like failure of the circulation is longer delayed, the arterial pressure upon unclamping the cava not infrequently, at first, ascends gradually to a level as high as, or even higher, than that obtaining before the clamp had been applied. The systemic venous pressure may rise slightly throughout the course of the experiment; and this rise may be indicative of some growing inefficiency of the heart (see below).

The portal pressure may be slightly above normal for a while after deocclusion, but it returns to, and then falls below, normal, it may be before the arterial pressure begins to give way. These changes in portal pressure obviously are modified somewhat by the coincident behavior of the peripheral resistance.

With deocclusion and the consequent improvement in conditions, the tone of the vasomotor center increases until, often, the inflow reaches the upper limit of normal. Unfortunately we have not made inflow observations in any of the instances in which the arterial pressure mounts above its pre-occlusion level and, therefore, do not know the condition of the peripheral resistance during the interesting stage of high arterial pressure. The vasomotor tone often continues to increase after the arterial pressure begins to fall, but eventually it begins to slowly decrease. This decrease presumably is to be attributed in part to the continuance of the animal on the table and in part to the diminishing supply of blood to the brain determined by the falling arterial pressure. After the arterial pressure has fallen to 50 mm. Hg., or thereabout, the tone of the center and the arterial pressure usually start on a more rapid decline. There almost always is some, indeed, often considerable, residual vasomotor tone up to the moment the animal dies.

We here point out that if, in an otherwise normal animal, the arterial pressure were lowered by some means to the level to which the arterial pressure rises shortly after deocclusion (often less than 80 mm. Hg.), a very marked constriction unquestionably would result. The fact, therefore, that the center under this low pressure does not succeed in increasing vascular tone beyond that which is normal for a normal arterial pressure, indicates that the center is depressed. Under the stimulus of the continuous low arterial pressure it cannot effect more than a normal grade of constriction. This is not due to the fact that the effort of the center at this time is maximal; for if the animal is asphyxiated, some further increase in tone can be elicited, but this response is not nearly so vigorous as is that given by a normal animal.

Although impairment of the vasomotor center undoubtedly is a factor in the failure of the circulation in this group of cases, it by no means is the only factor. There is evidence that the heart also suffers to a certain extent. In a long series of experiments of the same general character as those described in the present paper, but performed with another object in view, impaired heart action was not infrequently encountered. In such instances, at some time during the period of caval occlusion the heart became irregular in force and rhythm and sometimes stopped

despite removal of the caval clamp. When, in such cases, the heart did not stop and it was possible to carry the animal through the period of occlusion without a return of the cardiac symptoms, it was found that the average height attained by the arterial pressure after removing the clamp was lower than in the cases that had not exhibited these cardiac symptoms. This failure of the pressure to attain an average height during the post-occlusion period presumably is indicative of cardiac weakness. Whether the heart suffers as well in cases in which, during the clamping period, it does not become irregular, is a question we are not prepared to answer. The fact that in these cases adrenalin may raise the arterial pressure quite high does not necessarily mean that the capability of the heart is unimpaired. This is a question that could be answered only by determining the way the heart would hold up under a long continued high resistance.

In the description of the individual experiments, we have, in every instance, called attention to unmistakable evidence of a reduction in the effective blood volume. The blood supplied the heart did not seem to be sufficient in amount to permit that organ to maintain the pressure in the aorta. The condition of the veins gives no definite clue to the whereabouts of the blood that is out of circulation; if it is on the venous side of the circuit it is so held there that it does not materially increase the venous pressure.

The *second group* includes only two cases, both of which had abnormally high initial venous pressures, both systemic and portal. In both, occlusion of the cava caused some further elevation of the systemic, and an enormous elevation of the portal venous pressure. This state of affairs had the effect of markedly reducing the effective blood pressure and, therefore, the blood flow, especially, presumably, in the splanchnic area. The early failure of the respiratory center and the early turning of the preliminary vasoconstriction into a rapidly developing vasodilatation in both cases, and the temporary failure of the heart in one of them, all, presumably, are indicative of a blood supply to the tissues deficient out of all proportion to the arterial pressure of the clamping period. It is barely possible that this is to be attributed to the influence the unusually high venous pressure, exhibited by these two animals, must have had upon the effective arterial pressure. But these direct effects of the deficient blood supply to the tissues are not the sole cause of the failure of the circulation. For in both of the experiments there was a time (some 15 to 17 minutes after applying the clamp) when the heart was still efficient, when the vasomotor tone had not yet passed

below the normal range, and when the venous pressure (portal at least) had returned to its initial level; yet the arterial pressure was far below normal. Evidently a reduction in effective blood volume must be the factor that accounts for the low arterial pressure. This reduction in effective volume could not have been the result merely of static distension of the veins, because the venous pressure, in certain periods, was not any higher than the initial pressure. Loss of tone as a result of some action on a veno-pressor mechanism such as has been described by Hooker (4), loss of tone as a result of local nutritional disturbances, and concentration of the blood are other possible explanations. But further than to state that as rapid a concentration of the blood as would be necessary to effect a change of sufficient magnitude to cause the circulation to fail in 17 minutes, in our experience is not entirely without the realm of possibility, we would postpone the discussion of this question until certain other experiments bearing on the subject have been presented.

Pathological picture and its significance

In this connection the anatomical picture presented by these cases is of some significance. At autopsy the abdomen, in perfectly successful cases, contains no fluid. The small intestine often contains a little blood and the mucosa is usually of a deep bluish-red, almost hemorrhagic, color. This appearance is usually most marked in the upper parts of the small intestine. Below the ileocecal valve the congestion usually is not nearly so striking and may be entirely absent. The spleen often is enlarged and may contain hemorrhagic areas, though it may show no gross changes whatever. The liver as a rule does not seem to be enlarged nor especially full of blood, though Janeway and Jackson (2) state that the volume of the liver, measured plethysmographically, increases, at least until the animal dies. We have carefully weighed the liver in nine of our cases and have found the average to be 3.93 per cent of the body weight. This figure is at the lower limit of normal (3.85 to 5.9 per cent), as given by Ellenberger and Baum (5). Indeed, of the nine livers, seven actually were at or below Ellenberger and Baum's lowest figure, while none reached the uppermost figure. Microscopically, in the few livers we have examined, the venules do not seem to be especially full of blood. This is sometimes true also of the spleen, though more often it is the seat of hemorrhages. The most constant and the most striking change, though, is found in the intestine; almost invariably the capillaries and the venules of the villi are enormously

distended and solidly packed with red corpuscles. The appearance is quite the same as that presented by the intestines of animals dying of shock from exposure of the intestines (1).

This picture indicates that a part of the blood, or at least, of the blood corpuscles, is retained in the capillaries and venules of the villi of the intestines, and sometimes, a part in the spleen. If any excess is retained in the liver, kidneys or stomach, gross and microscopical examination usually fails to reveal it. Other organs have not been carefully examined. The significance of the amount of blood retained in the capillaries and venules will be considered in another connection.

In their experiments Janeway and Jackson found that the volume of the intestine, measured plethysmographically, did not increase, whereas that of the liver did, and they conclude that "there is certainly no special sequestration in the vessels of the small intestine. The experiments indicate rather that there is a special sequestration of the blood in the capillaries of the liver." Whether they made anatomical and histological examinations in addition to the plethysmographic observation is not stated. It is here, however, that our results are not in agreement with theirs. The plethysmograph in their hands has failed to show the concentration of the blood in the villi of the intestines, which we find almost invariably; has led them to conclude that the blood is sequestered in the capillaries of the liver, while we, as a rule, find no post-mortem increase in liver weight or distension of liver blood vessels; and has failed to show the loss of vasomotor tone, which in our experience is characteristic of all excepting the first stages of these experiments. We do not mean to say that the volume of the liver is not increased during life, but merely that the blood is not "*sequestered*" there; otherwise the weight of the liver would be increased and the capillaries distended post-mortem. Nor do we mean to say that the volume of the intestine does not decrease; but merely that a decrease in the volume of a hollow and muscular organ does not necessarily mean that there has been a diminution in the volume of blood its vessels contain. It is quite conceivable that the immediate vasoconstriction and fall in arterial pressure which occur upon clamping the cava decrease the intestinal volume, and that this decrease might be maintained throughout the rest of the experiment; the subsequent diminution in vasomotor tone failing to manifest itself as an increase in the caliber of the vessels, because of the falling arterial pressure and because of the falling venous pressure; the volume effect of the accumulation of blood in the capillaries and venules, being compensated in part by the disappearance of plasma and in part by the emptying of the other vessels of the bowel.

SHOCK BY TEMPORARY PARTIAL OBSTRUCTION OF THE AORTA

Obstruction of the inferior vena cava acts detrimentally upon the circulation in several ways, presumably, *a*, by mechanically causing blood to accumulate in the veins and capillaries, thus removing it from effective circulation; through asphyxial damage, *b*, to the walls of the veins and capillaries, *c*, to the heart and *d*, to the medullary centers. In an effort to determine the relative importance of these several possibilities we have studied the consequences of temporarily interfering with the blood supply to a large part of the body by another procedure, —namely, partial occlusion of the thoracic aorta. For this purpose the aorta was occluded in the chest just beyond the origin of the left subclavian artery. In these experiments, therefore, the blood supply to the upper parts of the body,—the medullary centers, the heart, etc.,—is not diminished; and there is no increase in back pressure in the veins. It was hoped that by thus dissociating the several factors the desired information might be gained.

Methods

The pressure was followed in the femoral artery as well as in the carotid, and the aorta so occluded that, in the case of the three experiments we have done, the femoral pressure at first fell to 45, 30 and 31 mm. Hg., but eventually, by the end of the clamping period, to 40, 16 and 15 mm. Hg., respectively. In two of the experiments therefore, the peripheral pressure for part of the time was held much lower than in the caval experiments. The period of occlusion lasted, as in the caval experiments, about 2 hours. It was necessary, of course, to give artificial respiration throughout the experiments. All three animals behaved essentially alike, yet the differences that occurred are so interesting that each experiment may profitably be considered separately, before an attempt is made to generalize.

Experiment 35 (*fig. 6*). Partial occlusion of the aorta, *a*, raised the carotid pressure from the normal of 90 to 130 to 140 mm. Hg. The increased blood supply to the brain thus effected, at first decreased the vasomotor tone, but then the latter immediately began to increase and before the end of the period of occlusion it had returned to normal.

With deocclusion, *b*, the arterial pressure fell at once to about the initial level and then slowly declined to attain (after 1 to 1½ hours) a pressure of 50 mm. Hg., though the pressure did not remain consistently below 50 mm. Hg. until over 2 hours had elapsed. The vasomotor tone increased sharply at first and then more slowly, so that an hour after deocclusion it was definitely above normal. In the

next period, 2:40 to 3:40, the arterial pressure ranged irregularly between about 40 and 60 mm. Hg. At the same time the inflow rate also fluctuated considerably, around, or somewhat below, the normal level. In the last stage, beginning at about 3:40, the arterial pressure slowly declined from about 50 to 36 mm. Hg., when heart failure supervened. The vasomotor tone in this stage diminished quite rapidly, reaching, to be conservative, the upper limit of normal at the time the heart failure and death led, after a slight asphyxial constriction, to complete failure of the vasomotor center. The animal required no ether during the last 40 minutes of the experiment. The eye reflex was not clearly obtainable.

At 4:00, *c*, the reactivity of the vasomotor center was tested by stopping the artificial respiration. Some constriction did occur. The heart then stopped, but was revived by massage, the artificial respiration being resumed at the same time.

After deocclusion, therefore, the arterial pressure falls despite increasing peripheral constriction. The low arterial pressure presumably at first causes this

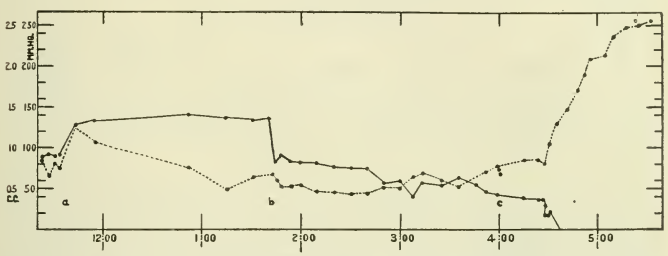

Fig. 6. Experiment 35. Temporary, partial occlusion of the aorta. Carotid pressure, —●—●—; femoral inflow, – – ● – – ● – –. *a*, aorta clamped; *b*, aorta unclamped; *c*, animal temporarily asphyxiated.

constriction through stimulation of the center. The subsequent diminution in peripheral resistance presumably is attributable to the giving way of the centers as a result of the continuance of the low arterial pressure.

In *experiment 36* (*fig. 7*), occlusion of the aorta, *a*, raised the carotid pressure from 102 to a maximum of 146 mm. Hg. by the end of an hour. The carotid pressure then steadily declined, despite the fact, previously referred to, that the femoral pressure steadily decreased. This decline, as well as the fall in femoral pressure, seems to be explained by the behavior of the vasomotor center; for the tone of this center is decreased not alone immediately upon occluding the aorta, as in the preceding experiment, but in addition, the tone decreases more or less consistently and markedly during the whole of the occlusion period.

With deocclusion, *b*, the arterial pressure falls to about 60 mm. Hg., where it remains for a period of 1 hour and 40 minutes (until 2:10). The inflow rate decreases sharply at first and then more slowly, eventually coming into, or slightly below, the initial range. In the last stage, beginning at about 2:10, the arterial

pressure falls to 50 mm. Hg. and then well below it. Vasomotor tone diminishes slowly at first and then more and more rapidly. The animal was finally killed by stopping the artificial respiration, *c*.

In this experiment, therefore, the inflow rate did not become faster than normal until the arterial pressure had been below 50 mm. Hg. for some time. The animal required no ether during the last hour. The eye reflexes were present practically to the end. The behavior of the arterial pressure and of the inflow rate during the period of deocclusion is so similar to that of the preceding experiment that no additional comment is necessary.

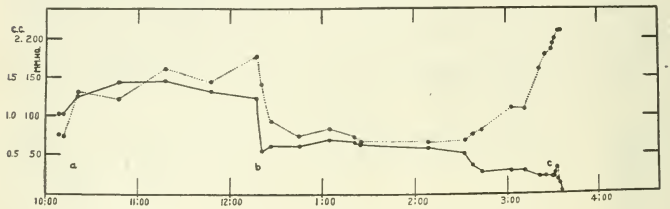

Fig. 7. Experiment 36. Temporary, partial occlusion of the aorta. Arterial pressure, —●— ●—; femoral inflow, – – ●– – ●– –. *a*, aorta clamped; *b*, aorta unclamped; *c*, asphyxiation.

In *experiment 52* (*fig. 8*), clamping the aorta (at *a*) raises the arterial pressure from 105 to 110 to 145 mm. Hg. in the course of about 50 minutes. The pressure then declines until, just before deocclusion, it measures 80 mm. Hg. The general trend of the femoral inflow rate parallels the arterial pressure; but the short, sharp fluctuations of the inflow rate are opposite in direction to those of the central arterial pressure. The general trend, presumably, is an expression of the effect upon centers of the improvement of the circulation through them, while the sharper changes, presumably, are referable to spontaneous changes of some kind in the activity of the vasomotor center, vasodilatation or vasoconstriction being accompanied by a fall or a rise in pressure, respectively. By the end of the occlusion period (at *f*) the inflow has returned almost to the initial rate, despite the fact that the arterial pressure is now well below normal. This fall in arterial pressure, therefore, is not referable to a vasodilatation.

Inasmuch as the circulation through the heart is improved by the rise in pressure in the aorta, weakness of the heart muscle cannot be invoked as a cause of the fall in pressure. It is possible, though, that the high pressure the heart had to work against may overtax its capacity, and cause some inefficiency through dilatation. A fall in arterial pressure due to dilatation of the heart should be associated with a striking rise in the jugular pressure. As a matter of fact the jugular pressure is increased, though somewhat less than 1.5 mm. Hg. The jugular pressure, however, does not increase as the arterial pressure falls, to the contrary, it actually falls somewhat. The fall in arterial pressure cannot, therefore, be explained upon the basis of an increase in the tax on the heart; rather, the fall

in arterial pressure actually relieves the heart. The portal pressure, as might have been anticipated, is lowered by the aortic obstruction, and remains low, with unimportant fluctuations, throughout the period. As, therefore, at the close of the occlusion period the peripheral resistance is practically normal, and as the efficiency of the heart is not impaired, the low arterial pressure can be accounted for only by a diminution in the effective volume of blood.

If occlusion of the aorta here works injuriously to the circulation by damaging the parts in which the blood flow has been cut down, removing the obstruction should increase still further the difficulty of maintaining the circulation. This is exactly what happens: with deocclusion the arterial pressure at once drops below the level of 50 mm. Hg. and continues to fall, the animal dying 15 minutes later through failure of the heart.

Fig 8. Experiment 52. Temporary, partial occlusion of the aorta. Carotid pressure, —●—●—; portal pressure, —○—○—; jugular pressure, —x—x—; femoral inflow – –●– –●– –. *a*, aorta clamped; *b*, clamp tightened; *c*, clamp loosened; *d* clamp loosened more; *f*, clamp off.

At the same time a tremendous peripheral constriction develops. Despite this constriction, which almost stops the inflow, the pressure in the arteries is only 45 mm. Hg. The fall in pressure cannot, therefore, be attributed to any inefficiency of the vasomotor mechanism either central or peripheral. This fact is of great importance in the interpretation of the results obtained; for although there is no reason for believing that the obstruction would injure the centers, it would not have been surprising if it had been found that the long lasting anemia had damaged the peripheral mechanism.

The extreme reduction in inflow rate gives way almost at once to a rapidly developing increase in rate which shows some further acceleration shortly after

the circulation stops. This increase in inflow rate unquestionably is attributable to failure of the center in consequence of the anemia associated with the failure of the circulation. It furnishes final proof of the previous perfect condition of the whole vasomotor mechanism.

With deocclusion, the portal pressure rose to 8 mm. Hg., and then fell as the arterial pressure fell. At no time was the portal pressure high enough to justify the slightest suspicion that an alteration in the portal circulation, through mechanical means, is the cause of the disturbance in the general circulation.

Discussion

The main results obtained in the foregoing experiments on occlusion of the aorta can be summarized in a few words. The fall of the arterial pressure to the level of 50 mm. Hg. is not due to inefficiency of the vasomotor mechanism, either central or peripheral, nor to inefficiency of the heart; it must therefore find its explanation in a reduction in the effective volume of blood.

We thus far have laid stress only upon the direct effects of the occlusion of the aorta as the cause of the failure of the circulation in these experiments. It is a well-known fact, however, that the circulation eventually will fail in animals merely kept on the table under an anesthetic. Thus, Morison and Hooker (6) show a record obtained in an experiment in which the animal, prepared by certain cannulations and by carefully removing from the abdomen one loop of the intestine and keeping this under salt solution, in which a pressure of 50 mm. Hg. was not reached until 11 hours had elapsed. The time required to lower the pressure to 50 mm. Hg. by mere exposure probably is shortened somewhat in our aortic experiments by the trauma of opening the thorax and of giving artificial respiration. The form of anesthesia also is a factor in the determination of the rate of onset of circulatory failure. Thus we have obtained evidence in the present experiments indicating that the administration of morphine increases very greatly the difficulty of bringing on the shock-like failure of the circulation. The morphine seems to have this influence indirectly through its action in sparing the ether necessary to maintain anesthesia. But all of these extraneous factors notwithstanding, there can be no question, in view of the very characteristic responses obtained, both physiological and anatomical, that failure of the circulation for the most part is referable to the effects of blocking the aorta.

Pathology

Our experience with aortic shock has been considerably more limited than with caval shock. But even this limited experience justifies the statement that the gross lesions found in animals dead of both types of shock are very much alike. In aortic shock the liver and kidneys show no certain changes; the stomach has occasionally shown some injected areas. The main changes, again, have been found in the intestines, which may contain bloody material, while the mucosa usually presents a bluish-red, hemorrhagic appearance, it may be, throughout the length of the small gut.

The microscopical picture also resembles very closely that seen after occlusion of the vena cava. Hemorrhages into the spleen pulp are sometimes to be seen. But the striking change is the tremendous distension of the capillaries and venules of the intestinal villi with solid masses of red corpuscles.

Comparison of caval with aortic shock, and discussion

In regard to the primary object of determining the effects of partial occlusion of the aorta it can now be stated definitely:

a. That exactly the same accumulation of blood in the venules and capillaries occurs after aortic occlusion as after caval occlusion; back pressure, therefore, is not the primary cause of the phenomenon, though, to be sure, there is the possibility that back pressure facilitates the process.

b. That failure of the circulation occurs as a result of aortic occlusion despite the absence of any evidences of damage to the heart such as seems, at times, to be caused by caval occlusion.

c. That in aortic occlusion the circulation fails despite (and contrary to what obtained in caval shock) a perfectly functionating vasomotor mechanism.

d. That, therefore, the accumulation and concentration of blood in the venules and capillaries and the failure of the circulation are primarily due to some local peripheral effect of the slowed blood stream.

The failure of the circulation in aortic shock and in caval shock, possibly also in intestinal shock, to us seems to be related to a process first observed by Mall and Welch. It is described by Welch in his classical article on "Embolism and Thrombosis" (7). When, in an animal, a mesenteric artery is partially or completely occluded, the smaller and the larger microscopic veins become more and more distended with red corpuscles and all of the phenomena of an intense venous hyperemia

appear. The red corpuscles accumulate in clumps or in solid columns. This change may become permanent, producing an evident obstacle to the forward movement of the blood. The same phenomena of distension with red corpsucles, clumping and stasis appear gradually in the capillaries. With the partial blocking of the venules and capillaries, red corpuscles begin to pass through the walls of these vessels by diapedesis; and after a time the hemorrhage becomes so great that it is difficult to observe the condition within these vessels. Mall and Welch found that the process begins to take place when the pressure in the artery is reduced to about one-fourth to one-fifth of the normal.

These are just about the conditions that obtain in our experiments during the period of aortic occlusion; and the similarity of the microscopical picture confirms the inference that the processes are similar in the two cases. Mall and Welch seem inclined to attribute the clumping of the corpuscles to the absence of pulsation. Their evidence does not, however, preclude mere slowing of the blood stream as a factor. If it is a factor, then a similar mechanism accounts for the condition found at autopsy in the capillaries and venules after partial occlusion of the cava.

The fact that after deocclusion both in cava[1] and aortic experiments, but especially in the former, the arterial pressure may rise to normal and the circulation show signs of failure only after some hours have elapsed, although explicable on the basis of asphyxial damage to vital functions, also can be accounted for upon the basis of the observation of Mall and Welch that the accumulation of corpuscles in clumps or in solid columns may become permanent.

The mechanism of the dilatation of the capillaries and venules is a question that has not been included in the scope of this investigation. It can, however, be assumed, on the basis of our observations, that the activity of neither the central nor the peripheral parts of the vasomotor mechanism need be affected in order that these processes may take place; the distention of the venules is not attributable to paralysis of nerve terminals. It, therefore, becomes highly improbable that failure of a veno-pressor mechanism (4) is responsible for the dilatation.

CONCLUSIONS

Properly graded temporary partial obstruction of the inferior vena cava above the liver or of the aorta beyond the origin of the left subclavian artery eventually results in the development of a condition closely resembling traumatic shock.

At the time the circulation gives evidence of failing the state of affairs is about as follows:

	AFTER CAVAL OCCLUSION	AFTER AORTIC OCCLUSION
Vasomotor mechanism...	Somewhat impaired	Efficient
Heart action.....	Possibly somewhat impaired	Unimpaired
Effective blood volume...	Evidences of reduction	Evidences of reduction

And at autopsy dilatation of the capillaries and venules of the intestinal villi by masses of red corpscles is found.

It is therefore concluded:

a, That the failure of the circulation after both manipulations is in part certainly due to the consequence of the sequestration of corpuscles in the capillaries and venules.

And *b*, that if back pressure in the veins, such as is produced by caval obstruction, is a factor in the development of this state of affairs in the capillaries and veins, it is not an essential one.

The fact that the vasomotor mechanism need not be involved (as for example in aortic obstruction) renders it unnecessary to invoke failure of a nervous veno-pressor mechanism in order to explain the fullness of the capillaries and venules. It can be explained upon the basis either of the change in the character of the blood stream, or of respiratory and nutritional changes in the walls of the vessels, or, and more probably, of both.

BIBLIOGRAPHY

(1) Erlanger, Gesell and Gasser: This Journal, 1919, xlix, 90.
(2) Janeway and Jackson: Soc. Exper. Biol. Med., 1915, xii, 193.
(3) Stewart and Pike: This Journal, 1907, xix, 328.
(4) Hooker: This Journal, 1918, xlvi, 591.
(5) Ellenberger and Baum: Anatomie des Hundes, Berlin, 1891.
(6) Morison and Hooker: This Journal, 1915, xxxvii, 86.
(7) Welch: Allbutt's System of medicine, 1910, vi, 779.

GASTRIC RESPONSE TO FOODS[1]

III. The Response of the Human Stomach to Beef and Beef Products

HAMILTON R. FISHBACK, CLARENCE A. SMITH, ÒLAF BERGEIM, ROBERT A. LICHTENTHAELER, MARTIN E. REHFUSS AND PHILIP B. HAWK

From the Laboratory of Physiological Chemistry of the Jefferson Medical College, Philadelphia

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Among the foods of man the meats hold a high place. Because of their high protein content and the presence of connective tissues which are digested more readily in an acid than in an alkaline medium the rôle of the stomach in the digestion of meats is one of greater importance than with certain other foods. For this reason it has been considered worth while to carry out a series of studies on the response of the stomach to meats. The beef products are among the most widely used of meats.

The digestion time of meats in the human stomach has been studied by Beaumont (1), Jessen (2) and Penzoldt (3). The values commonly quoted in the literature are the results of their investigation. Beaumont's experiments were carried out on a single man; the hunter, Alexis St. Martin, who possessed a permanent gastric fistula. Beaumont records some experiments on beef and beef products, in most cases the meat fed being but a part of the mixed meal which also included water *ad libitum*. The amounts of meat fed were ordinarily regulated only by the appetite of the subject. Beaumont's results were for a long time the only ones available. His findings on beef are shown in table 1.

Jessen's subject was a man thirty years old. . He was given 100 grams of meats of various kinds with 300 cc. of water. The stomach was emptied after a couple of hours. If meat fibers remained, the test was repeated on another day using a longer interval. The data given in table 2 were obtained as the result of five tests.

[1] The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

Penzoldt used two men as subjects, one being given 250 grams of meat, the other 100 gram portions. Water ingestion was not limited, as much as 1200 cc. being given in some cases. Toward the estimated end of digestion, samples were pumped out every 15 minutes, using a large stomach tube, until the stomach had been emptied. A little

TABLE 1

Digestion time of beef in the human stomach (Beaumont)

KIND OF MEAT	COOKING	HOURS	MINUTES
Beef	Roast rare	3:00	
Beef	Roast well-done	3:00	30
Beef steak	Broiled	3:00	
Beef boiled	Boiled	2:00	45
Beef liver	Broiled	2:00	

TABLE 2

Digestion time of beef in the human stomach (Jessen)

KIND OF MEAT	COOKING	EVACUATION TIME
		hours
Beef	Raw	2
Beef	Boiled medium	$2\frac{1}{2}$
Beef	Boiled well-done	3
Beef	Roast medium	3
Beef	Roast well-done	4

TABLE 3

KIND OF MEAT	COOKING	PRAEGER 100 GRAMS	GIGGLBERGER 250 GRAMS
		hours	*hours and minutes*
Beef sausage	Raw	$2\frac{3}{4}$	3:25
Beef steak	Fried warm	$3\frac{1}{2}$	4:15
Beef steak	Fried cold	$3\frac{1}{4}$	
Beef steak	Raw chopped	$3\frac{1}{4}$	3:10

meat and water was given after each sample to replace that removed. The results this author obtained are presented in table 3.

The present series of tests on beef were carried out on normal medical students. Over seventy complete experiments were made on twenty-five different subjects. The results of sixty-four of these experiments

are reported in the present paper. Most of these tests were started at 1:00 p.m., the residuum from breakfast being previously removed with the Rehfuss stomach tube. One hundred gram portions of the meats prepared in various ways were ingested, the stomach tube being usually kept in place, though in a few cases it was withdrawn during the period of mastication. The presence of the tube did not, in most cases, interfere with the chewing of the food. The subject was told to follow his regular habit as to mastication. From 15 to 25 minutes were ordinarily required. Samples of from 5 to 8 cc. volumes were withdrawn at 15 minute intervals until the stomach was empty. Determinations of free and total acid, pepsin and amino nitrogen were carried out according to the procedure previously described (4), the method for amino nitrogen being a formol titration method. Specimens were also examined as to amount and character of meat present, character of liquid and other features. The emptying time was shown by the disappearance of meat residues from the samples or by failure to aspirate anything from the stomach with the subject placed successively in four positions, on right side, left side, face downward and on the back. The emptying time was confirmed by giving at once 100 cc. of water as lavage and immediately withdrawing same. This lavage in all cases showed a very low acidity (from 1–10), was clear or slightly cloudy and contained no meat residues except occasionally traces adhering to the mucus present. The lavage water was recovered quantitatively within a few cubic centimeters in these cases.

Subjects were chosen in such a way that direct comparisons of the different methods of cooking, etc., could be made under similar conditions. The subjects were at rest, that is, reading magazines, playing cards, copying notes or studying.

Table 4 gives a summary of the results obtained with beef prepared in different ways. The subjects are classified as having rapid- or slow-emptying stomachs. This classification has been found necessary because of the fact that there are marked differences between the emptying times of the stomachs of different entirely normal individuals under exactly the same conditions, this rapid or slow emptying being characteristic of a given stomach and very constant. The classification into types as used in this table is based not only on experiments with beef but on numerous experiments with other foods. About half of the men observed fall into each class. Nothing but confusion can result from a disregard of the marked differences in the response of the stomachs of different entirely normal individuals to the same stimuli.

TABLE 4

Response of the human stomach to beef and beef products

NO.	SUBJECT	FOOD AND PREPARATION	TYPE OF STOMACH							
			Rapid-emptying type				Slow-emptying type			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				aver-age		*aver-age*		*aver-age*		*aver-age*
1	Tri	Roast beef rare	2:45		70					
2	Ri	Roast beef rare	2:00		110					
3	Joa	Roast beef rare	2:45		90					
4	Cru	Roast beef rare	2:30		130					
5	Gl	Roast beef rare	2:45	2:30	110	102				
6	Sim	Roast beef rare					3:30		120	
7	Re	Roast beef rare					2:45		130	
8	Wal	Roast beef rare					2:30		130	
9	Oa	Roast beef rare					3:00	3:00	112	120
10	Ara	Roast beef medium	1:45		122					
11	Bos	Roast beef medium	2:45	2:15	122	122				
12	Sim	Roast beef medium					3:15		120	
13	Ral	Roast beef medium					3:45		108	
14	Kon	Roast beef medium					3:15	3:30	110	113
15	Ri	Roast beef well-done	2:00		105					
16	Lun	Roast beef well-done	2:30	2:15	123	114				
17	Ca	Roast beef well-done					3:30		131	
18	Con	Roast beef well-done					3:45		126	
19	Joe	Roast beef well-done (250 grams)					3:15		128	
20	Ne	Roast beef well-done (250 grams)					3:45	3:30	164	138
21	Con	Roast beef well-done (250 grams)					5:45	5:45	138	138
22	Hou	Steak hamburger round raw					3:00		110	
23	Sim	Steak hamburger round raw					3:00	3:00	113	111
24	Mor	Steak hamburger round (well-done)	2:00	2:00	98	98				
25	Kon	Steak hamburger round (well-done)					3:00		118	
26	Lec	Steak hamburger round well-done					3:00	3:00	120	119
27	Ri	Steak hamburger chuck well-done	2:00	2:00	109	109				
28	Joa	Steak rump tough rare	2:00		95					
29	Tri	Steak rump tough rare	2:30	2:15	135	115				
30	Joa	Steak rump well-done	2:00		93					
31	Tri	Steak rump well-done	2:45	2:30	153	123				
32	Swe	Steak shank tough well-done	2:30	2:30	111	111				
33	Con	Steak shank tough well-done					3:45	3:45	126	126

TABLE 4—*Concluded*

NO.	SUBJECT	FOOD AND PREPARATION	TYPE OF STOMACH							
			Rapid-emptying type				Slow-emptying type			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				aver-age		*aver-age*		*aver-age*		*aver-age*
34	Ara	Steak sirloin medium	3:00	3:00	128	128				
35	Kna	Steak sirloin medium					3:45	3:45	135	135
36	Joa	Steak sirloin well-done	3:15		118					
37	Kli	Steak sirloin well-done	3:00	3:10	131	125				
38	Joa	Steak tenderloin medium	3:30	3:30	98	98				
39	Tri	Steak sirloin well-done	3:30	3:30	168	168				
40	Oa	Steak sirloin planked medium					3:00		151	
41	Mab	Steak sirloin planked medium					3:00	3:00	144	147
42	Cla	Steak sirloin planked well-done					3:15	3:15	79	79
43	Joa	Steak tenderloin planked medium	3:00	3:00	92	92				
44	Wal	Steak round boiled					3:45	3:45	139	139
45	Ne	Stewed beef					3:15		88	
46	Ral	Stewed beef					4:15	3:45	107	100
47	Ral	Stewed beef (250 grams)					6:00		147	
48	Ne	Stewed beef (250 grams)					5:30	5:45	117	132
49	Ara	Dried beef	2:15	2:15	120	120				
50	Bec	Dried beef					3:45	3:45	90	90
51	Bec	Beef tongue boiled					4:30		159	
52	Kar	Beef tongue boiled					4:00	4:15	131	145
53	Lun	Corned beef boiled	2:45	2:45	77	77				
54	Sim	Corned beef boiled					3:30	3:30	125	
55	Wal	Corned beef boiled					3:45	3:30	125	125
56	Cru	Bologna	2:45		130					
57	Bos	Bologna	2:30	2:45	105	118				
58	Ara	Liver, calves, fried	2:30		110					
59	Bos	Liver, calves, fried	3:15	2:45	135	122				
60	Gl	Frankfurters (80 grams)	2:15		118					
61	Ca	Frankfurters	2:45	2:30	109	114				
62	Spk	Sweetbreads	2:15	2:15	85	85				
63	Tri	Tripe	3:30	3:30	125	125				
64	Oa	Tripe					3:15	3:15	110	110

* Acidity expressed in cc. of N/10 alkali required to neutralize 100 cc.

This was clearly brought out by a comparison of the average evacuation times of the two types of subjects on beef. These were found to be 2 hours 35 minutes for subjects of the rapid type and 3 hours 25 minutes for subjects of the slow-emptying type. Note also the three tests on subject "Ri" (expers. 2, 15 and 27) with roast beef and beef steak, the evacuation time in each instance being 2 hours, acidities varying only from 105–110. Compare this with tests on one of the men of a slow-emptying type, e.g., subject "Con" who showed evacuation times of 3 hours 45 minutes each on roast beef and beef steak (expers. 18 and 33) and gave in each case an acidity of 126. The response of a normal subject as regards the forms of the acid curves and the evacuation times are constant within quite narrow limits when tests are carried out on different days using similar foods. The acidities vary somewhat more than the emptying times. It is of course possible in spite of the large number of subjects used and tests made and cross checking wherever practicable, that in some cases the results are influenced to a slight extent by uncontrolled variations in individual response, which in general show a remarkable constancy. Very seldom was an instance noted where a stomach of the slow type emptied sooner than one of the rapid type on a given food. Experiments 63 and 64 on tripe show such a condition and must be explained on the basis of a personal idiosyncrasy of one of the subjects for this particular food.

It is necessary to carefully distinguish rapid emptying with high acidity (a normal response), from rapid emptying with low or anacidity which is usually pathological, and is readily brought about, for example, by a cold. No experiments on the latter class of individuals are reported here.

The curve of acidity may be taken as a measure of a glandular response of the stomach. With meats this response gave the highest normal total acid (184 cc. N/10 KOH — 100 cc. gastric juice) that has been obtained on any class of food in this laboratory. The acidity due to free HCl in this case was 133. Carlson found that pure gastric juice from his one subject, Mr. V., did not rise above an acidity of 0.48 per cent HCl, which is equivalent to 132 cc. N/10 KOH required to neutralize 100 cc. of gastric juice. The average high point of the acid curves in our beef tests was 120 with many instances of acidities between 130 and 160. These acidities are far higher than the values usually accepted by physiologists and clinicians for the acidity of human gastric juice and gastric contents. Values which have been held to represent conditions of marked pathological hyperacidity are found to be within the average

range of normal subjects and require a revision of our ideas as to what constitutes "hyperacidity."

The total acid is the strength of the gastric juice as secreted minus the lowering produced by the neutralizing and diluting effect of ingested food and saliva and possible regurgitated duodenal contents. After taking the test meal all saliva was expectorated into a beaker furnished the subject and kept constantly by him, so that after eating the error introduced by the esophagus was probably small. The diluting effect produced by ingested material therefore grows smaller and smaller as digestion proceeds and the gastric juice continues to be poured out in increasing ratio to the original volume. In the meat tests especially the primary amount is comparatively small when the total volume of juice is considered. This has been estimated by Carlson (5) to be as much as 700 cc. for a single meal.

The neutralization effected by regurgitation of duodenal contents is inconstant, but fortunately when digestion is going on with chyme passing into the duodenum there is practically a constant flow also of pancreatic juice into the duodenum, so that regurgitation at this time is evidenced by trypsin in the stomach. The presence of bile with regurgitation is variable, with foods which do not stimulate the production of bile. Meats, however, do cause a flow of bile so that in most cases in which there was found a significant drop of the acid before the final downward curve, bile could be demonstrated in the sample.

No association could be found between visible evidence of regurgitation and the height of acidity in the stomach. There seemed to be a tendency for bile to appear oftener with the long-continued digestions. With this there was associated the factor of individuality in that some subjects constantly showed regurgitation at some point of the test meal, while others rarely did.

The total acid curves shown have, then, been influenced almost constantly by the amount of food ingested. The type of curve was further influenced by the constant flow of gastric juice and by inconstant regurgitation of duodenal contents which could be demonstrated by the presence of bile. The degree of total acidity given by the added gastric juice is the best measure of the response of the gastric glands.

The other chief factor in determining the curve given is the rate of passage of the food from the stomach. It is generally considered that this begins when the food at the pyloric end of the stomach has been prepared for the entrance into the duodenum. The rate of its passage is fixed by the rapidity of digestion in the stomach which in turn is deter-

mined by the activity of the stomach and the digestibility of the food. With a given acidity of the stomach, then, the digestibility of a food is indicated by the emptying time of the stomach. This relates principally to meats and other high protein foods which are subjected to considerable digestion in the stomach.

The motor function of the stomach which is a most important one is determined in a healthy man largely by individual characters. So with a given kind of food as a stimulus there are shown quick-emptying and slow-emptying stomachs. This time division is not absolute with different kinds of foods but is only relative to the types of stomachs.

Other analyses made upon the gastric juice were free acid, pepsin and the formol titration.

The free acid found in any fresh gastric sample may be influenced by the total acidity, the rapidity of secretion of the gastric HCl and the rate of combination of the acid with food materials in the stomach. In general the free acid follows fairly well the trend of the total acid curve. The difference between the total acid and free acid which represents mainly combined acids might be taken to indicate the rate of protein digestion in the stomach.

The formol titration of Sörenson has been performed with the hope that it might lead to valuable data upon the rate of protein cleavage in the stomach. This was used by Zunz (6) in working upon the human stomach contents and the stomach contents of cats and dogs. He found no definite relationship between the time the food was eaten, the degree of hydrolysis of the food, the amounts of ammonia or amino nitrogen split off and the free or total acid secreted.

Christiansen (7), however, working with human gastric contents found that the increase in bound (combined) HCl is equivalent to that of the free amino groups determined.

The formol titration includes not only the free amino groups of the gastric contents but also the ammonia of the gastric juice and of the meats fed, and a slight amount from the saliva. Carlson (8) reports that his gastric fistula subject showed an average of 3 mgm. ammonia nitrogen in 100 cc. of gastric juice. The amount of ammonia nitrogen in fresh raw beef ranges from 3 to 10 mgm. in 100 grams of meat (9), (10).

In general the curves show a tendency to rather high initial values of formol-titratable nitrogen. They then tend to follow the total acid curve upward, but break downward somewhat more quickly than the final drop of the total acid curve, and generally end low as compared with the initial point.

The high beginning is probably due to the rapid extraction of ammonia nitrogen from the finely divided meat ingested. The rise in the curve thereafter is due then to the splitting of proteins, with the liberation of more amino groups and slight amounts of ammonia. Since, however, most of the protein products are not final products the amount of amino groups formed is comparatively small, so that a slight rise in the amino curve probably indicates considerable protein cleavage. This rise of the curve is undoubtedly minimized also by the fact that the soluble products of digestion leave the stomach fairly rapidly. This last fact is also the explanation of the early downward break of the amino curve.

Pepsin was determined in a number of tests, but was not continued throughout the series. The charts show constantly that there was a rise of the pepsin curve which became more marked toward the end of digestion. Furthermore, consecutive samples may show pepsin values which

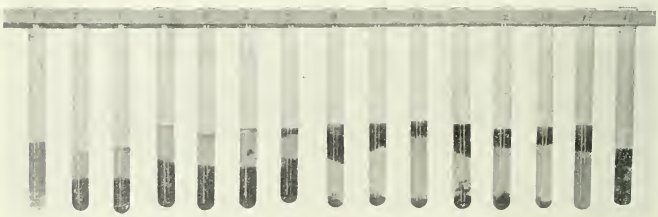


Fig. A1

vary widely out of the general course of the curve, so that no reliance can be placed in any sample as showing the true peptic strength of the gastric juice as secreted. The reason for this is found in the meat proteins which are so abundant and which adsorb pepsin easily, so that the juice as it is filtered leaves behind with the meat a large part of its pepsin content. As the meat is digested and leaves the stomach, the pepsin curve gradually rises due to the larger amount of that enzyme in proportion to the undigested proteins. A true test of the pepsin-secreting power of the stomach should be made with some stimulant which does not adsorb pepsin. Working with water and glucose solution, Fowler (11) showed in this laboratory that the pepsin content of the gastric juice is proportional to the height of total acidity up to a certain point. This would probably be found true also with meats were the difficulties of estimating the true value of pepsin to be overcome.

Passage of different constituents of meat from stomach. The meat which is taken as test meal is not all uniformly mixed, and disintegrated in the stomach, and passed into the duodenum as a homogeneous mass. As the characters of the samples were observed it was early noted that there is a separation of different parts of the meat which leave the stomach separately. The finely divided portions which settle to the bottom of a sample tube empty first, then the coarse fibrous material which is on top of the sample after standing, and finally the fatty material. The effect of gravity seems to play a considerable part in the emptying of the various constituents. This is illustrated by the photograph (fig. A1) and following description of samples obtained after a test meal of meat had been given to a normal subject.

Description of samples

1. 11 cc. Layer fat at top 2 mm. fine globules. Solids 0.7. White. Fluffy. Fibrous. Fibers held in bunches in mucus. Shows globules (fine) of fat in fibers. Liquid—water clear.

2. 9 cc. Fat up 2 mm. fine globules. Solids 0.5 down, very fine fibers which are separated. Mucus of previous sample all dissolved. Very fine fat globules mixed in. Light brownish pink. Liquid—very slightly turbid. Whitish.

3. 10 cc. Trace of fat up. Solids 0.5 down. Like no. 2 except no fat mixed in.

4. 13 cc. Solids 0.5 down. Like no. 2. Liquid—slightly turbid.

5. 12 cc. Like no. 4. Solids 0.5 down.

6. 13 cc. Solids 0.4 down. Material is more pinkish. Less of brown. More fluffy.

7. 12 cc. Solids 0.3 down and 0.1 up. Light brown.

8. 13 cc. Trace of solids down and 0.4 up and 2 mm. solids fat up. Material up is coarse fibers clumped in masses and light brown.

9. 12 cc. Solids 0.1 down and 0.3 up. Trace fat. Same as no. 8. Down more brownish. Liquid—clear.

10. 13 cc. Solids 0.1 down and 0.2 up. Like before. Sediment is fine yellowish. Liquid—turbid. Very light yellow (slight regurgitation).

11. 13 cc. Solids 0.3 up. Same as sediment of no. 10. 0.1 down. Trace of fat up.

12. 14 cc. Solids 0.3 up and 0.1 down. Trace fat up.

13. 13 cc. Solids 0.2 up ($\frac{1}{2}$ coarse fibers and $\frac{1}{2}$ fat). Trace down of fine yellow. Liquid—very slightly turbid. Very slight trace yellow.

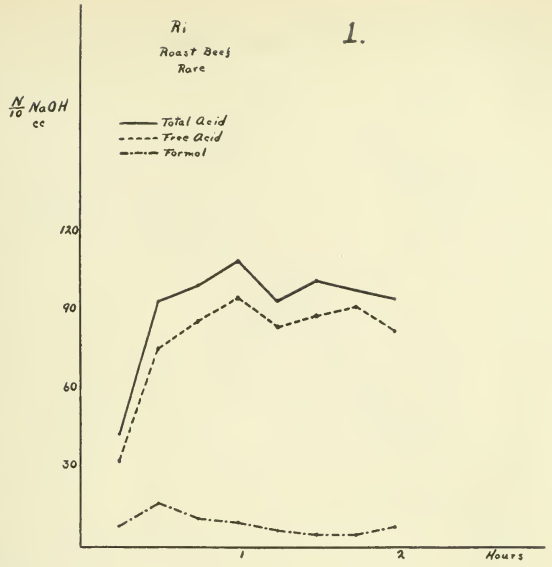
14. 14 cc. Solids 0.3 up ($\frac{1}{2}$ coarse fibers and $\frac{1}{2}$ fat). Trace down fine yellow. Liquid—very slightly turbid. Very slight trace yellow.

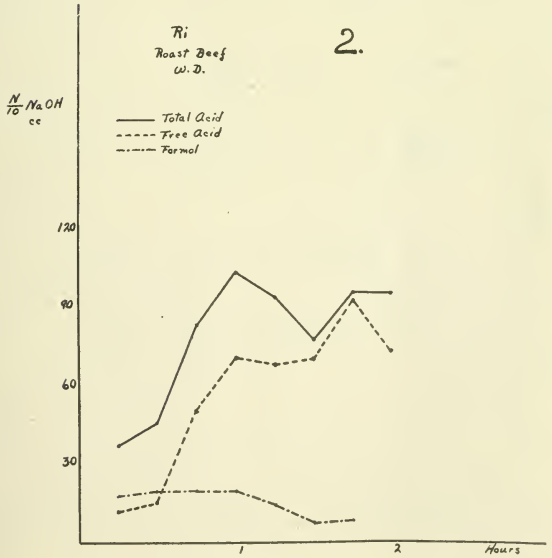
15. 9 cc. Considerable (about 0.2) very fine light brown granular material throughout the solution. Trace fat up. Probably washed from walls of stomach.

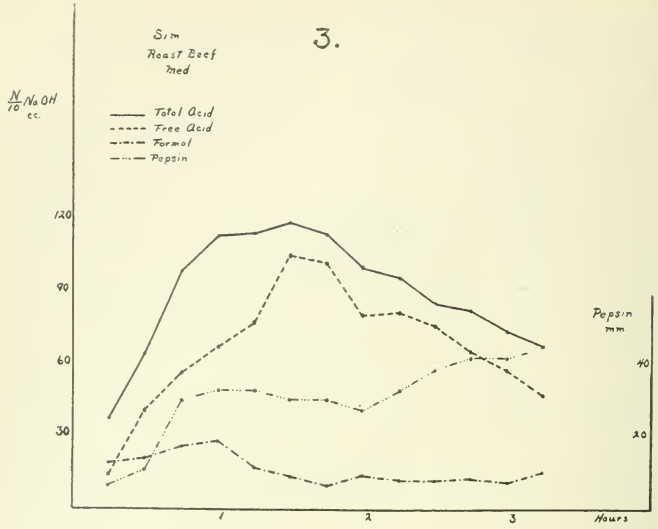
DISCUSSION OF RESULTS OBTAINED WITH DIFFERENT FORMS OF BEEF AND BEEF PRODUCTS

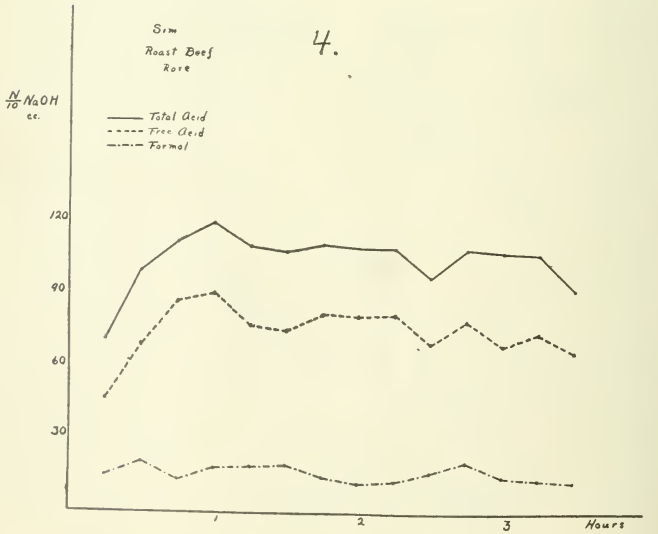
Roast beef. Figures 1 and 2 show the results obtained after feeding rare and well-done roast beef respectively to a man of the rapid-emptying type. Figures 3 and 4 show the comparative effects produced by rare and medium well-done roast beef in an individual of the slow-emptying type. It will be noticed that as far as maximum acidities developed and evacuation time were concerned the mode of cooking showed very little effect. The averages given in table 4 show the same tendency. The rare roast beef may be slightly more easily digested than the medium or well-done. Our figures for rare and well-done roast beef on the slower type of stomachs are the same as those obtained by Beaumont; 3 and $3\frac{1}{3}$ hours respectively. In no case however was a variation as great as that of Jessen found who noted in his subject an evacuation time of 3 hours for medium and 4 hours for well-done beef. Grindley found that different methods of cooking meat had no influence upon utilization in the human organism (12).

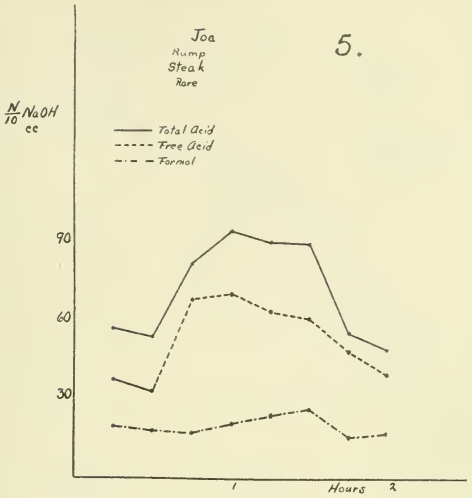
Beef steaks. Steaks from several different cuts of beef prepared in different ways were tested. For average results see table 4. The evacuation times for men of the rapid type varied from 2 to $3\frac{1}{2}$ hours and for the slow men 3 to $3\frac{3}{4}$ hours. Varying the degree of cooking seemed to have little effect. Neither rump, shank, sirloin nor tenderloin steaks showed more than 15 minutes difference in time of digestion of rare, well-done or medium-well-done products, with one exception which must be otherwise explained. Some differences were, however, noted with regard to the various cuts of beef used. The cheaper and tougher cuts of meat showed more rapid evacuation from the stomach than more expensive and more tender cuts such as tenderloin. This is brought out clearly by the summary of averages given in table 4. Curves showing the response of the same individual to rare and well-done steaks of the same cut (rump) are shown in figures 5 and 6. These should also be compared with figures 7, 8 and 9 showing the reactions of the same individual to a sirloin, a tenderloin and a planked tenderloin steak. It will be noted that these latter required from an hour to an hour and a half longer to leave the stomach and that planking reduced slightly the time required. The difference in favor of the cheaper and tougher cut is also clearly shown by a comparison of figures 10 and 11. The cheap cuts of meat used and the steaks prepared from them were extremely tough and were chosen particularly with a view to comparing the digestion of the toughest obtainable steak with the digestion of tenderloin

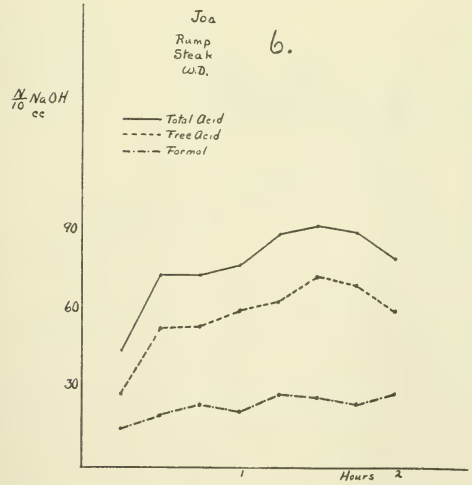

Ri
Roast Beef
Rare
1.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours


Ri
Roast Beef
W. D.
2.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours

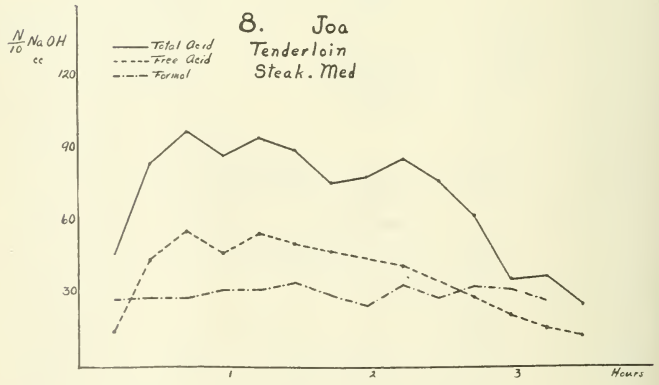

Sim
Roast Beef
Med
3.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
Pepsin
120
90
60
30
Pepsin mm
40
20
1
2
3
Hours

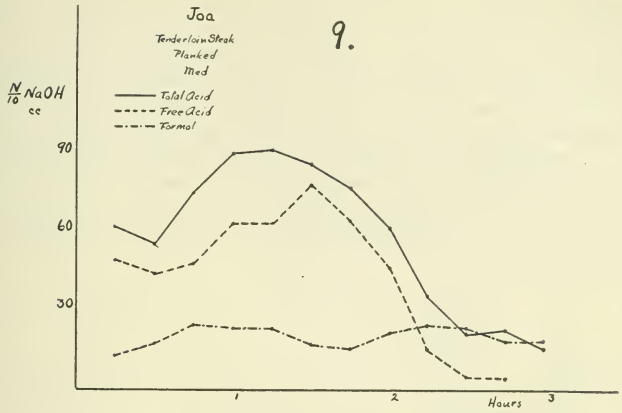

Sim
Roast Beef
Rare
4.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours

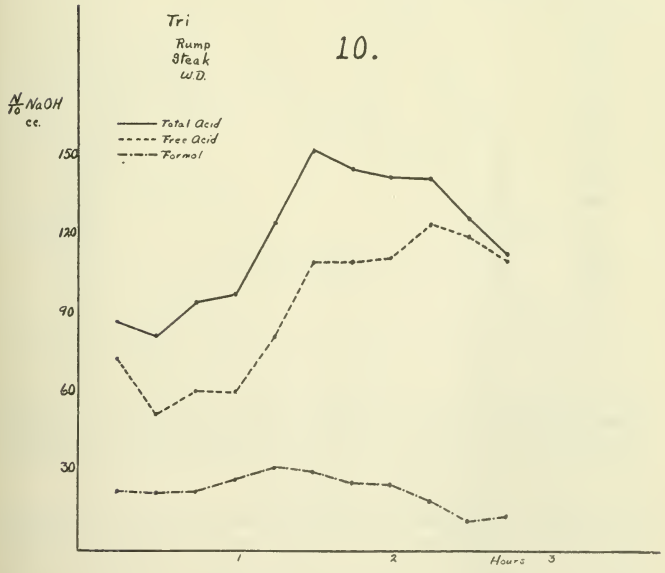

Joa
Rump
Steak
Rare
5.
N/10 NaOH
cc
Total Acid
Free Acid
Formal
90
60
30
1
Hours
2


Joa
Rump
Steak
W.D.
6.
N/10 NaOH
cc
Total Acid
Free Acid
Formal
90
60
30
1
Hours
2

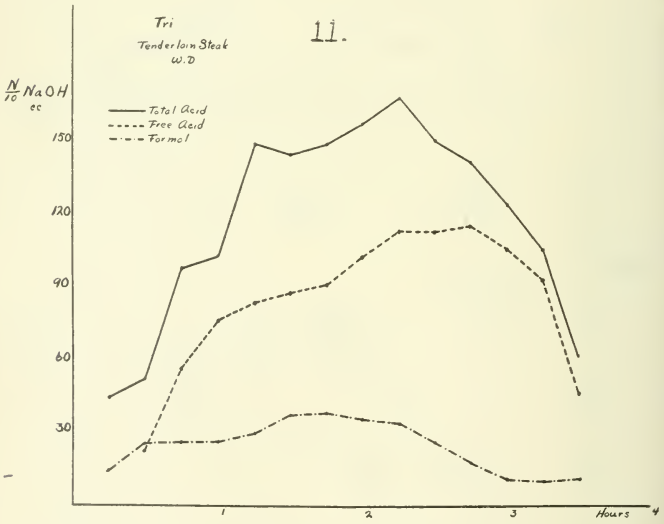

Joa
Sirloin
W.D
7.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


N/10 NaOH
cc
Total Acid
Free Acid
Formol
8. Joa
Tenderloin
Steak. Med
120
90
60
30
1
2
3
Hours


Joa
Tenderloin Steak
Planked
Med
9.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
90
60
30
1
2
3
Hours

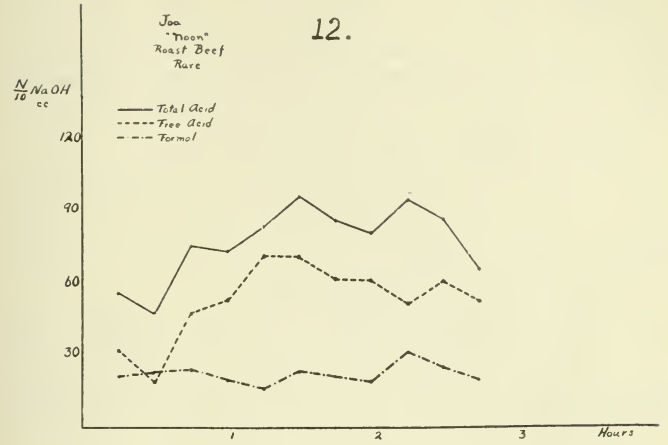

Tri
Rump
Steak
W.D.
10.
N/10 NaOH
cc.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

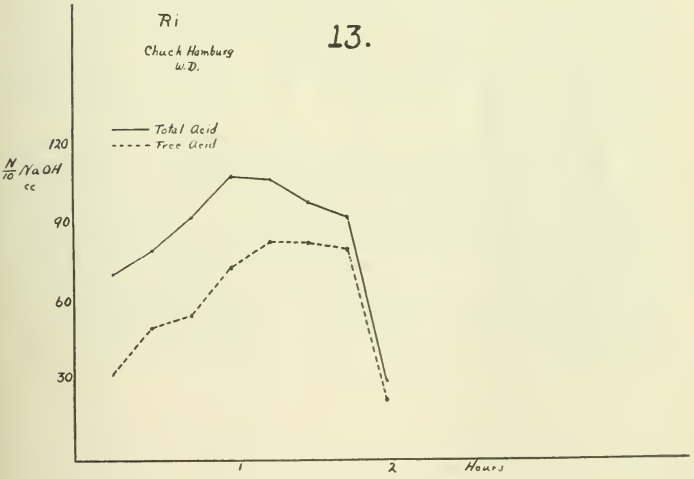
steaks. These latter were prepared by the chef of a high grade restaurant and were of course far more appetizing in appearance and taste as well as more tender than the others. That the cost of the different cuts of beef bears no relation to nutritive value has been shown particularly by Hall and Emmett (13). Cost is dependent upon such considerations as tenderness, grain, color, general appearance and convenience of cooking. According to the authors cited, the choice portion of beef forming about one-fourth its total weight represents nearly one-half its retail cost. From the standpoint of protein and energy, the cheaper cuts were found to be as valuable and in some cases more valuable than higher-priced cuts.



It is fortunate that our stomachs also give favor to the cheaper cuts of beef by digesting them more easily than expensive cuts. A typical comparison between roast beef and these steaks is obtained from figure 12. It will be seen that roast beef leaves the stomach sooner than tenderloin but more slowly than a rump steak.

Hamburger steaks. That a hamburger steak remains in the stomach about the same length of time as an equal amount of roast beef is illustrated by a comparison of these with the same individual (see figs. 3, 4 and 13). It may be that hamburger is handled a little more easily than roast beef but the difference is very slight.


Joa
"Noon"
Roast Beef
Rare
12.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Ri
Chuck Hamburg
W.D.
13.
Total Acid
Free Acid
120
N/10 NaOH
cc
90
60
30
1
2
Hours

Stewed beef. Figures 14, 15, 16 and 17 show comparisons between roast beef and stewed beef on the same individuals. In one case the stewed beef left the stomach the sooner while in the other case the reverse was true. In one case the acid stimulation was greater with roast than with stewed beef. Apparently the response of the stomach to stewed beef is not much different from that to roast beef.

Corned beef. Boiled corned beef shows little difference in gastric response from that of roast beef or stewed beef. Evacuation times are about the same. The boiled meats appear, however, to be somewhat less active in stimulating secretion as the rise in the curve is usually somewhat delayed. This may be due to loss of extractives. Compare figure 18 with figures 3 and 4 and figures 19 and 20.

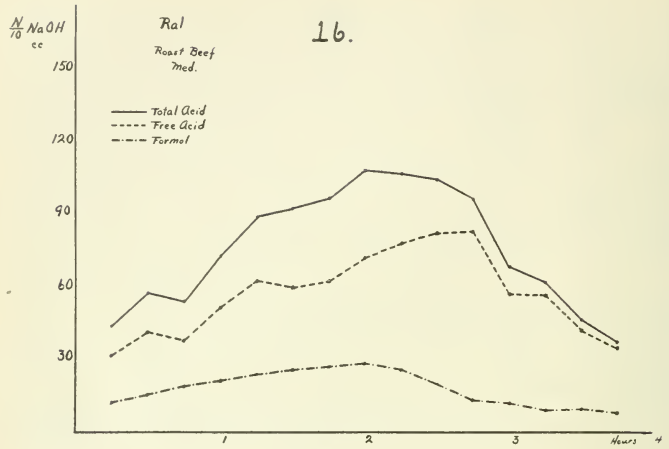
Dried beef. In the case of dried beef 70 grams were fed instead of 100 grams on account of the high solid content of this form of beef. Dried beef appears to be about as digestible as ordinary roast beef, but the lack of moisture where no water is taken with the meat appears to delay slightly the onset of digestion. See figures 21 and 22 for a comparison of dried and roast beef on a subject of the rapid type.

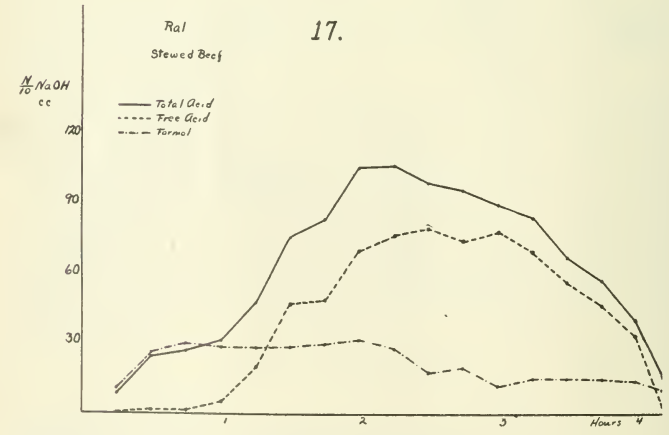
Beef bologna, calves' liver and beef tongue. Beef bologna was found in two cases to be digested in practically the same time as roast beef (see figs. 23 and 24). Calves' liver required a slightly longer time to leave the stomach than roast beef or dried beef. This is not in agreement with the finding of Beaumont (compare figs. 22 and 25). Boiled tongue was found in a single case to take over $4\frac{1}{4}$ hours as compared with $3\frac{3}{4}$ hours for dried beef.

Frankfurters, sweetbreads and tripe. Beef frankfurters left the stomach rather more quickly than most of the other forms of beef. For example, 80 grams of boiled frankfurter left the stomach in $2\frac{1}{4}$ hours in one case (fig. 26) as compared with $2\frac{3}{4}$ hours for 100 grams of roast beef (fig. 27). Calf sweetbreads required $2\frac{1}{4}$ hours to leave the stomach in our test, thus belonging to the class of more rapidly moving meats (see fig. 28). The so-called "honeycomb" tripe left the stomach a little more slowly than roast beef (see figs. 29, 30 and 31). The gastric digestion of tripe did not appear, however, to differ materially from the digestion of other beef products.

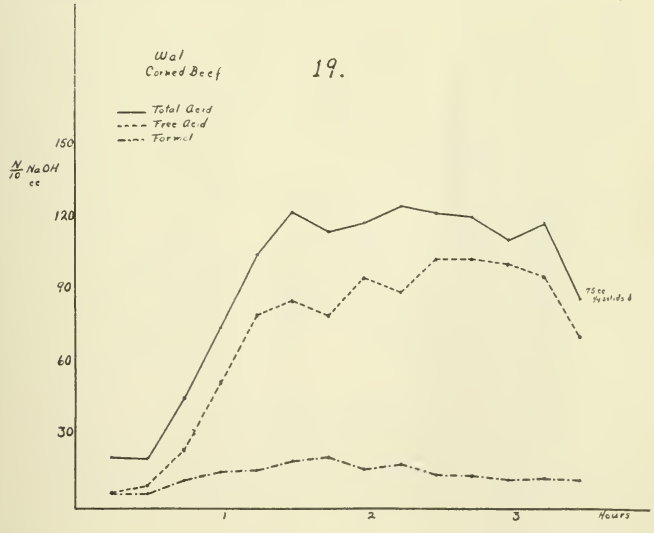

Ne
Stewed Beef
14.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours

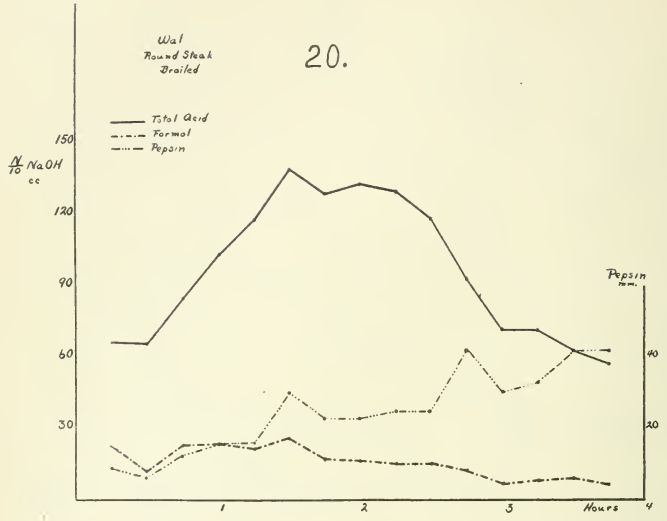

Ne
Roast Beef
W. D.
15.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

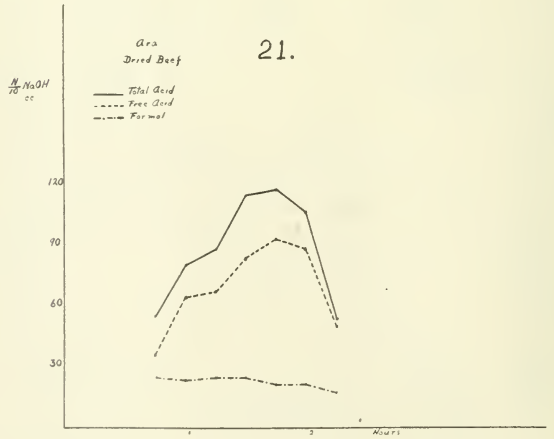

N/10 NaOH cc
Ral
Roast Beef
Med.
16.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours
4

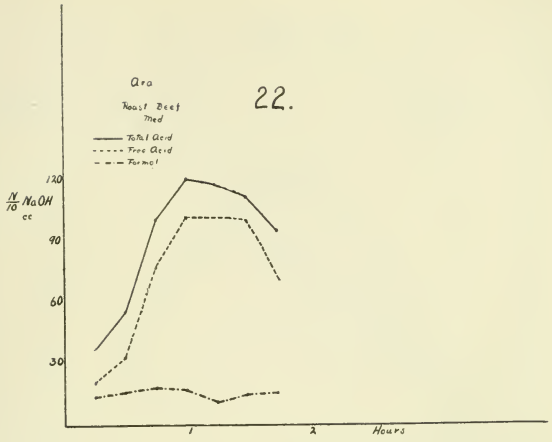

N/10 NaOH cc
Ral
Stewed Beef
17.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4


Sim
Corned Beef
18.
Total Acid
Free Acid
Formol
150
N/10 NaOH cc
120
90
60
30
1
2
3
Hours

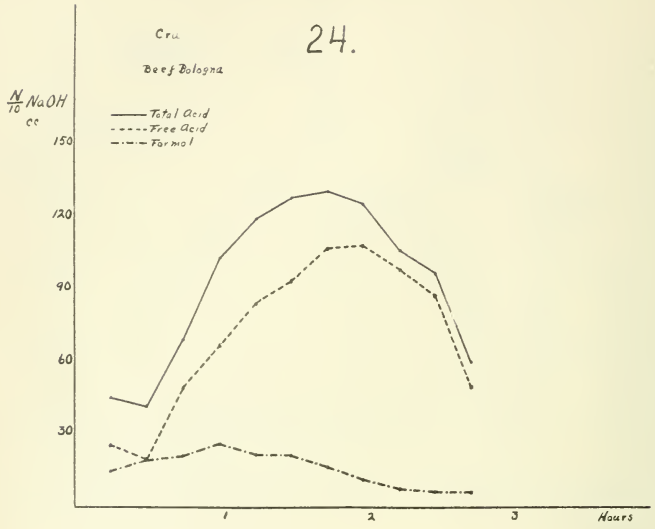

Wal
Corned Beef
19.
Total Acid
Free Acid
Formol
150
N/10 NaOH cc
120
90
60
30
75cc hydrochloric acid
1
2
3
Hours

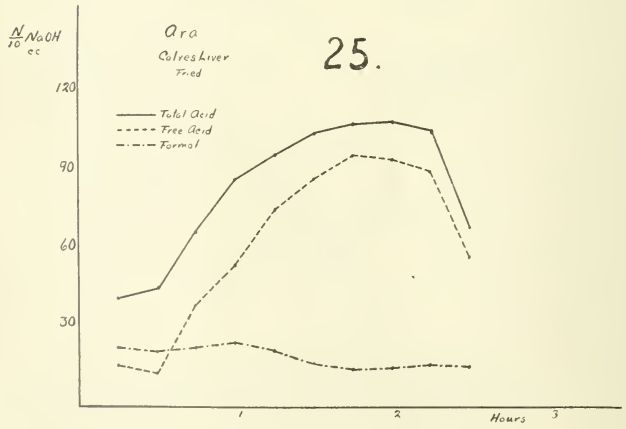

Wal
Round Steak
Broiled
20.
Total Acid
Formol
Pepsin
N/10 NaOH cc
150
120
90
60
30
Pepsin mm.
40
20
1
2
3
Hours
4

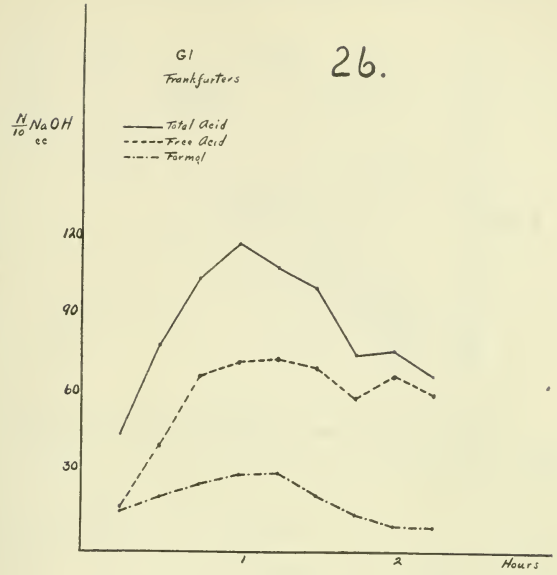

Ara
Dried Beef
21.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
Hours

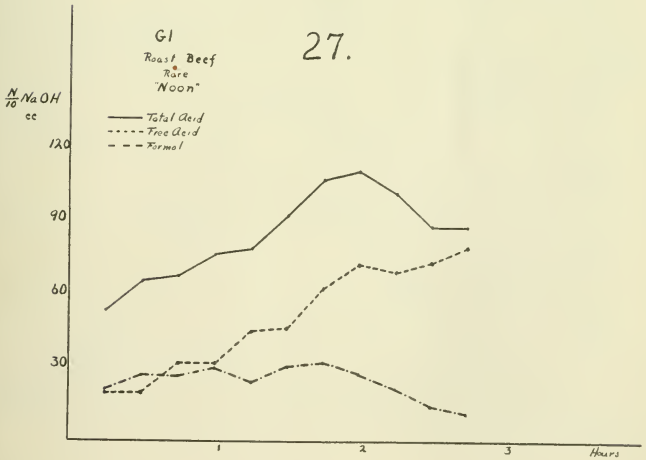

Ara
Roast Beef
Med
22.
Total Acid
Free Acid
Formol
N/10 NaOH cc
120
90
60
30
1
2
Hours

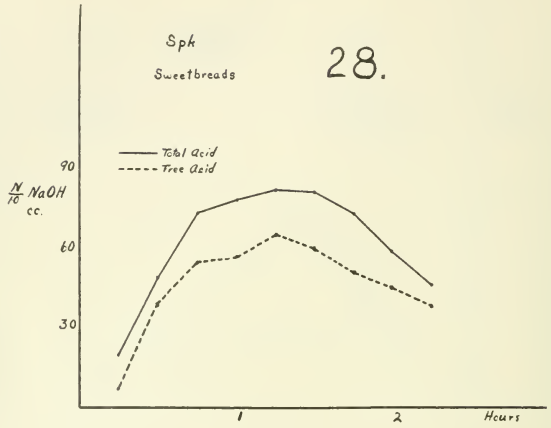

N/10 NaOH cc
Cru
Roast Beef
Rare
23.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
Hours
3

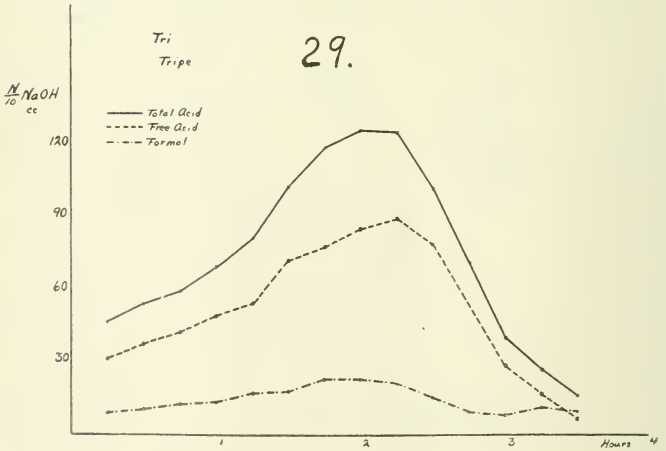

Cru
24.
Beef Bologna
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

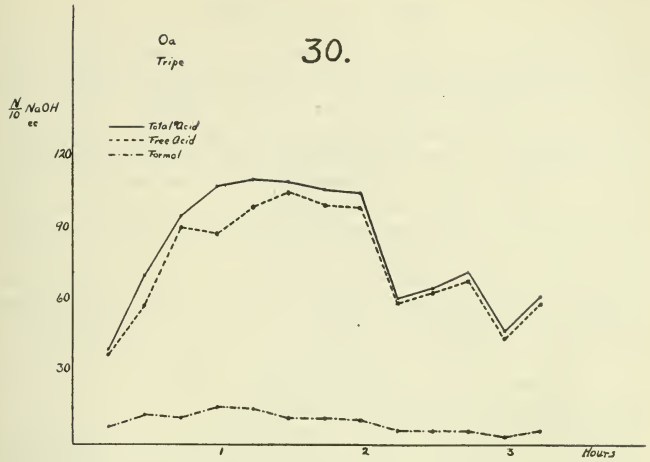

N/10 NaOH cc
Ora
Calves Liver
Fried
25.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours
3

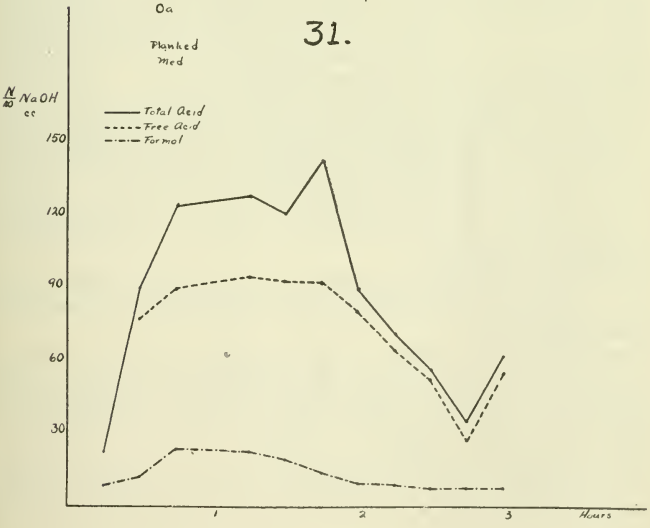

GI
Frankfurters
26.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours


GI
Roast Beef
Rare
"Noon"
27.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Spk
Sweetbreads
28.
Total Acid
Free Acid
N/10 NaOH cc.
90
60
30
1
2
Hours


Tri
Tripe
29.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Oa
Tripe
30.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Oa
Planked
Med
31.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

SUMMARY AND CONCLUSIONS

The digestion of various forms of beef and beef products in the normal human stomach was studied by the fractional method. Over seventy complete experiments were made on twenty-five different subjects. It was found that all normal stomachs do not respond alike to the entrance of the same food. Some respond very promptly and decidedly to the entrance of food, whereas others respond very slowly and indifferently to the same food. Also one type of normal stomach empties very quickly whereas a second type evacuates slowly under like dietary conditions. The average results for individuals of the rapid- and slow-emptying types have been tabulated (see table 4) and typical comparisons of different meats or different modes of cooking have been illustrated by curves showing acidities developed and emptying times.

Roast beef appeared to be handled by the stomach with about the same ease whether rare, medium or well-done, although the rare beef had perhaps a slight advantage in this respect.

Beef steaks appeared to be just as readily digested if cooked rare as if medium or well-done. Very tough steaks from the cheaper and tougher cuts of meats such as rump and shank showed distinctly more rapid evacuation than sirloin or the best tenderloin steaks, in the same individuals. Roast beef was found to lie between these two classes of steaks in gastric response and evacuation time.

Hamburger steaks were found to leave the stomach in about the same time as an equal weight of roast beef under the same conditions.

Stewed beef was handled by the stomach in practically the same time as roast beef, but with a less rapid development of acidity.

The response to boiled corned beef was similar to that of roast beef or stewed beef. Acid productions may, however, be slightly delayed in the case of corned beef.

Dried beef was digested with almost the same ease as ordinary roast beef. There may be a slight delay due to the low moisture content if the meat is eaten dry.

Beef bologna was handled by the stomach in the same length of time as roast beef. Calves' liver required a slightly longer time. Beef tongue was less readily digested than dried beef. Frankfurters left the stomach rather quickly, as did also sweetbreads. Tripe was digested a little less rapidly than roast beef.

For 100 grams of the beef products tested an average evacuation time of 2 hours and 35 minutes was obtained on subjects of the rapid-

emptying type and of 3 hours and 25 minutes on subjects of the slow-emptying type. Total acidities as high as 184 were obtained on meats. The average total acidity at the height of digestion was in the case of beef products 120. These high acid values regularly shown by normal men necessitate a revision of older ideas of "hyperacidity."

The amino acid nitrogen values (which include ammonia) are moderately high at the beginning of digestion due to the ammonia of the meats, show a secondary rise as digestion proceeds and fall to a low level at the end of digestion as the soluble products leave the stomach.

Pepsin values attain their highest point toward the end of digestion.

The authors wish to express their appreciation of the coöperation of the students of Jefferson Medical College, who kindly acted as subjects of these tests.

BIBLIOGRAPHY

(1) Beaumont: Physiology of digestion, 2nd Ed., 1847, Burlington, Vt.
(2) Jessen: Zeitschr. f. Biol., 1883, xix, 129.
(3) Penzoldt: Deutsch. Arch. klin. Med., 1893, li, 535.
(4) Bergeim, Rehfuss and Hawk: Journ. Biol. Chem., 1914, xix, 348.
(5) Carlson: This Journal, 1915, xxxvii, 70.
(6) Zunz: Internat. Beitr. Path. Therap., 1910, ii, 372.
(7) Christiansen: Biochem. Zeitschr, 1913, xlvi, 24.
(8) Carlson: This Journal, 1915, xxxviii, 248.
(9) Hoagland, McBride and Powick: U. S. Dept. Agric., Bull. 433, 1917.
(10) Falk and McGuire: Journ. Biol. Chem., 1919, xxxvii, 547.
(11) Fowler: Unpublished data from this laboratory.
(12) Grindley, Majonnier and Porter: U. S. Dept. Agric., Bull. 193, 1907.
(13) Hall and Emmett: Ill. Agric. Exper. Sta. Bull. 158.

GASTRIC RESPONSE TO FOODS[1]

IV. The Response of the Stomach to Pork and Pork Products

CLARENCE A. SMITH, HAMILTON R. FISHBACK, OLAF BERGEIM, MARTIN E. REHFUSS and PHILIP B. HAWK

From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia

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This study on the digestion of pork and pork products is one of a series (1) of studies on the response of the human stomach to various foods. There are comparatively few investigations that bear directly on the gastric digestion of pork and its products.

Beaumont (2) gives the following results for the evacuation time of pork products, the tests being carried out on the subject, Alexis St. Martin. Roast beef values of the same investigator are given for comparison.

TABLE 1

Digestion time of meats in the human stomach (Beaumont)

MEAT	COOKING	HOURS	MINUTES
Pork	Roast	5	15
Sausage	Broiled	3	20
Pigs' feet	Soused	1	
Beef	Roast rare	3	
Beef	Roast well-done	3	30

Jessen (3) on his subject obtained a value of three hours for raw pork as compared with two hours for raw beef.

Penzoldt (4) obtained the following values for pork and beef steak on his two subjects.

[1] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

TABLE 2

Digestion time of meats in the human stomach (Penzoldt)

MEAT	COOKING	PRAEGER 100 GRAMS	GIGGLBERGER 160 GRAMS
		hours	*hours and minutes*
Ham	Boiled	$3\frac{1}{4}$	3:20
Ham	Raw	$3\frac{3}{4}$	3:46
Beef steak	Fried warm	$3\frac{1}{2}$	4:15 (250 gm.)

The present series of tests on the digestion of pork and pork products in the stomachs of normal men was carried out in the same manner as the tests on beef described in a preceding article (1).

Table 3 gives a summary of the results as to evacuation time and highest total acidities attained for a number of these meats, the results obtained with slow- and rapid-emptying types of stomachs being averaged separately. The results of 31 separate tests on 10 different subjects are recorded.

Figures 1 to 24 illustrate the digestive response of the stomach to the various pork products as compared with each other and with the response of certain types of beef. The comparative tests were made upon the same individuals under as nearly identical conditions as possible and the illustrations given are typical of the results obtained. One hundred grams of meat were fed unless otherwise stated.

Pork has usually been considered a rather indigestible kind of meat. In explanation of this it is said that the high proportion of fat between the meat fibers forms a coating which the gastric juice can penetrate only with difficulty. Hence convalescents, or people with digestive disturbances of any kind, are warned to leave all pork products out of their diet. In spite of this fact, however, and in spite of the fact that a great mass of people of the United States abstain from pork on principle, the per capita consumption of pork in this country is estimated at a little more than 88 pounds per year, nearly 10 pounds more per capita than beef, and almost as much as all the other forms of meat combined—including beef.

In reality the normal human stomach finds no extraordinary difficulty in digesting pork. It is true that, as shown in table 3, the emptying time of pork averages somewhat longer than that of other common meats, and this relative slowness of digestion applies also to pork products. If pork taken with a mixed meal, however, retards stomach digestion appreciably, then it may well be avoided by weak stomachs.

TABLE 3

Gastric digestion of pork and pork products

NO.	SUBJECT	MEAT	TYPE OF STOMACH							
			Rapid emptying				Slow emptying			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				average		*average*		*average*		*average*
1	Ara	Roast pork	3:30		108					
2	Cru	Roast pork	2:15		112					
3	Dal	Roast pork	2:00		102					
4	Ev	Roast pork	3:00	2:45	155	119				
5	Wat	Roast pork					3:45		118	
6	Wr	Roast pork					3:30		160	
7	Spn	Roast pork					3:45	3:45	120	130
8	Spn	Roast pork (250 grams)					5:30	5:30	145	145
9	Oa	Sausage	2:45	2:45	104	104				
10	Gl	Sausage					3:15		108	
11	Fal	Sausage					2:45	3:00	104	106
12	Joa	Ham, fried	3:00	3:00	65	65				
13	Wal	Ham, fried					4:15	4:15	150	150
14	Bos	Ham, boiled	3:15	3:15	137	137				
15	Kru	Ham, boiled					3:00	3:00	140	140
16	Km	Ham, minced	2:00	2:00	115	115				
17	Bos	Ham, minced					3:00	3:00	120	120
18	Mes	Ham, baked					4:15		118	
19	Ra	Ham, baked					3:45	4:00		118
20	Joa	Ham, bologna	3:15	3:15	68	68				
21	Wel	Bacon, fried					4:30	4:30	115	115
22	Gl	Pigs' feet, soused					3:45		120	
23	Ca	Pigs' feet, soused					2:30	3:15	135	125
24	Ara	Liver and bacon, fried	3:00		94					
25	Bos	Liver and bacon, fried	2:30	2:45	137	116				
26	Kru	Liver and bacon, fried					3:30	3:30	125	125
27	Wel	Ham sandwich, fried					2:45	2:45	101	101
28	Gl	Scrapple, fried					3:45		85	
29	Oa	Scrapple, fried					3:45	3:45	85	85
30	Gl	Pork chops					4:00		129	
31	Ca	Pork chops					3:45	4:00	144	134

*Acidities are expressed as cc. of N/10 alkali required to neutralize 100 cc.

Roast pork. The response of the stomach to 100 grams of roast pork is charted in figure 1. The evacuation time, as shown, was $3\frac{3}{4}$ hours.

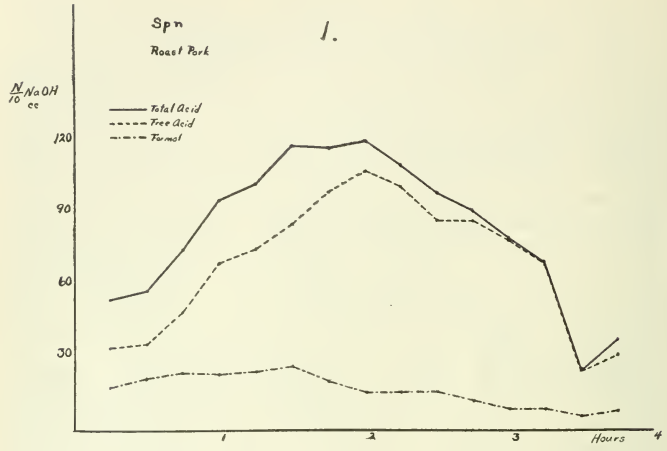
Figure 2 shows the response of another individual to roast pork. By comparing this chart with figure 22 in the preceding article on beef products (1) it will be seen that whereas roast beef required $1\frac{3}{4}$ hours to leave the stomach, roast pork required $3\frac{1}{2}$ hours. A comparison of the average results likewise shows for the rapid type of individual an emptying time for beef of $2\frac{1}{4}$ hours and for pork $2\frac{3}{4}$ hours, while for the slow-emptying type the periods were $3\frac{1}{2}$ hours and $3\frac{3}{4}$ hours respectively. Thus roast pork requires appreciably longer to digest than roast beef although the differences are not nearly so great as those found by Beaumont (see table 1). In fact certain individuals appear to handle roast pork very readily; fully as well as roast beef. Compare figure 3 with figure 23 in our paper on beef products showing an evacuation time for pork of $2\frac{1}{4}$ hours as compared with $2\frac{1}{2}$ hours for beef.

Figures 13 and 24 illustrate the digestion of pork chops. Compare figure 24 with figure 4 showing digestion of roast beef and note that practically the same time was required for each. In the same way compare figures 13 and 27 in the article on beef (1), noting the markedly longer time required for pork in the case of this individual. Pork chops and roast pork appear to require about the same periods for digestion in the stomach.

Fried ham in our experiments required considerably longer to digest than beef (compare figs. 5 and 6, also fig. 20 in the paper on beef products (1)).

A comparison of boiled ham and minced ham may be obtained from figures 7 and 8. The minced ham shows but slight advantage although the acidity is more rapidly developed in this case than where mincing was not employed. Likewise a difference of only fifteen minutes was noted in favor of roast beef as compared with the minced ham or a difference of thirty minutes as compared with boiled ham (see fig. 9 as compared with figs. 7 and 8).

The digestion of liver and bacon is illustrated by figures 10 and 11. If figure 10 is compared with figure 22 of the earlier paper on beef products (1), it will be noted that liver and bacon require appreciably longer for gastric digestion than roast beef. Comparisons of liver and bacon with liver alone are shown by figures 11 and 12, and by figure 10 as compared with figure 25 in paper on beef products. It will be noted that from $\frac{1}{2}$ to 1 hour longer was required for the fried liver without bacon in one case while in the other case the bacon slightly prolonged the evacuation time.


Spn
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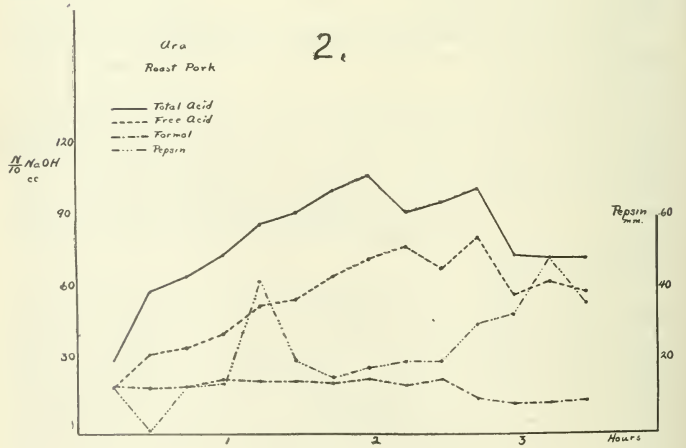
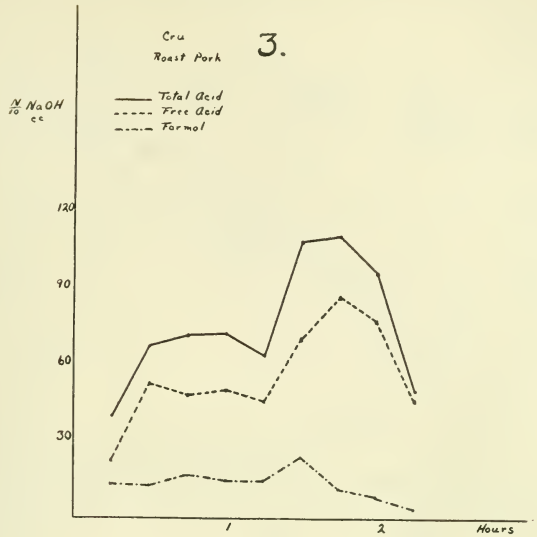
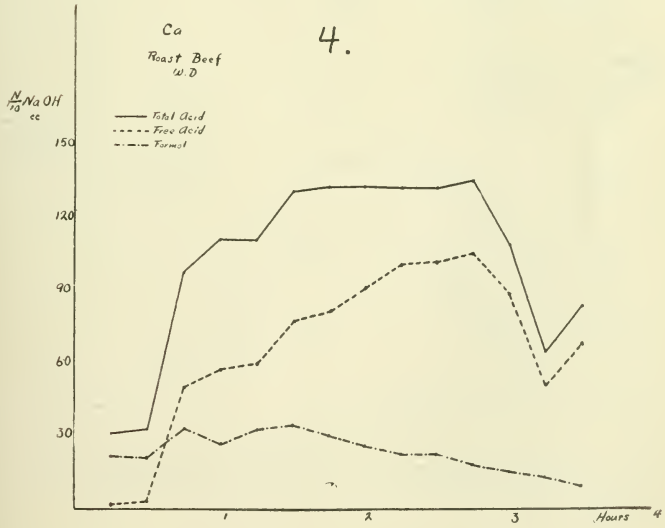
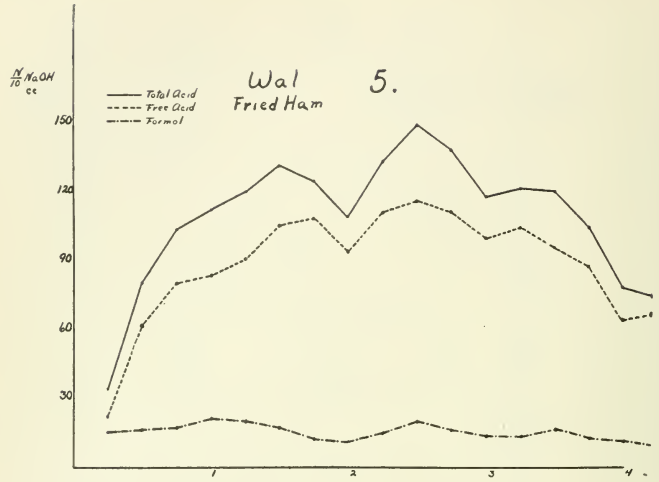
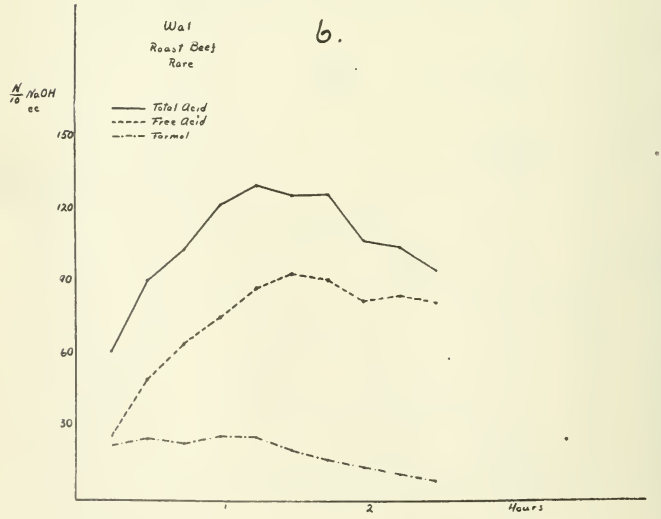
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4


Ura
Roast Pork
2.
Total Acid
Free Acid
Formol
Pepsin
N/10 NaOH cc
120
90
60
30
Pepsin mm.
60
40
20
1
2
3
Hours


Cru
Roast Pork
3.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours


Ca
Roast Beef
W. D
4.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours


N/10 NaOH cc
Total Acid
Free Acid
Formol
Wal
Fried Ham
5.
150
120
90
60
30
1
2
3
4


Wal
Roast Beef
Rare
6.
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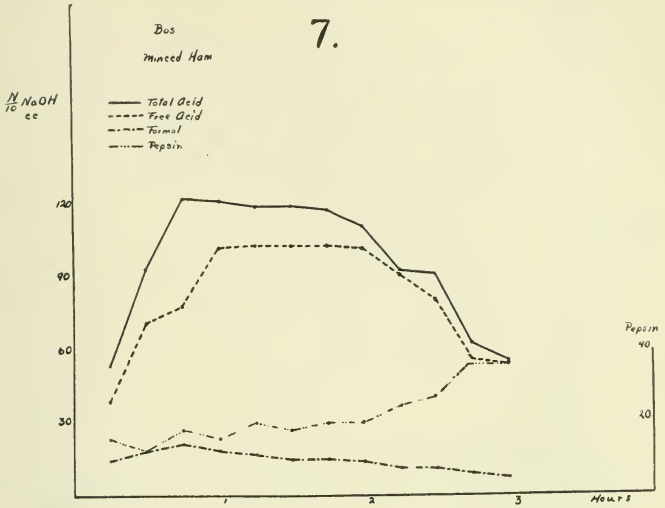
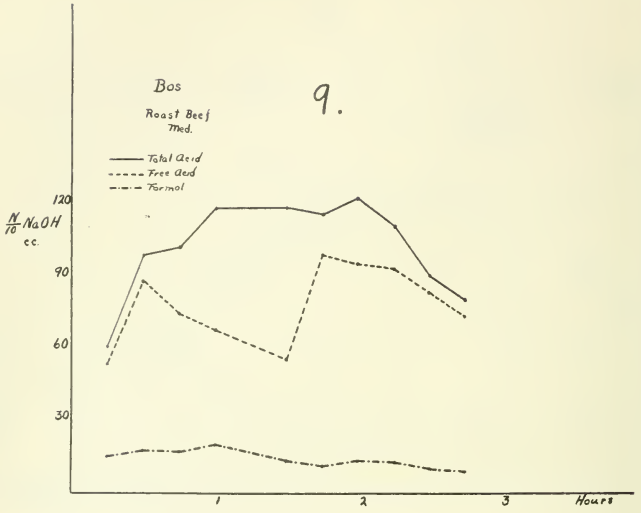
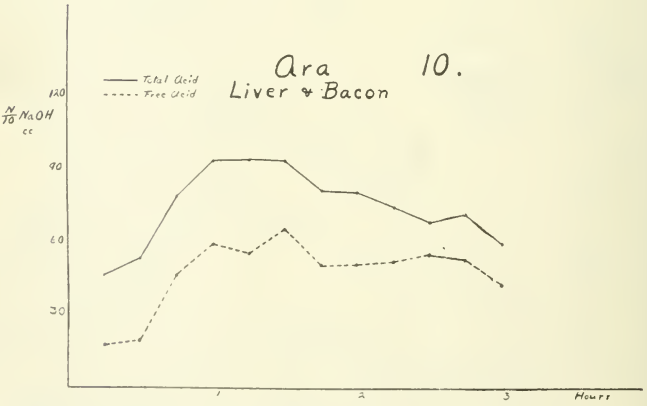
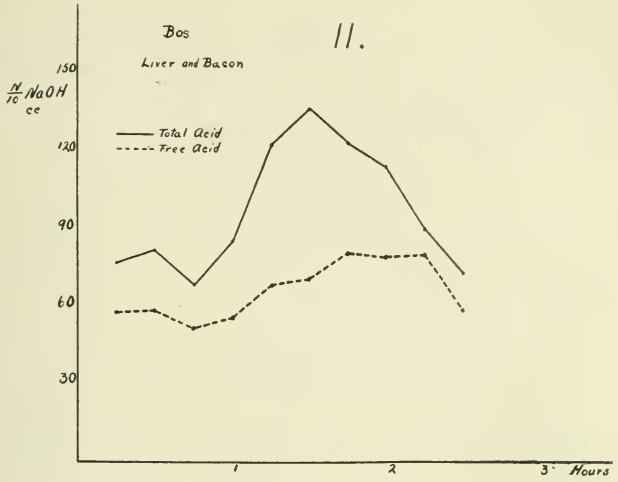
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
Hours


Bos
Minced Ham
7.
N/10 NaOH cc
Total Acid
Free Acid
Formol
Pepsin
120
90
60
30
Pepsin
40
20
1
2
3
Hours


Bos
Boiled Ham
8.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
Pepsin
150
120
90
60
30
Pepsin mm
40
20
1
2
3
Hours

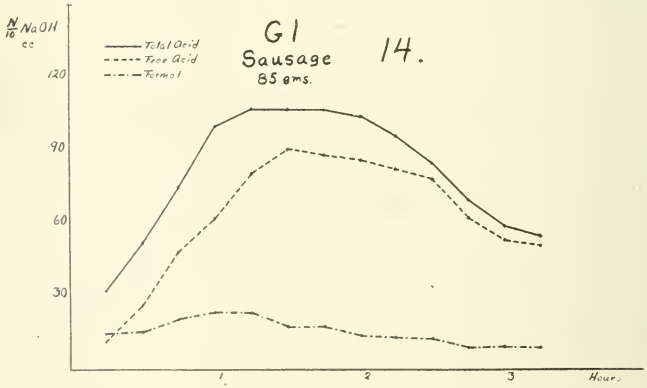

Bos
Roast Beef
Med.
9.
Total Acid
Free Acid
Formol
N/10 NaOH
cc.
120
90
60
30
1
2
3
Hours

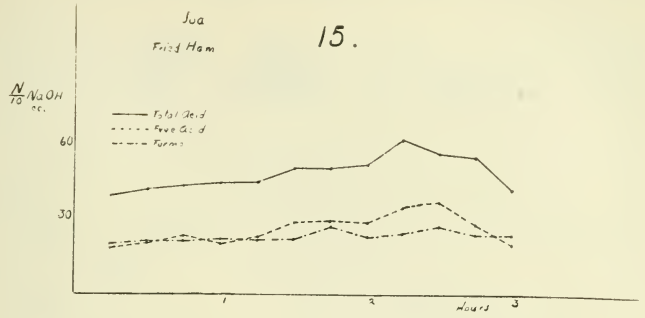

Ara
Liver & Bacon
10.
Total Acid
Free Acid
N/10 NaOH
cc
120
90
60
30
1
2
3
Hours


11.
Bos
Liver and Bacon
N/10 NaOH cc
150
120
90
60
30
Total Acid
Free Acid
1
2
3
Hours


12.
Bos
Calves Liver
Fried
N/10 NaOH cc
150
120
90
60
30
Total Acid
Free Acid
Formol
1
2
3
4
Hours


13.
GI
Pork Chops
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


N/10 NaOH
cc
Total Acid
Free Acid
Formol
GI
Sausage
85 gms.
14.
120
90
60
30
1
2
3
Hours


Joa
Fried Ham
15.
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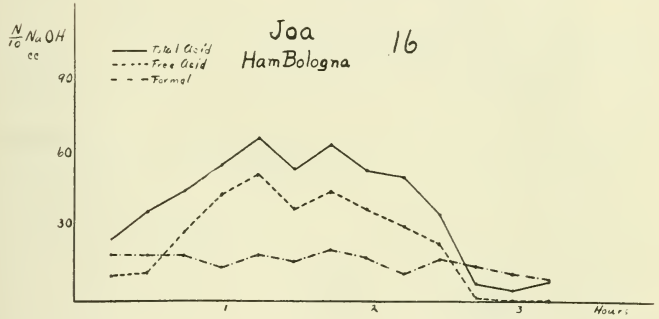
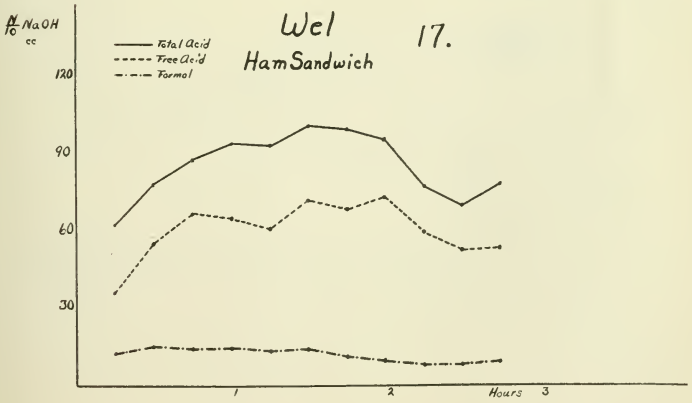
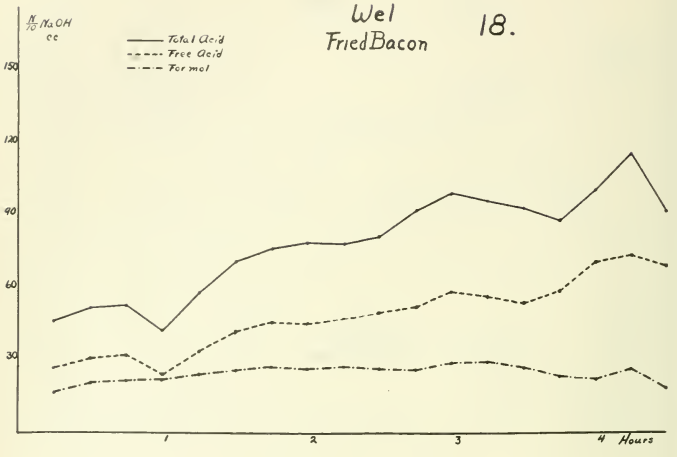
Total Acid
Free Acid
Formol
60
30
1
2
Hours
3


N/10 NaOH cc
Total Acid
Free Acid
Formal
Joa
Ham Bologna
16
90
60
30
1
2
3
Hours

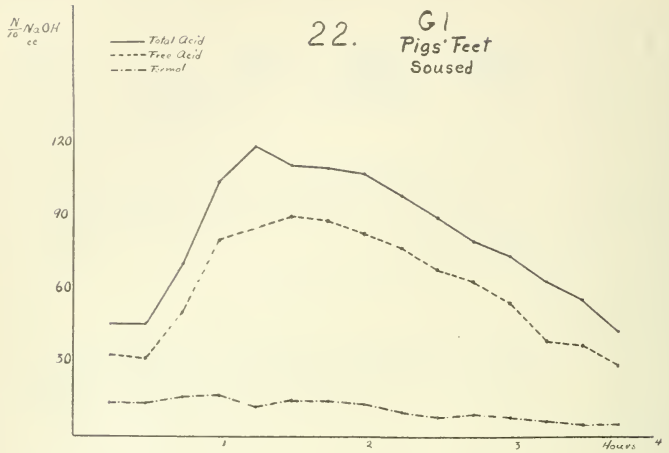

N/10 NaOH cc
Total Acid
Free Acid
Formal
Wel
Ham Sandwich
17.
120
90
60
30
1
2
Hours
3

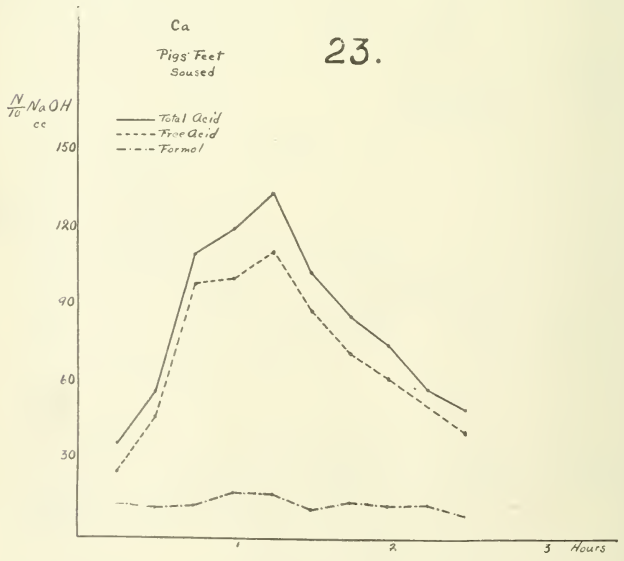

N/10 NaOH cc
Total Acid
Free Acid
Formol
Wel
Fried Bacon
18.
150
120
90
60
30
1
2
3
4 Hours


GI
Scrapple
19.
N/10 NaOH
Total Acid
Free Acid
Formol
90
60
30
1
2
3
Hours
4


N/10 NaOH cc
Total Acid
Free Acid
Formal
Oa
Scrapple
20.
120
90
60
30
1
2
3
Hours

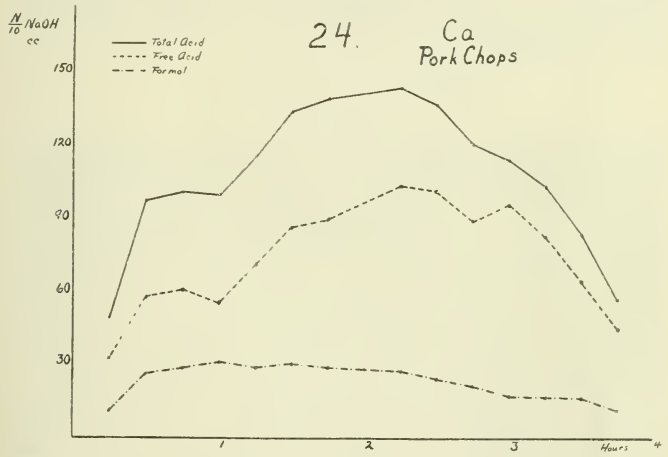

Oa
Sausage
21.
N/10 NaOH cc
Total Acid
Free Acid
Formal
120
90
60
30
1
2
Hours


N/10 NaOH cc
Total Acid
Free Acid
Formol
22.
G1
Pigs' Feet
Soused
120
90
60
30
1
2
3
Hours
4


Ca
Pigs' Feet
Soused
23.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3 Hours

The comparative responses of pork sausage and pork chops are illustrated by figures 13 and 14. The averages given in table 3 also indicate that the sausage is somewhat more easily handled. Compare also with roast beef (fig. 27) and beef frankfurters (fig. 26, beef article) and note that the beef has an advantage over sausage.

A comparison of fried ham with ham bologna is given by figures 15 and 16, little difference being shown as to response. These charts should be compared with figures 5, 6, 7 and 8 in preceding paper on beef products. The results obtained indicate that ham remains in the



stomach somewhat longer than steaks of the tougher cuts and about the same time as a sirloin or tenderloin steak.

The slow evacuation of bacon when ingested in as large amounts as 100 grams is illustrated by figure 18. The comparatively slow development of acidity may also be noted. The high fat content is presumably responsible for these effects. In contrast with this note the fairly rapid evacuation where two ham sandwiches were given (100 grams bread and 23 grams boiled ham) (fig. 17), also the more rapid development of free acidity.

The so-called "scrapple" is a preparation made from pork and cereal, whose "habitat" is more or less restricted to certain localities. The

head and jowls of the pig are sometimes used as well as heart, liver and meat trimming. The cereals used most commonly are wheat flour, buckwheat flour and cornmeal mixtures. That scrapple leaves the stomach rather slowly is indicated by the charts (figs. 19 and 20) as compared with roast beef (fig. 27, beef article), pork chops (fig. 13) and sausage (fig. 14). The greater delay of digestion of "scrapple" as compared with pork sausage is shown also by figures 20 and 21.

Soused pigs' feet were also tested. In one case the pigs' feet required practically as long to leave the stomach as pork chops (compare figs. 22 and 13). In the other case the pigs' feet were digested much more rapidly (figs. 23 and 24). The latter type of response is perhaps the more characteristic.

SUMMARY AND CONCLUSIONS

A series of studies of the response of the normal human stomach to various pork products prepared in various ways was carried out using the fractional method of gastric analysis. The average results for evacuation times and acidities developed have been tabulated. Comparative responses of the same individuals to these various products as well as to certain beef products have been charted. For individuals with stomachs of the rapid-emptying type a general average evacuation time for pork products of $2\frac{3}{4}$ hours was found. Subjects of the slow-emptying type showed a general average of 3 hours and 40 minutes. The average total acidity observed at the height of digestion was 117.

Pork products in general were comparatively slow to leave the stomach as would be expected from their high fat content. The differences were not as great, however, as some figures in the literature indicate. Thus roast pork was retained appreciably longer than roast beef in most instances. Pork chops required about the same period of gastric digestion as roast pork. Fried ham also required considerably longer to digest than roast beef.

Minced ham showed a slight advantage over boiled ham as to evacuation time. Acidities were also more rapidly developed in the former instance. Roast beef was handled somewhat more quickly than either.

Liver and bacon required about the same period of digestion as roast beef. In certain instances liver and bacon were handled more readily than liver alone. In one case the contrary was found.

Pork sausage was somewhat more easily handled than were pork chops but less readily than roast beef.

Ham bologna required about the same time to digest as fried ham or as the less readily digestible beef steaks.

The evacuation of bacon was found to be slow and low gastric acidities were developed. Ham sandwiches were more readily handled than most other pork products tested.

"Scrapple" left the stomach more slowly than pork sausage and belongs to the less readily evacuated pork products.

Pigs' feet gave variable results but appear ordinarily to be handled more easily than pork chops.

The authors wish to extend their thanks to Dr. R. A. Lichtenthaeler who assisted them in carrying out these tests and also to those Jefferson men who sacrificed time and convenience in acting as subjects.

BIBLIOGRAPHY

(1) Fishback, Smith, Bergeim, Lichtenthaeler, Rehfuss and Hawk: This Journal, 1919, xlix, 174.
(2) Beaumont: Physiology of digestion, 2nd Ed., 1847, Burlington, Vt.
(3) Jessen: Zeitschr. f. Biol., 1883, xix, 129.
(4) Penzoldt: Deutsch. Arch. klin. Med., 1893, li, 535.

GASTRIC RESPONSE TO FOODS[1]

V. The Response of the Stomach to Lamb and Lamb Products

HAMILTON R. FISHBACK, CLARENCE A. SMITH, OLAF BERGEIM, MARTIN E. REHFUSS and PHILIP B. HAWK

From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia

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The present series of tests on the response of the normal human stomach to lamb and lamb products was carried out in the same manner as our experiments on beef (1) and pork (2) products and with a similar object. Beaumont (3) reported on his subject an evacuation time of $3\frac{1}{4}$ hours for roast lamb as compared with 3 to $3\frac{1}{2}$ hours for roast beef. Jessen (4) reports two experiments with raw lamb as compared with raw beef and found both to leave the stomach in two hours.

In the present paper are reported the results of fourteen tests with roast lamb and other lamb products on several different normal subjects as well as certain tests on beef and pork products for direct comparison. One hundred grams of meat were ingested in each case.

The results of these experiments are averaged in table 1. Values obtained for men whose stomachs in general empty rapidly are given separately from the values obtained with men whose stomachs were of the slow-emptying type. The distinction between the two types of stomach is not so marked in the case of lamb products as it is with certain other foods. As the table indicates from $2\frac{1}{2}$ to $2\frac{3}{4}$ hours were required for lamb to leave the stomach in the one case and 3 to 4 hours in the other.

The charts showing the results of fractional gastric analyses in a number of cases where these meats were fed enable us to compare the responses of the same individual to various meats. In certain cases reference is made to preceding papers upon beef and pork products for such comparisons. The acid curves enable us to distinguish between

[1] The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

certain pathological responses where evacuation is rapid although little acid is secreted and hence little gastric digestion occurs, and a normal type of rapid-emptying stomach where acid secretion is normal and considerable protein digestion takes place in this organ.

The digestion of roast lamb is illustrated by figures 1 to 5. The extreme variation in evacuation times of these normal subjects (compare, for example, figure 1 showing an evacuation time of $3\frac{3}{4}$ hours with

TABLE 1

Gastric digestion of lamb and lamb products

NUMBER	SUBJECT	MEAT	TYPE OF STOMACH							
			Rapid-emptying				Slow-emptying			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity	
				average		*average*		*average*		*average*
1	Riv	Roast lamb	1:45		114					
2	Ara	Roast lamb	3:15		138					
3	Bos	Roast lamb	3:45		147					
4	Don	Roast lamb	2:30		147					
5	Han	Roast lamb	2:45	2:45	142	137				
6	Wel	Roast lamb					3:45		156	
7	Cop	Roast lamb					3:00	3:30	123	140
8	Cop	Stewed lamb					4:00		122	
9	Ber	Stewed lamb					4:00	4:00	150	136
10	Tri	Lamb chops	2:30	2:30	140	140				
11	Cop	Lamp chops					3:30		115	
12	Oa	Lamb chops					2:30	3:00	115	115
13	Hou	Sheep brains					2:15		97	
14	Sim	Sheep brains					2:45	2:30	73	85

*Acidities are expressed in terms (cc.) of N/10 alkali required to neutralize 100 cc. of sample.

figure 3 where the stomach is emptied in $1\frac{1}{2}$ hours) is of interest. The subject of experiment 3 is however unusual in this respect.

For comparisons of roast lamb with roast beef, observe figure 1 showing an evacuation time of $3\frac{3}{4}$ hours and figure 9 in preceding paper on pork products which shows an evacuation time for roast beef of $2\frac{3}{4}$ hours. Also compare figure 3 showing an emptying time of $1\frac{1}{2}$ hours with experiments on roast beef with the same individual recorded in preceding paper on beef products (figs. 1 and 2) which show the beef leaving in 2 hours. A more marked difference in favor of roast beef is

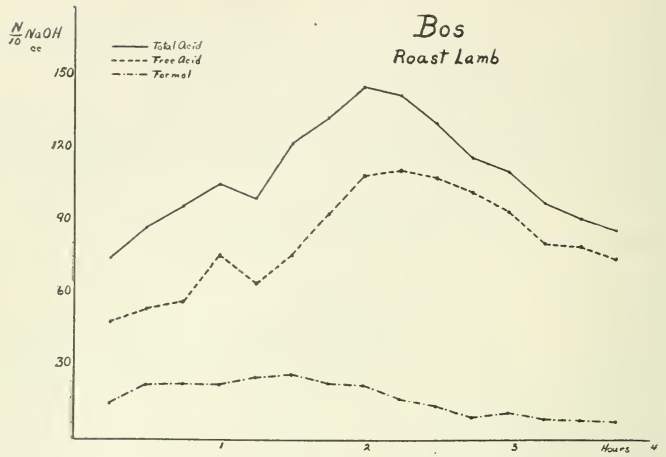
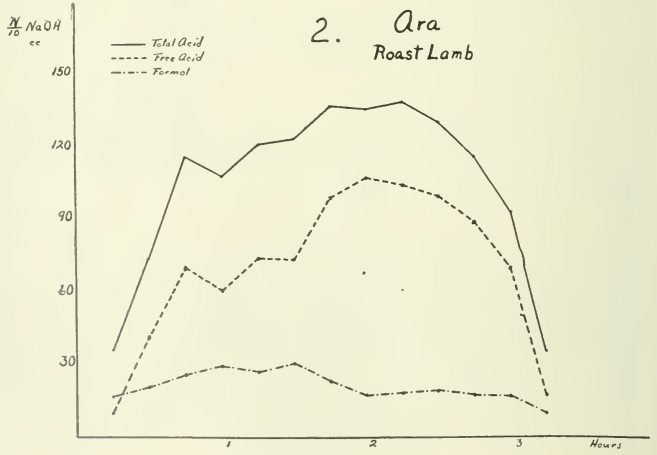
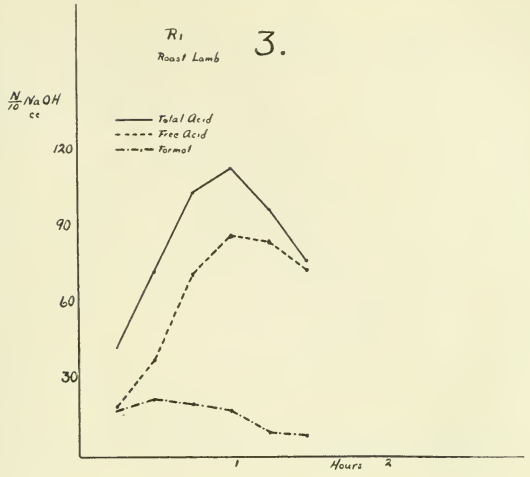
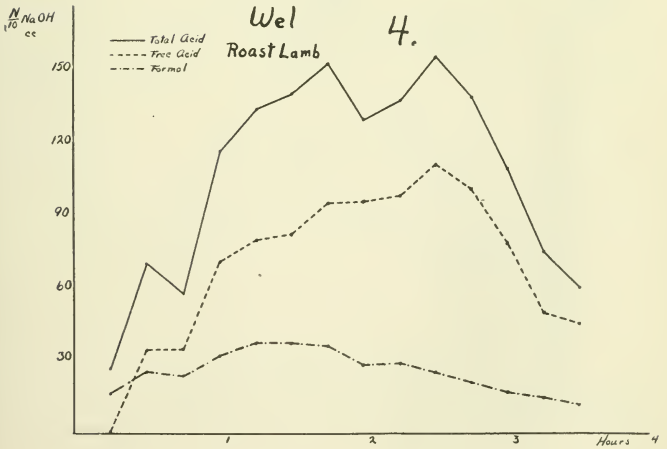

N/10 NaOH cc
Total Acid
Free Acid
Formol
Bos
Roast Lamb
150
120
90
60
30
1
2
3
Hours
4

Fig. 1


N/10 NaOH cc
Total Acid
Free Acid
Formol
2. Ara
Roast Lamb
150
120
90
60
30
1
2
3
Hours


R1
Roast Lamb
3.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
Hours
2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Free Acid
Formol
Wel
Roast Lamb
4.
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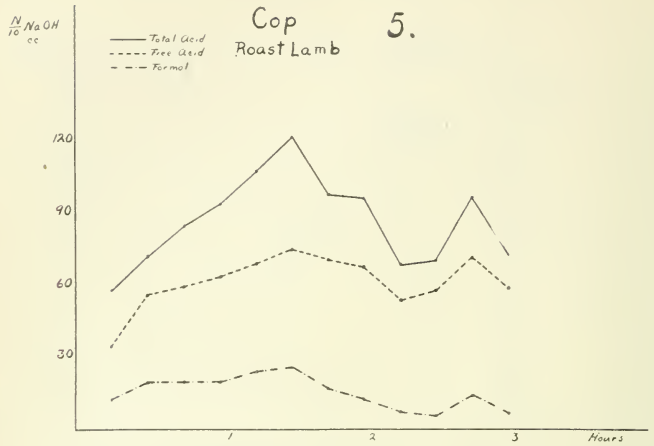
2
3
Hours
4


N/10 NaOH cc
Total Acid
Free Acid
Formol
Cop
Roast Lamb
5.
120
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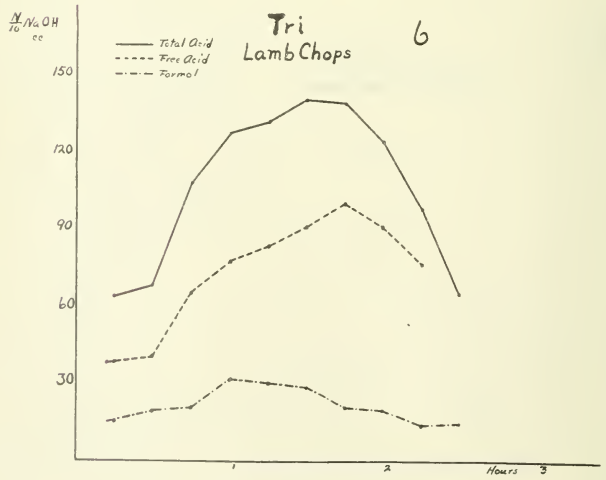
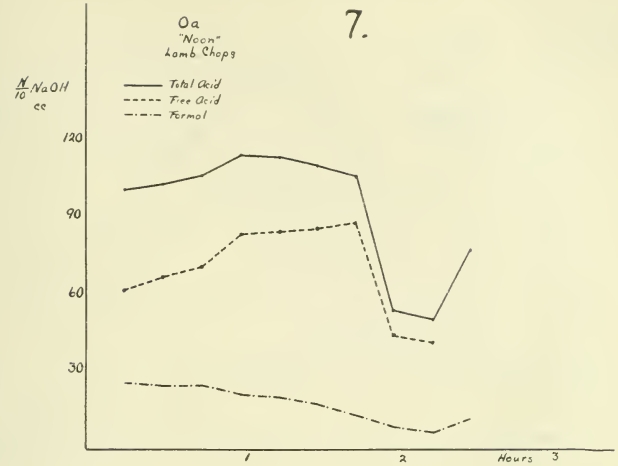
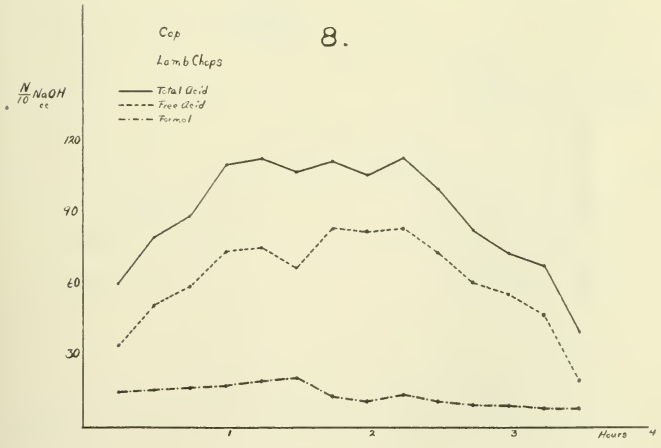
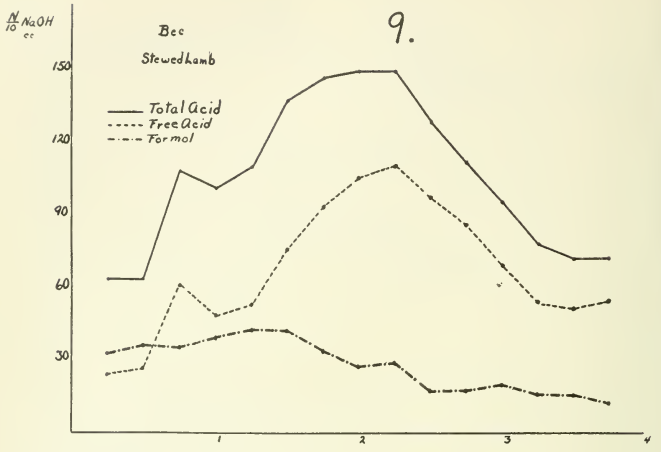
1
2
3
Hours

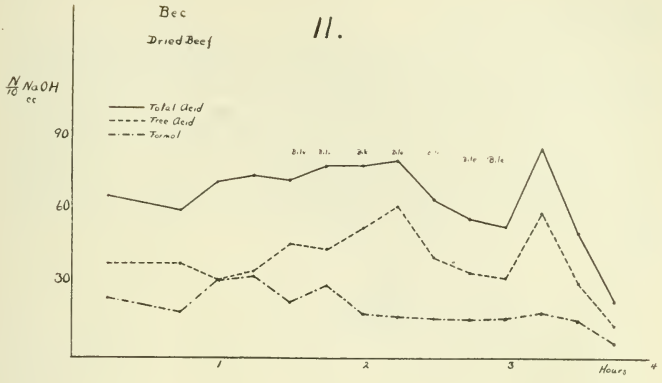

N/10 NaOH cc
Total Acid
Free Acid
Formol
Tri
Lamb Chops
6
150
120
90
60
30
1
2
Hours
3

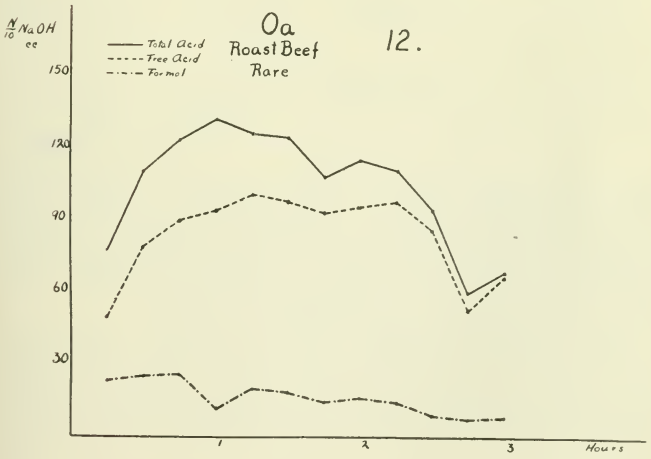

7.
Oa
"Noon"
Lamb Chops
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours
3


8.
Cop
Lamb Chops
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4


N/10 NaOH cc
Bee
Stewed Lamb
9.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
4

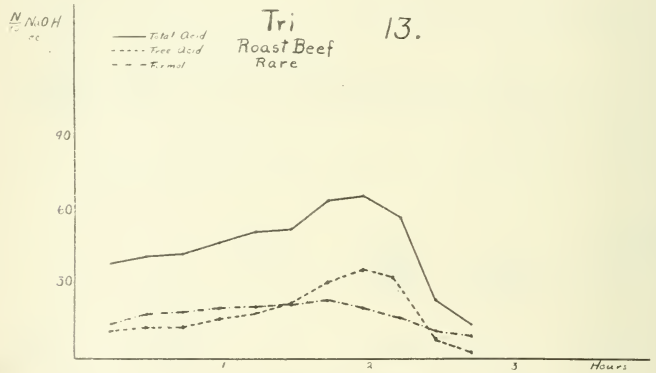

N/10 NaOH cc
Cop
Stewed Lamb
10.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4


Bec
Dried Beef
11.
N/10 NaOH cc
Total Acid
Free Acid
Formol
90
60
30
1
2
3
Hours
4


N/10 NaOH cc
Total Acid
Free Acid
Formol
Oa
Roast Beef
Rare
12.
150
120
90
60
30
1
2
3
Hours

shown by figure 2 as compared with figure 22 in article on beef, the latter leaving in $1\frac{3}{4}$ hours.

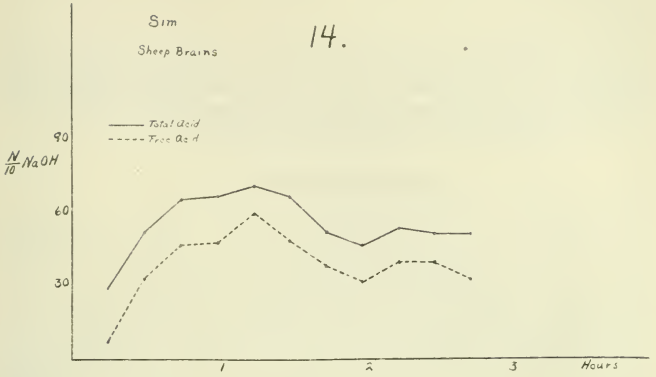
A comparison of roast lamb and roast pork is obtained from figure 2 and figure 12 in preceding paper on pork products, the roast pork requiring $3\frac{1}{2}$ hours digestion as compared with $3\frac{1}{4}$ for lamb chops. Roast lamb may also be compared with boiled ham, figure 1 showing evacuation time for roast lamb of $3\frac{3}{4}$ hours while figure 8 in preceding article on pork shows $3\frac{1}{4}$ hours for the boiled ham. As would be expected, roast lamb (fig. 4, $3\frac{1}{2}$ hours) leaves the stomach sooner than bacon (fig. 18 in preceding article on pork showing evacuation time of $4\frac{1}{2}$ hours).

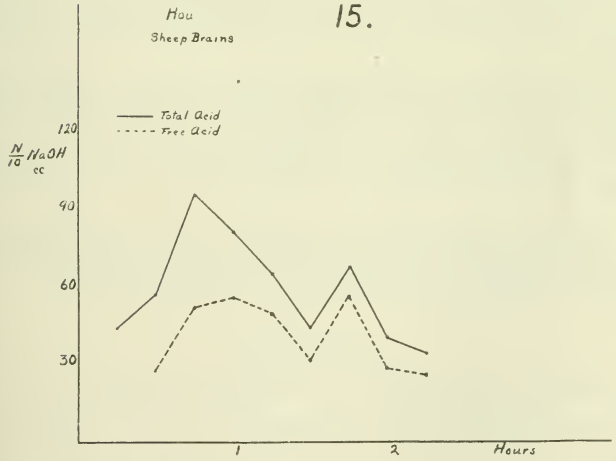


Lamb chops appear to require about the same period of gastric digestion as roast lamb (see table 1). A direct comparison of the two on the same individual may be obtained from figure 5 (roast lamb, 3 hours) and figure 8 (lamb chops, $3\frac{1}{2}$ hours). Compare also figure 6, lamb chops, $2\frac{1}{2}$ hours, with figure 13, roast beef, $2\frac{3}{4}$ hours and also with rump steak, $2\frac{3}{4}$ hours (figure 10 in preceding paper on beef).

Further comparisons of lamb chops with roast beef are given by figure 7, lamb chops, $2\frac{1}{2}$ hours and figure 12, roast beef, 3 hours and by figure 31 in beef article, sirloin steak, 3 hours. The same individual shows an evacuation time of $2\frac{1}{2}$ hours for pork sausage (fig. 21 in article on pork).

Stewed lamb (fig. 10) left the stomach in 4 hours as compared with $3\frac{1}{2}$ hours for lamb chops (fig. 8) and 3 hours for roast lamb (fig. 5). In


Sim
Sheep Brains
14.
Total Acid
Free Acid
90
N/10 NaOH
60
30
1
2
3
Hours


Hau
Sheep Brains
15.
Total Acid
Free Acid
120
N/10 NaOH
cc
90
60
30
1
2
Hours

another case (fig. 9) stewed lamb required the same time as dried beef (fig. 11).

Sheep brains left the stomach in moderate time. In one case $2\frac{3}{4}$ hours were required for sheep brains (fig. 14) as compared with $3\frac{1}{4}$ and $3\frac{1}{2}$ hours for roast beef (figs. 3 and 4 in preceding paper on beef). An individual who showed an evacuation time of $2\frac{1}{2}$ hours for sheep brains (fig. 15) required 3 hours for raw hamburg steak.

SUMMARY AND CONCLUSIONS

The response of the normal human stomach to roast lamb, stewed lamb, lamb chops and sheep brains was studied using the fractional method of gastric analysis. The results have been tabulated as averages (table 1). Comparisons of lamb cooked in different ways as well as comparisons of lamb with pork and beef have been charted.

Lamb was found to require from 2 to 3 hours (average $2\frac{1}{2}$ hours) for individuals possessing the rapid-emptying type of stomach to digest and from 3 to 4 hours (average 3 hours 20 minutes) for individuals with the slow-emptying type of stomach. Roast lamb and lamb chops required practically the same period of gastric digestion and stewed lamb a little longer than the other two. Sheep brains left the stomach rather rapidly ($2\frac{1}{2}$ hours) and developed a lower acidity than the other meats.

Lamb stimulated acid production fully as much as any other class of meats and apparently to a slightly greater extent than beef or pork. The average total acidity at the height of digestion was 134.

On the average, roast lamb remained in the stomach a few minutes longer than roast beef but not as long as roast pork.

The authors wish to extend their thanks to Dr. R. A. Lichtenthaeler who assisted them in carrying out these tests, and also to those Jefferson men who sacrificed time and convenience in acting as subjects.

BIBLIOGRAPHY

(1) Fishback, Smith, Bergeim, Lichtenthaeler, Rehfuss and Hawk: This Journal, 1919, xlix, 174.

(2) Smith, Fishback, Bergeim, Rehfuss and Hawk: This Journal, 1919, xlix, 204.

(3) Beaumont: Physiology of digestion, 2nd Ed., 1847, Burlington, Vt.

(4) Jessen: Zeitschr. f. Biol., 1883, xix, 129.

THE ELECTRICAL CONDUCTIVITY METHOD OF DETERMINING THE RELATIVE VOLUME OF CORPUSCLES AND PLASMA (OR SERUM) IN BLOOD

G. N. STEWART

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

Received for publication April 28, 1919

Many years ago (1) I worked out a method of determining the percentage volume of plasma (or serum) in blood, based upon the fact that the corpuscles may be assumed to be non-conducting particles suspended in the plasma.

Although the method unquestionably gives more accurate results than the haematocrite and requires little time, it has not been greatly employed even in the laboratory, still less in the hospital. P. Fraenkel (2) recommended it for scientific purposes, after comparing it with Bleibtren's chemical method, and Wilson (3) compared it with the haematocrite method. However, he did not keep up the rotation of the haematocrite until the column of corpuscles had assumed a constant volume, but rotated for a fixed time (five minutes). This was done purposely because frequently the haematocrite is employed in this way. But although useful comparative results may be obtained on the same blood with a fixed time of rotation, this is not the case where bloods differing greatly in the percentage of corpuscles are to be compared.

As the measurement of the conductivity of physiological liquids is a much more familiar operation among biologists and clinicians than it was twenty years ago, and as we have been recently making use of the method again in calculating epinephrin concentrations in serum from the concentrations in the corresponding blood, and have had the opportunity to compare it rather extensively with the haematocrite method, I am confirmed in the belief that its advantages have not been sufficiently appreciated. To be sure, a larger quantity of blood is needed than that required to fill a haematocrite tube. I use a conductivity tube holding about 3 cc. and, therefore, must have 3 cc. of serum. But it is not difficult, as Wilson did, to employ a tube which requires only 4 to 5

drops of blood. The principle of the method permits the assumption that the formulae worked out on dog's blood will also be applicable to other bloods, since the corpuscles can always be taken as practically non-conducting.

In the previous paper the percentage volumes of serum as determined by the electrical method in thirty dogs taken at random are given. The range is from 39.6 to 74.0, and the average 55 per cent. The average for blood specimens taken from six of the dogs used in the recent work is 52 per cent of serum, the range 36.5 to 66.5. The results are compared with haematocrite readings in table 1.

TABLE 1

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1	52.3	48 (5 minutes), 49 (10 minutes), 50 (15 minutes)
2	36.5	29.5 (40 minutes), 33 (60 minutes), 35 (70 minutes)
263	66.5	62 (15 minutes), 65 (23 minutes)
297	51.0	25.5 (10 minutes), 36 (20 minutes), 41 (30 minutes), 45 (40 minutes) 48 (50 minutes)
306	46.6	10 (10 minutes), 25 (20 minutes), 33 (32½ minutes)
307	62.0	

It will be observed that while great differences exist between the haematocrite readings with a given blood, according to the time of rotation, in no case does the percentage of serum with the longest time of rotation quite equal the percentage determined by the electrical method. The latter obviously constitutes a limit toward which the haematocrite readings approach more nearly the longer the rotation is continued. With some bloods the approach is extraordinarily slow (dogs 2 and 306, e.g.). Where the serum is scanty this is usually the case. In dog 306 after 32½ minutes rotation the end point had not been nearly reached. The percentage of serum as determined by the haematocrite at this time would have been less than two-thirds of the real percentage. The speed of the haematocrite was about 4000 turns a minute. A very high speed was purposely avoided so as to permit such differences to be readily detected. But with higher speeds they would still exist, although the end point would be reached sooner.

In table 2 is given a similar comparison on bloods from eighteen cats.

TABLE 2

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1		73 (5 minutes), 73.5 (10 minutes), 74 (15 minutes), 74.5* (20 minutes)
2	62.5	57 (8 minutes), 60.5 (16 minutes), 62 (21 minutes)
8		71 (5 minutes), 71.5 (10 minutes), 72* (15 minutes)
9		51.5 (5 minutes), 53.5 (10 minutes), 54.5 (15 minutes), 55* (20 minutes)
91		87*†
214	71.0	
239	70.0	
259	76.5	65 (8 minutes), 70 (15 minutes), 73.5 (20 minutes)
284		50.5 (15 minutes), 51 (22 minutes), 52 (34 minutes)
285	56.5	46 (15 minutes), 49.5 (22 minutes,) 52 (34 minutes)
286	67.3	32 (5 minutes), 57 (15 minutes), 60 (22 minutes), 62 (30 minutes)
287	44.7	30 (5 minutes), 40 (15 minutes), 41 (22 minutes), 42 (30 minutes)
288	67.1	
289	72.3	69 (15 minutes), 70 (25 minutes)
290	57.0	53 (10 minutes), 56 (20 minutes)
298	82.0	
305	53.8	43 (10 minutes), 48.5 (20 minutes), 51.5 (32½ minutes)
308		65 (10 minutes), 68 (15 minutes), 69.5 (20 minutes) 70* (25 minutes)

* With these bloods rotation was continued until the increment of the column of serum in successive rotation periods became negligibly small. It was seldom that an absolutely constant length was reached.

† This blood as it sedimented in the test tubes obviously consisted mainly of serum.

The average for the eighteen cats, including those where only haematocrite determinations were made, is 66.2 per cent of serum, the range 44.7 to 87 per cent. The average for all the cats used by us would certainly be distinctly higher, for a number of old animals with an abnormally low serum content for a cat are included in the table.

In accordance with the fact that the cat's corpuscles in general sediment much more rapidly on standing than the dog's, and also because of the lower proportion of corpuscles, the haematocrite readings approximate sooner to the percentage determined electrically. The latter still, however, constitutes a limit which is not exceeded by the haematocrite determinations. I have found the same to be true for human blood, for example, in a case of diabetes insipidus with anaemia, studied along with Christie (4). Wilson (3) invariably obtained lower serum

percentages by the haematocrite than by the electrical method in pathological cases. Probably the difference in these cases would have been reduced by longer centrifugalization. That the electrical method gives results which are approximately correct was established by comparison with two other methods in the former paper (1). It is strong corroborative evidence that the haematocrite readings come nearer and nearer to the values determined by the electrical method, the longer the rotation, without quite attaining them, since some serum is inevitably left in the sediment. In the electrical method no alteration whatever can be produced in the corpuscles, the measurement being made while they are normally suspended in the serum.

Since the viscosity of blood is influenced greatly by its content of corpuscles, it is easy to see that very considerable differences may exist in the coefficient of viscosity of the blood of healthy individuals of the same species and in the average viscosity of the blood of different kinds of animals. Burton-Opitz has shown this in his elaborate investigations on the viscosity of blood. He found that the viscosity of dog's blood was on the average five times greater than that of distilled water (at 37°C.), that of rabbit's blood only 3.4 times greater, while cat's blood possessed an intermediate value.

Lewy (6) also observed a great difference in the viscosity of blood from different animals of the same species as well as in animals of different species. Welsh (7) found a considerable range in healthy human beings and of course very great variations in disease.

The fact that great variations in the viscosity are compatible with a perfectly efficient circulation, suggests that the emphasis which has been placed on the superiority of such substances as gum or gelatine as constituents of transfusion liquids is scarcely warranted. In so far as they may attract water from the tissues and retain it in the circulation, they may have some advantage. But when it is argued that since gum or gelatine solutions by increasing or maintaining the viscosity of the blood enable a greater arterial pressure to be attained, they must be superior to simple salt solutions, the physiological basis for the conclusion seems open to question.

Why should it be necessarily advantageous to artificially increase the resistance which the heart must overcome in order to drive blood through the tissues? When the viscosity is increased the resultant rise of blood pressure, provided the response of the heart is adequate, does not mean as a matter of course that the tissues are getting more blood than before but may merely mean that the heart by working harder

is able to deliver to them as much blood as before. It is true that the physiologist or the physician who is estimating the blood pressure may have the satisfaction of seeing it mount toward what is considered a normal level, but beyond this it is not clear that there would be any necessary advantage. If the response of the heart is inadequate and the rise of blood pressure is insufficient to overcome the extra resistance, the blood flow in the tissues may be diminished although the pressure has been increased. Of course, it may be argued that a certain minimum blood pressure is essential and that if this were not reached the organs, including the heart, would not function properly, even if a sufficient blood flow through them could be maintained. This, however, is one of the moot points of physiology, while there is general agreement that the important thing is a sufficient flow of blood. There are certainly conditions in which this essential blood flow might be promoted by diminishing the viscosity of the blood. If no other change occurred the blood pressure might be expected to fall. But where the blood viscosity is diminished by the injection of salt solutions the volume of the circulating liquid is at the same time increased, and if the pressure does not fall (it is not necessary that it should rise) the blood flow will be increased. When blood corpuscles are injected the viscosity is necessarily increased and the flow to that extent rendered more difficult. But there is the compensating advantage, which is most obvious in haemorrhage, that the increased viscosity is due to the addition of essential elements to the blood, an advantage which, of course, does not exist in the case of gum. These remarks are not intended in any way to prejudge the question whether clinical experience or physiological experiments may not have demonstrated the superiority of solutions containing such substances as gum.

BIBLIOGRAPHY

(1) Stewart: Journ. Physiol., 1899, xxiv, 357.
(2) Fraenkel: Zeitschr. f. klin. Med., 1904, lii, 476.
(3) Wilson: This Journal, 1905, xiii, 139.
(4) Christie and Stewart: Arch. Int. Med., 1917, xx, 10.
(5) Burton-Opitz: Journ. Amer. Med. Assoc., 1911, lvii, 353; Pflüger's Arch., 1900, lxxxii, 447; Journ. Physiol., 1905, xxxii, 8.
(6) Lewy: Pflüger's Arch., 1897, lxv, 447.
(7) Welsh: Heart, 1911, iii, 118.

THE EFFECT OF FEEDING PARS TUBERALIS AND PARS ANTERIOR PROPRIOR OF BOVINE PITUITARY GLANDS UPON THE EARLY DEVELOPMENT OF THE WHITE RAT

CARLETON J. MARINUS

From the Research Laboratories of Parke, Davis and Company

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INTRODUCTION

The pituitary body (hypophysis) is usually described as made up of two component parts, the anterior and posterior lobes (1).

Under the general term posterior lobe are included the pars nervosa, the pars intermedia and the neural stalk. Functionally these structures are very closely related. Extracts of each of them are capable of producing similar characteristic immediate effects when injected into the blood stream. It is believed that the active principles of the posterior lobe are elaborated in the pars intermedia and traverse the pars nervosa and neural stalk to the third ventricle, thus reaching the cerebrospinal fluid (2). The pars intermedia is histologically distinct from the pars nervosa and neural stalk, and traces its embryological origin to an entirely separate anlage. It is, however, very closely joined to the pars nervosa and is an essential part of the posterior lobe.

Injection of extracts prepared from the anterior lobe produce none of the effects characteristic of posterior lobe extracts. Long-continued feeding with the anterior lobe tissue, however, induces certain alterations in development, while the use in a similar way of posterior lobe preparations gives negative results.

Robertson in 1916 published a report in which he states that when fed to growing mice, anterior lobe produced a definite retardation of growth in the period immediately following puberty, this retardation being succeeded by a marked stimulation of growth (5). Goetsch, working with a much smaller number of white rats, stated that anterior lobe induced a uniform increase of growth-rate, denying the period of retardation (6). Later, Robertson and Delprat reported that anterior lobe feedings definitely increased the prepubertal growth rate of white mice.

The anterior lobe is also known to exert a certain control over the development and proper functioning of the reproductive system. Clinically, pituitary disease is always associated with more or less sexual abnormality, both anatomical and functional (9). Partial extirpation of the anterior lobe in experimental animals is followed by marked hypoplasia of the genitalia (3). Goetsch noted a marked hypertrophy of the genitalia in his pituitary-fed animals (6), while Robertson noted an increase in pugnacity and general virility in his pituitary-fed males (5).

Recently it has been shown that in addition to the structures mentioned above, the hypophysis contains a third epithelial lobe, the pars tuberalis, closely investing the neural stalk (10). The question arises whether this newly described structure is a part of the posterior or of the anterior lobe, or whether it possesses an entirely separate function. From its structural relations it might at first be considered a part of the posterior lobe complex for it is apparently continuous with the pars intermedia which has been proven to be a part of the posterior lobe mechanism. Atwell (11) has shown, however, that, in the rabbit at least, the pars tuberalis is embryologically quite distinct from the posterior lobe elements, arising as two lateral thickenings of the anterior lobe anlage and only later surrounding the developing neural stalk.

In a recent paper Atwell and Marinus (12) have compared the blood pressure and oxytocic reactions of pars tuberalis extracts with the reactions of pars intermedia, neural stalk and pars nervosa extracts. The reactions elicited by pars tuberalis extracts were so small that they were evidently due to unavoidable contamination of the extracts with active posterior lobe constituents, and not to any inherent activity on the part of pars tuberalis. It was concluded by the authors that the pars tuberalis is not a functional part of the posterior lobe.

Owing to its close anatomical relationship to the anterior lobe it was suspected that the pars tuberalis might be included in the ordinary anterior lobe preparations. Investigations showed that such was the case. As the anterior lobe is rolled from its capsule a small bit of the pars tuberalis is usually left attached to the pars anterior proprior. This fact opens the possibility that some of the effects of the anterior lobe feeding may be due to the inclusion of small portions of the pars tuberalis in the preparation. In the present study, white rats have been fed upon carefully separated pars tuberalis and pars anterior proprior, in an attempt to answer this question.

MATERIAL AND METHODS

In this experiment albino rats were fed portions of the pituitary gland, and carefully observed for any changes in growth rate and sexual development. The rats used were secured at the age of two to three weeks (shortly after weaning). They were immediately weighed and grouped according to weight and sex. Three separate series of experiments were

TABLE 1

Experiment 1. Gain in body weight (in grams)

SEX		AFTER 6½ WEEKS OF FEEDING			AFTER 12 WEEKS OF FEEDING		
		Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat.
Very young females	Number in group	6	2	4			
	Average final weight	72.6	68.0	73.1	Not weighed because of pregnancies		
	Average initial weight	16.0	16.7	17.0			
	Average gain	*56.6*	*51.3*	*56.1*			
Older females	Number in group	4	3	3			
	Average final weight	98.2	83.1	97.8	Not weighed because of pregnancies		
	Average initial weight	36.4	35.3	38.8			
	Average gain	*61.8*	*47.8*	*59.0*			
Very young males	Number in group		4	2		4	2
	Average final weight		74.7	84.2		90.5	97.0
	Average initial weight		18.2	16.7		18.2	16.7
	Average gain		*56.5*	*67.5*		*72.7*	*80.3*
Older males	Number in group	3	3	3	3	3	3
	Average final weight	113.6	107.1	113.5	130.3	121.3	134.3
	Average initial weight	38.3	38.6	46.0	38.3	38.6	46.0
	Average gain	*75.3*	*68.5*	*67.5*	*92.0*	*82.7*	*88.3*

conducted, one group comprising fifty-three rats, the second, thirty-seven, and the third, ten. The third series contained a single litter born at this laboratory.

During the entire experiment the rats were kept on a standard diet consisting of water and cracked mixed grains in abundance, stale (but not mouldy bread) once a week, and a small quantity of fresh milk daily. The rats were kept in metal cages, without litter except for the

grain they scattered about. The cages were cleaned weekly, using a coal-tar disinfectant. Individual rats were distinguished by spotting with stains—methylene blue, acid fuchsin and picric acid proving the most satisfactory. Each rat was weighed twice weekly.

In addition to the standard diet the rats were daily given the experimental foods. A small portion of the fresh gland was presented to the rat in forceps, whereupon it was invariably seized and swallowed. At

TABLE 2

Experiment 2. Gain in body weight (in grams)

SEX		AFTER 5½ WEEKS OF FEEDING			AFTER 11 WEEKS OF FEEDING		
		Group I. Anterior lobe.	Group II. Pars tuberalis	Group III. Meat.	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat
Very young females	Number in group	3	3	4			
	Average final weight	61.3	61.3	63.2	Not weighed because of pregnancies		
	Average initial weight	22.5	27.5	26.1			
	Average gain	*38.8*	*33.8*	*37.1*			
Older females	Number in group	5	4	6			
	Average final weight	94.9	91.2	94.3	Not weighed because of pregnancies		
	Average initial weight	44.8	47.1	45.5			
	Average gain	*50.1*	*44.1*	*48.8*			
Very young males	Number in group	7	5	5	7	5	5
	Average final weight	76.4	62.9	73.9	116.4	94.0	109.0
	Average initial weight	29.5	30.1	30.0	29.5	30.1	30.0
	Average gain	*46.9*	*32.8*	*43.9*	*86.9*	*64.1*	*79.0*
Older males	Number in group	4	4	3	4	4	3
	Average final weight	109.4	90.0	101.8	148.5	119.5	138.5
	Average initial weight	46.9	44.0	45.3	46.9	44.0	45.3
	Average gain	62.5	46.0	56.5	101.6	75.5	93.2

each feeding it was definitely ascertained that the portion allotted was completely devoured. The glands used were taken indiscriminately from steer, cow and heifer heads. As soon as possible after the animal was slaughtered the head was split in the sagittal plane and the pituitary gland dissected out. The glands were immediately wrapped in oiled paper and packed in ice. In every case the glands were dissected into their component parts and fed to the rats within six hours after the

TABLE 3

Experiment 3. Gain in body weight (in grams). After 6½ weeks of feeding

	GROUP I. ANTERIOR LOBE			GROUP II. PARS TUBERALIS			GROUP III. MEAT		
	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain
1. Female	32.0	105.5	73.5	32.0	80.5	48.5	32.5	96.0	63.5
2. Female	35.0	118.5	83.5	34.0	88.5	54.5	33.0	94.0	61.0
3. Male	36.5	129.5	93.0	32.0	100.0	78.0	37.0	112.5	75.5
4. Male				33.5	87.5	74.0			
Total	103.5	353.5	250.0	131.5	350.5	255.0	102.5	302.5	200.0
Average	34.5	117.8	*83.3*	32.9	89.1	*63.7*	34.1	100.8	*66.6*
Relative gain			125.0			95.0			100.0
Normal valued at 100				(cf. table 5)					

TABLE 4

Experiment 3. Body lengths in centimeters after 6½ weeks of feeding

	GROUP I. ANTERIOR LOBE	GROUP IV. PARS TUBERALIS	GROUP VII. MEAT
1. Female	15.2	13.8	13.6
2. Female	16.2	14.8	13.9
3. Male	16.3	14.6	15.2
4. Male		13.6	
Total	47.7	56.7	42.7
Average	15.9	14.2	14.2

TABLE 5

Experiment 3. Weights of genital organs in milligrams

	GROUP I. ANTERIOR LOBE		GROUP II. PARS TUBERALIS		GROUP III. MEAT	
	Male	Female	Male	Female	Male	Female
1		160		120		115
2		220		100		140
3	1530		885		910	
4			865			
Average	1530	190	875	110	910	135
Relative variation	168		96		100	
Normal valued at 100		140		81		100

death of the animal. During this time there was no actual contact between the ice or ice water and the glands. These precautions were taken in order to make certain that the material should lose none of its activity by autolysis, by decomposition or by solution of its constituents in the ice water.

The partes tuberales were dissected out as described by Atwell and Marinus. The material available was then divided into equal portions sufficient to supply all the rats to be fed. Each rat was given a quantity of pars tuberalis which varied from day to day but which approximated one-fourth of an entire pars tuberalis.

TABLE 6

Experiment 2. Birth of first litter and weight of mother at conception

	GROUP I. ANTERIOR LOBE		GROUP II. PARS TUBERALIS		GROUP III. MEAT	
	Litters	Weight	Litters	Weight	Litters	Weight
7th week of feeding	1	101.0				
8th week	2	94.0				
		90.0				
9th week						
10th week	1	107.5				
11th week	2	91.5				
		121.5			1	107.0
12th week			1	122.0	2	94.0
						89.5
13th week			2	94.5	2	127.0
			(2 preg.)	85.5	(3 preg.)	121.5
Average weight		*100.9*		*100.6*		*107.8*

The anterior lobes were secured by splitting the glands in the midsagittal plane and rolling the entire anterior lobe out of its capsule, leaving the posterior lobe attached to the capsule and dura mater. The portion of the anterior lobe at the point of origin of the pars tuberalis was snipped off, making certain that no pars tuberalis fluids or tissues were included with the anterior lobe as fed. Each rat was given a portion equal to about one-sixth of the entire lobe (approximately 250 mgm.). As a control certain of the rats were fed beef muscle. A portion of the muscle was freed from all loose fat and was then divided into portions approximately equal in weight to the portions of anterior lobe.

All the rats in series 3 (see above) were killed with ether after six and one-half weeks of feeding. The body weights and lengths were recorded. The entire female genital tract was dissected out down to the trigone and preserved intact. The sex organs were then fixed in Bouin's fluid and dehydrated in alcohols. When in 80 per cent alcohol each testis (together with the corresponding epididymis) was weighed separately. The female genital tract was weighed as a whole. The tissues were completely dehydrated, cleared with xylol, imbedded in paraffin and cut 5 micra thick. The sections were stained with Heidenhain's iron haematoxylin and eosin.

DISCUSSION

Puberty occurs in the white rat from sixty to ninety days after birth (13). The rats of experiments 1 and 2 were then barely approaching the normal time of puberty after six and one-half and five and one-half weeks of feeding. This stage corresponds roughly to the end of the second or beginning of the third growth cycle described by Robertson for white mice. Comparison of the weights at this stage (tables 1 and 2) shows a small but well defined increase in weight in the anterior-lobe-fed groups over all other groups. This is in agreement with Robertson and Delprat who conclude that anterior lobe feeding stimulates growth during the second development cycle.

The rats of experiment 3 were born in the same litter and allow more exact comparisons. Here also the anterior-lobe-fed surpassed the other animals at the age of nine weeks (after six and one-half weeks of feeding) (table 3). The difference is even more marked in this experiment owing to the greater uniformity of the animals.

After twelve weeks of feeding all the females had given birth to at least one litter or were in some stage of pregnancy. The rats had, therefore, all passed puberty. At this stage, also, the anterior-lobe-fed males were heavier than any of the others, the difference being greater than at the age of puberty. This observation is contrary to Robertson's conclusions that after puberty the administration of anterior lobe to white mice produces a preliminary retardation of growth. The seeming inconsistency may be due to a possible difference in the relative lengths of the growth cycles of white mice and white rats.

The nose-to-anus lengths recorded in table 4 show that the anterior-lobe-fed rats were considerably larger than either the controls or the pars-tuberalis-fed rats. The increased weights noted in the case of the

anterior-lobe-fed rats are, then, due to an increased skeletal growth. This conclusion is in keeping with the increased skeletal growth noted in cases of pathologic hyperpituitarism.

While the anterior-lobe-fed rats were growing faster than the controls, the pars-tuberalis-fed animals were losing ground. In all three experiments the pars-tuberalis-fed rats were markedly smaller than the controls. This was even more constant and more marked than the overgrowth of the anterior-lobe-fed animals. It should be remarked, however, that the pars-tuberalis-fed rats were not strictly controlled. The rats of the other groups received daily a quantity of muscle or glandular tissue weighing approximately 250 mgm. while the tuberalis-fed rats were given only a minute portion of tissue weighing but 5 to 10 mgm.

It is evident that the accelerated growth reported by previous observers as following anterior-lobe-feeding has not been due to any admixture of pars tuberalis substance, inasmuch as the same effects are produced by anterior lobe material known to be free from other parts of the gland, and inasmuch as no overgrowth is induced by feeding pars tuberalis alone. The apparent retardation of growth must be further studied before it is to be definitely ascribed to pars tuberalis feeding.

In addition to its action on the process of growth, the anterior lobe is known to control, to some extent at least, the development of the reproductive system. The effects of anterior lobe feedings on the sexual organs may be evaluated by *a*, study of the organs themselves, or *b*, date of birth of the first litter. Both methods have been used in this study.

The testes of the anterior-lobe-fed male (experiment 3) were markedly larger than those of the other males, weighing nearly twice as much as the controls. Upon section it was found, as reported by Goetsch, that all of the tissues in the testicle are involved in the hyperplasia but that the increase in size and weight is chiefly attributable to changes in the seminiferous tubules. The female organs were also better developed although the gross difference was not so marked.

The genital organs of the pars-tuberalis-fed rats could not, however, be distinguished from those of the controls. The slightly greater weight of the control organs is within the limits of individual variation and is explained as concomitant to the larger size of the control animals. Microscopical examination of both testes and ovaries revealed no difference between those of the pars-tuberalis-fed and control rats.

In normal healthy rats the first copulation occurs shortly after the sex glands become histologically mature. Thus the date of birth of the first litter is a good criterion of the relative maturity of two groups of animals. During the ninth week of feeding it became evident that several of the anterior-lobe-fed animals were pregnant, while none of the controls were showing signs of pregnancy. This observation was borne out by the fact that three anterior-lobe-fed females gave birth to the first litters two weeks before any of the control litters were born. The females evidencing this sexual precocity were not larger than the slower controls at the calculated date of impregnation, i.e., the sexual development had been more rapid than the somatic.

The pars-tuberalis-fed and control females dropped their litters during the same period of time, averaging more than two weeks after the anterior-lobe-fed females. In keeping with the microscopical evidence afforded by experiment 3, the births in this experiment show that the pars-tuberalis-feedings do not alter the sexual development or function of the white rat.

SUMMARY AND CONCLUSIONS

One hundred young rats were separated into three groups. The first group was fed upon pars anterior proprior of the pituitary gland, the second upon pars tuberalis, and the third or control group upon beef muscle. During twelve weeks of feeding the rats of the first group exhibited increased growth rate accompanied by a more rapid development of the reproductive system, evidenced by gross and microscopical hypertrophy of the organs and by the earlier birth of young. In the second (pars tuberalis fed) group there was no change in the sexual development as compared with that of the control group. The growth rate was slightly slower in the second group, owing perhaps to the smaller amount of meat fed.

This study has not shown that any of the functions ascribed to the anterior lobe as a whole are due to the pars tuberalis.

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BIBLIOGRAPHY

(1) Biedl: Innere Sekretion, 1913, 2 Aufl., Heft. ii.
(2) Herring: Quart. Journ. Exper. Physiol., 1908, i, 121.
(3) Crowe, Cushing and Homans: Bull. Johns Hopkins Hosp., 1910, xxi, 127.
(4) Aldrich: This Journal, 1912, xxx, 352.
Wulzen: This Journal, 1914, xxxiv, 127.
Schafer: Quart. Journ. Exper. Physiol., 1912, v, 203.
Lewis and Miller: Arch. Int. Med., 1913, xii, 137.
(5) Robertson: Journ. Biol. Chem., 1916, xxiv, 385.
(6) Goetsch: Bull. Johns Hopkins Hosp., 1916, xxvii, 29.
(7) Robertson and Delprat: Journ. Biol. Chem., 1917, xxxi, 567.
(8) Barney, E. L.: Journ. Lab. and Clin. Med., 1918, iii, 480.
Robertson and Burnett: Journ. Exper. Med., 1916, xxiii, 631.
(9) Falta and Meyers: The ductless glandular diseases, 2nd ed., 1916.
(10) Tilney: Internat. Monatschr. Anat. and Physiol., 1913, xxx, 258.
(11) Atwell: Amer. Journ. Anat., 1918, xxiv, no 3.
(12) Atwell and Marinus: This Journal, 1918, xlvii, 76.
(13) Donaldson: The rat, Memoirs of Wistar Inst., no. 6.

THE RELATION OF HYPOPHYSIS TO GLYCOGENOLYSIS

ROBERT W. KEETON AND FRANK C. BECHT

From the Laboratories of Pharmacology, University of Illinois College of Medicine, and Northwestern University Medical School, Chicago

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In a previous report it was shown by the authors (1) that stimulation of the hypophysis produced a hyperglycemia, which did not occur if the stimulus was applied contiguous to but not on the gland, and that this rise in sugar was absent in animals whose splanchnic nerves had been previously sectioned. The present study was undertaken to determine the significance of this apparent nervous relation of the hypophysis to the glycogenolytic process.

The same general plan of experimentation previously adopted was followed. The blood sugar however was estimated by the Benedict method as modified by Meyers and Bailey (2) and by Benedict (3) rather than by the Bertrand (4) titration after the removal of proteins in acid sodium sulphate solutions.

Stimulation of gland. It was soon observed that under etherization alone the colorimetric methods gave a continuous persistent rise in glycemia despite the use of precautions to rule out all influences other than ether. A study of this mechanism was undertaken and has been recently reported by one of us (5).

Stimulation of the hypophysis gave in some of the animals a glycemic level which was no higher than that attained by ether stimulation alone. In other cases the level was distinctly above that ever obtained by simple etherization. Responsiveness of different animals to such experimental procedure shows rather wide individual variations, so that it is difficult to establish a normal. Consequently a quantitative value cannot always be assigned to the ether hyperglycemia. However, the character of the curves under the two experimental conditions differ. Ether shows a slow rise with a maximum attained in the interval between second and third hour. With constant ether administration the curve appears within these ranges to be a simple function of the time. Following the excitation of the hypophysis, whether it be done mechanically (as in

drilling the bone out over the gland or manipulating it in its removal), or electrically, there is an immediate step up in the blood sugar. The quantity of sugar so liberated may be greater than would appear under prolonged etherization but of this we have no conclusive proof. However, the evidence furnished on stimulating the gland in animals with denervated livers is strongly suggestive that an increase above the ether level occurs. Certainly it is clear that there is an immediate acceleration of the glycogenolytic process.

TABLE 1

Stimulation of hypophysis in normal animals

ANIMAL	BEFORE ETHER	AFTER				
		Ether	Drilling†	Stimulation	Rest	Time of experiment in minutes
*1	0.094	0.091	0.088	0.100	0.106	73
2	0.088	0.113	0.182	0.232	0.183	75
3		0.124	0.176	0.193	0.169	55
4		0.115	0.136	0.260	0.273	63
5		0.145	0.201	0.243		105
6	0.096	0.133	0.146	0.155	0.160	80

* Attempt had been made to section cord in this animal previously. Appetite good but he never regained complete health.

† Gland exposed by using a dental drill.

The results are the average of duplicate estimations, figured to the percentage of dextrose in the whole blood.

Doubly splanchnectomized animals. In the previous report it was shown that when the splanchnic nerves were cut the rise in sugar did not follow the hypophyseal stimulation. This finding was confirmed in two animals:

Dog 25. Splanchnics were sectioned 17 days previously; the blood sugar after relaxation under surgical anesthesia 0.128 per cent; 1 hour and 43 minutes later during which gland was exposed and stimulated, 0.093.

Dog 26. Splanchnics cut 7 days previously; after surgical anesthesia, 0.087; 45 minutes later during which the gland was exposed and stimulated, 0.091.

Section of nerve in hepatic pedicle. In the first two cases (animals 7 and 8) only the nerves about the hepatic artery were cut. In the others (9, 10, 11) all of the structures in the hepatic pedicle were severed. The coats of the artery vein and common bile duct were cauterized lightly with hot iron. This precaution, as had been shown by Macleod (6),

must be taken if one is to be certain of a complete separation of liver from its nervous connections.

Animals so operated have been shown previously (5) to give a lower hyperglycemic response to ether than normal animals. The last column of table 2 shows these ether control values reproduced from the former study. Table 2 indicates quite clearly that the blood of these animals attains a high grade of hyperglycemia following excitation of the hypophysis. This rise in comparison with the control experiments suggests further that stimulation of the gland probably not only initiates and accelerates the glycogenolysis, but also raises it to higher level than ether alone does under similar experimental conditions.

TABLE 2

Effect of stimulation of the hypophysis after section of nerves in the hepatic pedicle

ANIMAL	BEFORE ETHER	AFTER				
		Ether	Drilling	Stimulation	Rest	Control after 3 hours ether anesthesia
Hepatic artery denervated*						
7	0.101	0.137	0.208	0.216	0.234	
8		0.171	0.201	0.225	0.234	
Section of all nerves in pedicle†						
9	0.105	0.148	0.167	0.248	0.277	0.132
10	0.083	0.106	0.161	0.168	0.163	0.124
11	0.090	0.095	0.133	0.164		0.117

* Nerves removed from artery alone and coats cauterized.

† Entire pedicle sectioned except hepatic artery, portal vein and common bile duct. Coats of these were cauterized.

Transection of cord. The cord was transected by removing the second dorsal spine and severing it with a sharp-pointed probe. It was suggested in our former study that the alleged hormone, which Weed, Cushing and Jacobsen (7) believed they had liberated in the stimulation of the hypophysis and the superior cervical ganglion, might really exist and might find its site of action on the terminations of the pre-ganglionic splanchnic fibers which we had destroyed in our splanchnectomy.

In order to test out this hypothesis, the experiments on animals with transected cords were performed. Reference to table 3 shows that such animals were used in all stages of recovery (from four to thirty-six

days), so as to eliminate the vasomotor instability associated with spinal shock and that in none of these was there a suggestion of hyperglycemia.

TABLE 3

Stimulation of hypophysis in animal with cord transected at the level of second thoracic vertebra

ANIMAL	DAYS AFTER TRANSECTION	BEFORE ETHER	AFTER			
			Ether	Drilling	Stimulation	Rest
12	4	0.108	0.111	0.117	0.076	0.088
13*	6		0.112	0.060	0.076	0.082
14	16	0.077	0.093	0.077	0.071	0.063
15	21	0.102	0.091	0.098	0.089	0.073
16*	25	0.069	0.087	0.084	0.112	0.111
17	36	0.104	0.097		0.088	0.071

* Sugar estimated by sodium sulphate precipitation: Bertrand titration; others by colorimetric method.

TABLE 4

Glycemia following hypophysectomy

ANIMAL	AFTER ETHER	AFTER DRILLING	AFTER REMOVAL OF GLAND	
			Immediately	Hours expressed as exponents
Normal animal				
18	0.143	0.227	0.277	0.093^{3}; 0.055^{6}; 0.083^{24}; 0.062^{47}; 0.067^{100}
19	0.143	0.142	0.137	0.093^{3}; 0.099^{5}; 0.072^{23}; 0.062^{46}
20	0.143	0.273	0.311	0.1001^{8}; 0.092^{23}
21	0.153	0.146	0.153	0.107^{4}; 0.092^{22}; 0.083^{46}; 0.103^{72}; 0.103^{44}
Complete denervation of hepatic pedicle				
22	0.143	0.168	0.330	0.134^{5}; 0.090^{23}
23	0.106	0.161	0.156	0.136^{4}; 0.090^{27}
24	0.090	0.134	0.167	0.085^{4}; 0.069^{23}; 0.089^{29}
Splanchnic nerves on both sides sectioned				
25	0.128	0.124	0.102	0.095^{5}; 0.094^{24}

Hypophysectomy. Cushing and associates (8) have reported that in dogs a transitory glycosuria follows hypophysectomy, manipulation of gland or compression of stalk with a metal clip. The literature has been

critically reviewed by Goetsch (9) but these workers are apparently the only ones who have investigated the relation of hypophysis to carbohydrate metabolism using the methods of glandular removal and electrical stimulation. We desired to know the duration and persistence of this hyperglycemia, so glands were removed in several animals as shown in table 4. Examination of the data shows that within three to five hours after the removal, the sugar level is normal and remains so until death.

In animal 19 there appeared to be some evidence of hypoglycemia but this we attributed to his moribund condition. No attempt was made to determine the completeness of the removal by microscopic section, since the animals died with all the evidences of insufficiency within the usual time limits.

DISCUSSION

The data presented demonstrate clearly that if the stimulus be prevented from reaching the level of the adrenals no hyperglycemia results from stimulation of the hypophysis. A hormone[1] if liberated by the stimulation does not act on any of the peripheral mechanism of glycogenolysis. For section of the cord leaves the splanchnic nerves (both pre- and post-ganglionic fibers), all their terminations in the adrenals and liver intact, and yet, following this, the experiment does not give a hyperglycemia. Such a hormone would have to find its site of action on some central cranial structure.

If the pathway be assumed to be a nervous one, then section of splanchnics removes the block a little further peripheralward, a procedure which prevents the hyperglycemia. Section in hepatic pedicle beyond the adrenals does not interfere with the rise in blood sugar. These findings are all analogous to those of Macleod (10) on stimulating splanchnic nerves, and Keeton and Ross (5) in their study of ether hyperglycemia. It appears then that excitation of the hypophysis has originated a stimulus which is directed peripheralward by nervous connections and falls upon the splanchnic-adrenal-hepatic mechanism controlling the ultimate partition of glycogen.

The transitory nature of the hyperglycemia following the removal of the hypophysis coupled with the maintenance of a fairly normal sugar level until death we believe points to the fact that the rôle played by the hypophysis in carbohydrate metabolism deals not with the

[1] Under the term "hormone" is included any chemical product formed in one organ which may stimulate any other organ to activity.

process of glycogenolysis, but with some other phase. Indeed the well established alteration of sugar tolerance in cases of acromegaly and pituitary tumors (11) as well as the studies of Cushing in carbohydrate tolerance after removal of the posterior lobes indicate that future investigation should be directed toward the influence of the hypophysis on the utilization of the sugar by the organism.

CONCLUSIONS

1. Stimulation of hypophysis in dogs causes hyperglycemia independently of the ether used in the anesthesia.

2. This hyperglycemia is absent after transection of cord at level of second thoracic vertebra and after section of the splanchnics. It persists after section of the nerves in the hepatic pedicle.

3. Following hypophysectomy a transitory hyperglycemia occurs, and persists three to five hours. After this the sugar level remains normal until death.

4. If a hormone is liberated by stimulation of the gland, it must have a central action.

5. The view is favored that the pathway is a nervous one mediated through the splanchnic nerves to their terminations in the adrenals and liver.

6. The physiological rôle played by the hypophysis in carbohydrate metabolism does not deal with transformation of glycogen into sugar, but more probably with the utilization of the sugar by the organism.

BIBLIOGRAPHY

(1) Keeton and Becht: This Journal, 1915, xxxix, 109.
(2) Meyers and Bailey: Journ. Biol. Chem., 1916, xxiv, 147.
(3) Benedict: Journ. Biol. Chem., 1918, xxxiv, 203.
(4) Sonntag: Biochem. Zeitschr., 1913, liii, 501.
(5) Keeton and Ross: This Journal, 1919, xlviii, 146.
(6) Macleod and Pearce: This Journal, 1912, xxix, 419.
(7) Weed, Cushing and Jacobsen: Johns Hopkins Hosp. Bull., 1913, xxiv, 40.
(8) Crowe, Cushing and Homans: Johns Hopkins Hosp. Bull., 1910, xxi, 127.
(9) Goetsch, Cushing and Jacobsen: Johns Hopkins Hosp. Bull., 1911, xxii, 165; 1910, xxi, 260.
Goetsch: Quart. Journ. Med., 1914, vii, 173.
(10) Macleod: Diabetes, 52.
Macleod and Pearce: This Journal, 1912, xxix, 419.
Macleod: This Journal, 1908, xxii, 373.
(11) Borchardt: Zeitschr. f. klin. Med., 1908, lxvi, 332.

THE GASTRIC RESPONSE TO FOODS[1]

VI. Digestion in the Normal Human Stomach of Eggs Prepared in Different Ways

RAYMOND J. MILLER, HARRY L. FOWLER, OLAF BERGEIM, MARTIN E. REHFUSS and PHILIP B. HAWK

From the Laboratory of Physiological Chemistry of Jefferson Medical College, Philadelphia

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Eggs like meats are particularly rich in protein. Hence their digestion is carred out to a considerable extent in the stomach and is of more than usual interest not only from the standpoint of normal nutrition but also from that of clinical dietetics, inasmuch as eggs in the raw state or prepared in different ways are frequently used to replace meat in the diet of invalids.

Beaumont (1) found that raw egg white left the stomach of his subject (Alexis St. Martin) very quickly, in fact sooner than any other food investigated. Pavlov (2) noted in dogs that such egg white produced no stimulation of gastric secretion. Bateman (3) found the utilization of raw egg white to be poor, particularly when large amounts were fed to dogs, and that this indigestibility was due to the ovalbumin which the egg white contains. Penzoldt (4) studied the evacuation times of eggs in the human stomach, using a large stomach tube, and obtained as the result of five experiments the values given in table 1.

Jaworski and Gluzenski (5) reported that it required one and a quarter hours for the white of one egg, finely chopped and taken with two and one-half ounces of water, to leave the stomach. Water has, however, in itself a stimulatory effect upon secretion (6).

The present study was made to determine the response of the normal human stomach to eggs prepared in different ways. The fractional method of gastric analysis was employed to follow the course of digestion. The experimental conditions and the methods employed were in general those used in the earlier work carried out in this laboratory upon meats

[1] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

(7). The subjects were normal medical students who came to the laboratory at 8:00 a.m. without breakfast. Residuums were not removed. The number of eggs given to each man was two except where otherwise specified. The subjects were permitted to eat these in their ordinary manner. The time required was about ten minutes with not more than two or three minutes variation. Most of the subjects of these tests swallowed the tube without assistance and showed no signs of discomfort. They remained seated most of the time and at rest, i.e., talking, reading and copying notes. Samples were withdrawn at 15 minute intervals and kept in ice water for the short period preceding analysis. Lavage was used in every case to determine conclusively whether the stomach was empty or not. In practically every case the results of lavage were confirmatory, showing that the stomach can be completely emptied by means of the tube used. In a few cases it was necessary to discontinue the experiment after a period of hours before digestion was complete.

TABLE 1

Evacuation times of eggs from the stomach (Penzoldt)

PREPARATION	EVACUATION TIME
	hours
2 lightly boiled eggs	$1\frac{3}{4}$
2 raw eggs	$2\frac{1}{4}$
2 poached eggs, 5 grams butter	$2\frac{1}{2}$
2 hard boiled eggs	3
2 eggs in omelet	3

In such cases the stomach was emptied and the volume of contents determined. All experiments were carried to completion except where otherwise indicated on the charts. Subjects showing any indication of pathological response were rejected. Free acidities are distinguished in charts from total acidities by using the prime mark as A′.

Among the egg preparations tested were raw white, raw yolk, raw whole eggs (strained and unstrained), soft boiled, hard boiled, scrambled (with and without excess of fat), fried (with and without excess of fat), plain omelet, Spanish omelet, deviled eggs, poached eggs, pickled eggs, shirred eggs and soft cooked eggs. Frozen eggs and cold storage eggs were compared with fresh eggs. Duck and turkey eggs were also used as well as the Chinese preserved duck egg called pidan. Eggs with milk, French toast or eggs with bread, scrambled eggs with frizzled beef, and bacon and eggs were food combinations tested. A few tests were made on raw eggs with water and egg albumin with orange juice.

TABLE 2

Response of the normal human stomach to eggs prepared in different ways

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity*		Evacuation time		Highest acidity*	
			Average	Average			Average	Average	
	Hens' eggs								
1	Raw white	1:30		37					
2	Raw white	1:00		83					
3	Raw white	2:00	1:30	90	70				
3a	Raw white					2:15	2:15	6.0	6.0
4	Raw yolk	2:15	2:15	94	94				
5	Raw yolk					3:00		84	
6	Raw yolk					2:45			
7	Raw yolk					2:00	2:35	105	95
8	Raw eggs (whole)					2:15		107	
9	Raw eggs (whole)					3:30	2:55	43	75
10	Raw eggs (strained)	3:00		81					
11	Raw eggs (strained)	3:10							
12	Raw eggs (strained)	2:15		80					
13	Raw eggs (strained)	2:00	2:35	71	77				
14	Raw eggs (strained)					3:00	3:00	53	53
15	Soft boiled	2:00		94					
16	Soft boiled	1:45		106					
17	Soft boiled	2:30		68					
18	Soft boiled	2:00		63					
19	Soft boiled	1:45		94					
20	Soft boiled	2:45	2:00		85				
21	Soft boiled					2:45		122	
22	Soft boiled					2:50			
23	Soft boiled					3:00		79	
24	Soft boiled					2:00		48	
25	Soft boiled					4:05			
26	Soft boiled					3:30		58	
27	Soft boiled					3:00	3:00		77
28	Hard boiled	2:00		92					
29	Hard boiled	3:00		132					
30	Hard boiled	1:30		82					
30a	Hard boiled	2:30		101					
31	Hard boiled	2:15		70					
32	Hard boiled	1:45	2:10	93	95				
33	Hard boiled					3:15			
34	Hard boiled					3:25			
35	Hard boiled					3:30			

TABLE 2—*Continued*

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity		Evacuation time		Highest acidity	
			Average	Average			Average	Average	
36	Hard boiled					3:00		107	
37	Hard boiled					3:30	3:20	97	102
38	Scrambled	2:30		86					
39	Scrambled	2:15	2:25	100	93				
40	Scrambled					2:45		81	
41	Scrambled					3:50			
42	Scrambled					3:15	3:15	94	85
43	Fried	2:00		106					
44	Fried	2:30	2:15		106				
45	Fried					2:00	2:00	108	108
46	Fried (excess fat)	2:45	2:45	91	91				
47	Fried (excess fat)					3:00	3:00	90	90
48	Omelet plain	2:15		83					
49	Omelet plain	2:30	2:25	80	82				
50	Omelet Spanish	2:30	2:30	100	100				
51	Deviled eggs	2:45	2:45	103	103				
52	Deviled eggs					3:40	3:40		
53	Poached eggs	1:30	1:30	117	117				
54	Poached eggs					3:30	3:30	61	61
55	Shirred eggs	2:00	2:00	95	95				
56	Shirred eggs					2:30	2:30	85	85
57	Pickled eggs					3:30		85	
58	Pickled eggs					3:30	3:30	65	75
59	Scrambled (excess fat)'	2:30	2:30	122	122				
60	Scrambled (excess fat)					3:30	3:30	58	58
61	Soft cooked	1:45	1:45	52	52				
62	Soft cooked					3:15	3:15	63	63
	Duck eggs								
63	Soft boiled					3:15	3:15	73	73
64	Hard boiled					3.00	3:00		
	Turkey eggs								
65	Hard boiled	2:45	2:45	103	103				
66	Hard boiled					3:15	3:15	88	88
	Frozen hens' eggs								
67	Scrambled	2:00	2:00	76	76				
68	Scrambled					2:30	2:30	79	79
69	Sponge cake (frozen eggs)	2:30		84					
70	Sponge cake (frozen eggs)	2:15	2:20	40	62				
71	Sponge cake (frozen eggs)					3:00	3:00	14	14
72	Sponge cake (fresh eggs)	2:00	2:00	42	42				
73	Sponge cake (fresh eggs)					2:30	2:30	15	15

TABLE 2—*Continued*

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity		Evacuation time		Highest acidity	
			Average	Average			Average	Average	
	Cold storage eggs								
74	Soft boiled	1:45	1:45	91	91				
75	Soft boiled					2:30		93	
76	Soft boiled					3:30	3:00	66	80
77	Hard boiled					3:15	3:15	99	99
78	Fried (excess fat)	2:45	2:45	124	124				
	Egg combinations								
79	Milk and eggs	3:00		96					
80	Milk and eggs	2:45	2:50	41	68				
81	French toast					3:30		99	
82	French toast					3:30	3:30	67	83
83	Bacon and eggs	2:15	2:15	97	97				
84	Bacon and eggs					3:30	3:30	83	83
85	Frizzled beef and scrambled eggs	2:30	2:30	67	67				
86	Frizzled beef and scrambled eggs					2:45	2:45	146	146
87	Chinese pidan eggs	3:15	3:15	99	99				
88	Chinese pidan eggs					4:45	4:45	30	30
89	Raw hens' eggs and water					3:15	3:15	67	67
90	Raw white and water					2:15	2:15	85	85
91	Raw white and orange juice	1:15	1:15	86	86				
92	Raw white and orange juice					2:15	2:15	109	109

* Acidities expressed as cubic centimeters of N/10 alkali required to neutralize 100 cc. of sample.

Evacuation times and highest total acidities are recorded in table 2 which also gives average responses for individuals of the rapid- and slow-emptying types. The necessity for this division of subjects into classes is clearly apparent from an examination of the charts for certain of these individuals. Compare, for example, subjects Se and Da (figs. 1, 10 and 20) showing average evacuation times of about two hours for eggs with subjects Me and Ka (figs. 7, 19, 14 and 16) whose gastric digestion was not usually completed in three hours. Similarly the grand average evacuation time for 44 experiments on subjects of the rapid type was 2 hours 15 minutes and for 48 experiments on the slower type, 3 hours and 5 minutes.

In order that comparisons of the digestion of eggs prepared in different ways might be made as direct as possible, several experiments were made

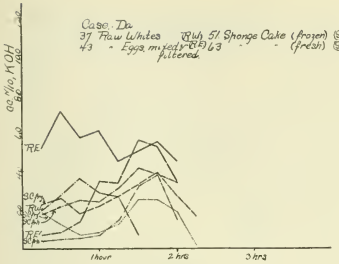

Fig. 1

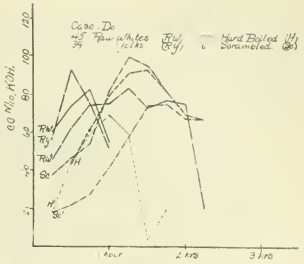

Fig. 2

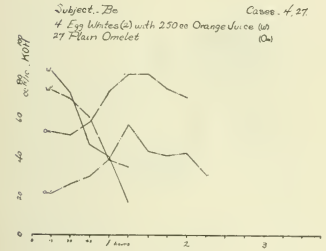

Fig. 3

Fig. 4

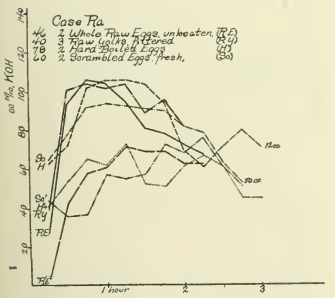

Fig. 5

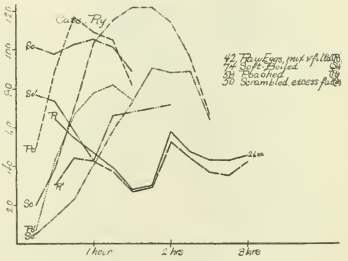

Fig. 6

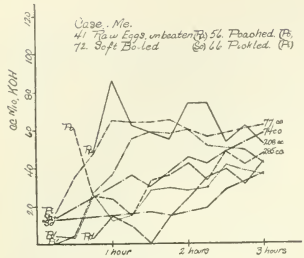

Fig. 7

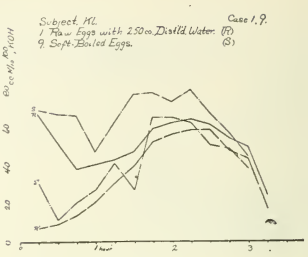

Fig. 8

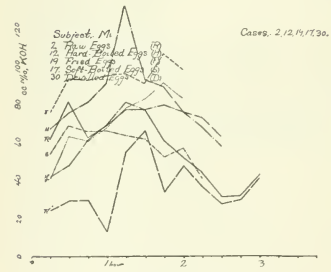

Fig. 9

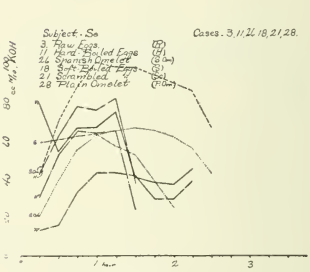

Fig. 10

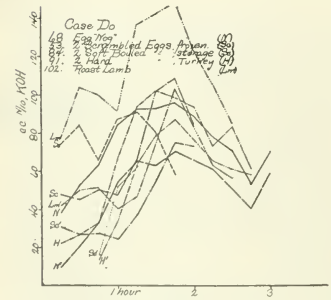

Fig. 11

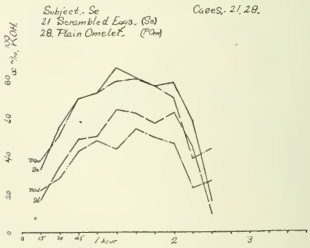

Fig. 12

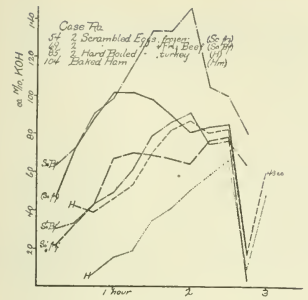

Fig. 13

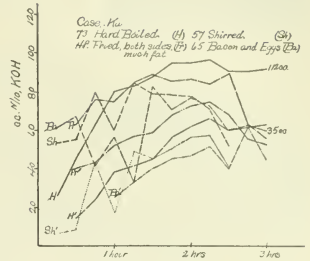

Fig. 14

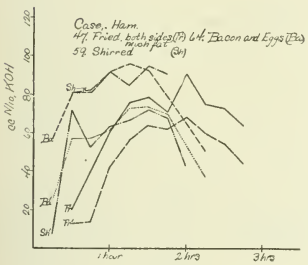

Fig. 15

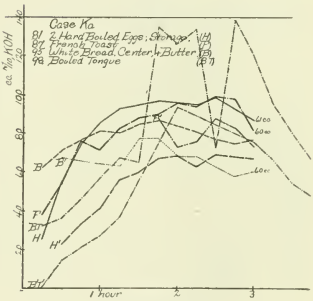

Fig. 16

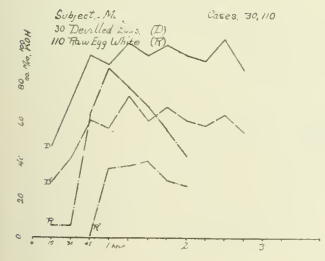

Fig. 17

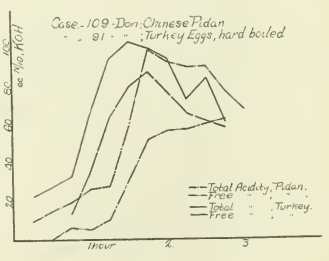

Fig. 18

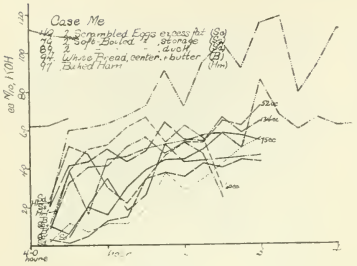

Fig. 19

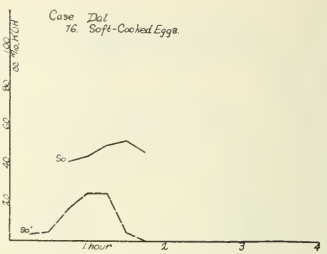

Fig. 20

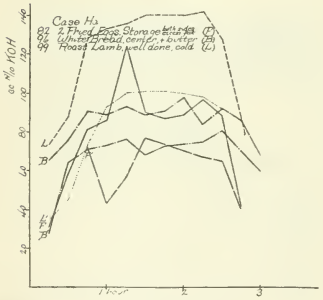

Fig. 21

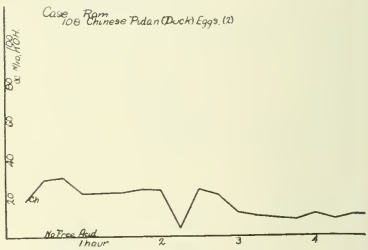

Fig. 22

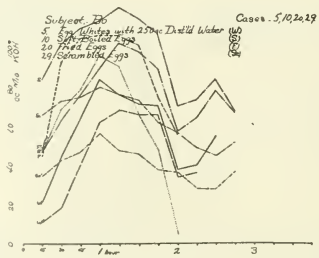

Fig. 23

upon most of the subjects used. About 75 of these experiments are charted in figures 1 to 23, which illustrate all of the most important facts brought out by this investigation.

DISCUSSION

The response of the stomach to raw eggs, raw white and raw yolk. In the experiments on raw egg white, the whites of three eggs were strained through cloth and given without any addition. This egg white left the stomach rather rapidly, i.e., in from 1 to 2¼ hours. (See table 2 and figs. 1, 2 and 17). In one case egg white showed hardly any stimulation of secretion, in another case the acidity was rather low, while two other cases showed a moderately high acidity. It appears that the stimulatory power of egg white is insufficient to cause any material secretion in individuals of a low acid type while subjects who respond readily to most food stimuli will show a response to egg white. Thus in cases Do (fig. 2) and Mi (fig. 17) a distinct stimulation of secretion by egg white occurred, although the secretion was not nearly as pronounced as with most cooked eggs. The finding of Pavlov (2) that egg white produced no stimulation of gastric secretion in dogs cannot be applied to man without qualification. The experiment on egg white charted in figure 17 was repeated the following day but instead of withdrawing samples at 15 minute intervals the entire gastric contents were aspirated after 45 minutes. Twenty cubic centimeters of liquid with a total acidity of 42 and a free acidity of 18 were withdrawn. Of the original 100 cc. of egg white given, not 10 per cent remained. The bulk of the egg white thus left very quickly but not without giving rise to a secretion of gastric juice which continued for an hour longer. Where 250 cc. of water were given with the egg white (exp. 90, fig. 23) gastric secretion was distinctly stimulated, not more so however than is usual with water alone (6). The addition of 200 cc. of orange juice (expts. 91 and 92 and figs. 3 and 4) led to a distinct acid secretion and rapid evacuation of the stomach. Experiment 92 (fig. 4) showing an evacuation time of 2¼ hours for albumin with orange juice should be compared with experiment 34 on the same subject showing an emptying time of 3½ hours for hard boiled eggs and experiment 41 on scrambled eggs which required 3 hours and 50 minutes to leave the stomach. The use of egg albumin with orange juice in the diet of invalids would appear therefore to be supported from the standpoint of gastric digestion.

In the tests on egg yolk the yolks of three eggs were given after being strained through cheese cloth. Egg yolk shows a distinctly different response from the white. The time required for gastric digestion is notably longer. For instance subject Do (fig. 2) required 1 hour for egg white and $2\frac{1}{4}$ hours for the yolk. Higher total and free acidities were also developed by egg yolk. The high fat content of egg yolk (33 per cent) may well account for the delayed evacuation. In one case more or less pathological and therefore not included in the present series, a high total but no free acidity was developed. The acidity in this case must have been due to the action of regurgitated lipase on the fat of the yolk.

Two whole raw eggs were fed in certain cases. A number of tests were also made on such eggs after being mixed and strained through cloth. It might be expected that the response of the mixed white and yolk would be intermediate between that of yolk and white taken separately. It was found, however, that the response was much the same as that of yolk alone. In fact the average evacuation time for two whole eggs was greater than that for three yolks. Note the very similar response of egg and yolk on subject Ra (fig. 5). Note also the slower evacuation and higher acidity developed by whole raw egg as compared with egg white in the case of subject Da (fig. 1). Observe likewise the response of raw eggs as charted in figures 6, 7, 8, 9 and 10. The acidities developed (especially the free acidities) are lower than for most cooked eggs, but not markedly so.

Soft boiled and hard boiled eggs. The "soft boiled" eggs were placed in boiling water for $3\frac{1}{2}$ minutes, the "hard boiled" eggs for 8 minutes. On the average the hard boiled eggs required a few minutes longer to digest than soft boiled eggs. Compare the two methods of cooking on subjects Mi (fig. 9), Se (fig. 10), Do (figs. 2 and 11), and note the similarity of response, in one case the evacuation times being the same, in another case the hard boiled having a slight advantage, while in the third the soft boiled left 15 minutes sooner. The acid response to hard boiled eggs was more marked in one case (fig. 9) but was the same as for soft boiled in the two other cases just mentioned.

Comparisons of raw eggs with boiled eggs are found in figures 5, 9 and 10. In cases 9 and 10, raw eggs took distinctly longer than either soft or hard boiled eggs. Apparently the stomachs of the rapid-emptying type handle the cooked eggs more readily than raw eggs.

Fried eggs and scrambled eggs. Scrambled eggs required on the average a little longer to digest than boiled eggs. Direct comparisons may be

obtained from figures 2 and 11, showing scrambled eggs to take $\frac{1}{4}$ to $\frac{1}{2}$ hour longer to leave than either hard or soft boiled eggs; from figures 10 and 12, showing scrambled eggs to take $2\frac{1}{2}$ hours as compared with $1\frac{1}{2}$ and 2 hours for soft and hard boiled eggs respectively; from figures 5 and 13, which indicate that the boiled eggs had a very slight advantage; and from figure 23, showing scrambled and soft boiled eggs each to leave the stomach in $2\frac{3}{4}$ hours.

The effect of scrambling the eggs with a large excess of bacon fat was tested in two cases. In one case (fig. 19) (that of a slow-emptying stomach) the response was similar to that for soft boiled eggs, while in the other case, that of a rapid-emptying stomach, the scrambled eggs with excess fat remained an hour longer in the stomach.

Six tests were made on fried eggs particularly to determine the effect of frying on both sides with excess of bacon fat. This was done in view of the common opinion as to the indigestibility of greasy foods. Eggs fried in the ordinary way and not turned over left the stomach fully as soon as eggs cooked in any other manner. Compare experiments 21, 32, 40 and 45 on the same individual (fig. 23) showing evacuation times for fried eggs (2 hours), scrambled eggs ($2\frac{3}{4}$ hours), for hard boiled ($3\frac{1}{4}$ hours) and soft boiled ($2\frac{3}{4}$ hours). Observe also figure 9, showing fried eggs to leave in 2 hours as compared with $2\frac{1}{2}$ hours for hard boiled and $2\frac{1}{4}$ hours for soft boiled eggs. When excess of fat was used the emptying period was somewhat longer than for shirred eggs (fig. 15) but not as long as for hard boiled eggs (fig. 14). It appears that an excess of fat may delay the digestion of eggs appreciably in individuals of the rapid-emptying type but has little effect in the case of slowly evacuating stomachs. The common opinion that fried foods are less digestible was not borne out in the case of eggs. We have previously shown that fried meats are readily handled by the stomach (7).

Omelets, poached, shirred and soft cooked eggs. The digestion of plain and Spanish omelets was compared. Figures 10 and 12 show that the response of the stomach of this individual was identical in the two cases. The omelets required the same period of digestion as scrambled eggs and a longer period than hard or soft boiled eggs. That a plain omelet may remain in the stomach an hour longer than egg white with orange juice is indicated by figure 3.

Poached eggs were found to leave the stomach in the same time as soft boiled eggs in the case of a rapid-emptying individual (see fig. 6) and to require a little longer in the case of a slow-emptying individual (fig. 7). Shirred eggs appear to leave the stomach more rapidly than hard boiled

eggs or eggs fried with excess of fat (figs. 14, 15 and 16) and belong with the more readily digestible forms of eggs. Soft cooked eggs appear also to be digested in medium time (expts. 61 and 62 and figs. 1 and 20).

Pickled and deviled eggs. Eggs pickled in a vinegar extract of beets appeared to require about as long to digest as the hard boiled eggs from which they were derived (expts. 57 and 58 and fig. 7). Deviled eggs required $2\frac{3}{4}$ hours to leave the stomach as compared with $2\frac{1}{2}$ hours for hard boiled eggs and $2\frac{1}{4}$ for soft boiled eggs (figs. 9 and 17).

Duck and turkey eggs, Chinese preserved eggs. Soft and hard boiled duck eggs and hard boiled turkey eggs were compared with hens' eggs prepared in the same ways. Two eggs were given to each subject. A comparison of experiments 26 and 63 (figs. 7 and 19) and experiments 36 and 64 show that duck eggs were handled by the stomach in about the same time as hens' eggs. This was perhaps to be expected from the similarity in composition of ducks' and hens' egg.

Hard boiled turkey eggs were compared with hard boiled hens' eggs in two cases. In the completed experiments (figs. 2 and 11) the subject Do required $2\frac{3}{4}$ hours for the turkey eggs as compared with $1\frac{3}{4}$ hours for hens' eggs prepared in the same way. The difference is probably due to the larger size of the turkey eggs, whose contents weighed 77 grams each.

The Chinese preserved egg called "pidan" was tried out in two cases. These eggs have dark greenish yolks and yellow brown "whites" of a firm gelatinous consistency and possess distinct odors of ammonia and hydrogen sulphide. The period of retention of these eggs in the stomach we believe to have been influenced by their unappetizing character and in one case at least (fig. 18) to a prejudice against them. In one case (fig. 18) a single egg only was given. This remained in the stomach for three hours as compared with $1\frac{3}{4}$ hours for hard boiled hens' eggs (fig. 2). In the other case (figs. 13 and 22) two "pidan" eggs were given and remained in the stomach $4\frac{3}{4}$ hours as compared with 3 hours for hard boiled hens' eggs. The composition of these eggs has been studied by Blunt and Wang who have also carried out tests on the digestibility of these eggs in vitro (8). We desire to thank them for supplying us with the Chinese eggs used in our tests.

Cold storage and frozen eggs. An attempt was made to determine whether the keeping of eggs in cold storage for eight months had any effect upon their gastric digestibility when cooked in different ways. When the cold storage eggs were fried and compared with fresh eggs fried in the same way (figs. 15 and 21), it was found that both remained

in the stomach the same length of time and developed similar acidities. Another individual (figs. 2 and 11) evacuated hard boiled fresh eggs and soft boiled storage eggs in the same period ($1\frac{3}{4}$ hours) and the highest acidity in each case was about 90. No differences in the responses of the stomach to fresh and cold storage eggs were demonstrated.

The commercial frozen egg mixture was given to our subjects in the form of scrambled eggs and also made up into sponge cakes inasmuch as such eggs are mainly used in baking. Our two subjects who were given scrambled eggs both showed evacuation times a few minutes shorter for preparations made from frozen eggs than for the fresh egg preparations. The acidities developed were also practically identical (figs. 2, 11, 5 and 13).

Sponge cakes into which fresh and frozen eggs had been incorporated were likewise compared on two subjects, 100 gram portions being used. One of these comparisons is charted in figure 1. It shows a few minutes more rapid emptying where the fresh eggs were used but this difference is within the limit of experimental error. No appreciable differences in the action of the stomach on the two cakes could therefore be demonstrated nor does there seem to be any objection to the use of such eggs when canned under proper conditions.

Eggs used in combination with other foods. Several popular combinations of eggs with other foods were fed to certain of our subjects and all were found to be readily taken care of by the stomach. The combinations tested were: *a*, milk and eggs; *b*, bacon and eggs; *c*, French toast; *d*, frizzled beef with scrambled eggs.

The egg-milk combination used was an egg-nog consisting of 1 egg, $\frac{3}{4}$ cup milk, $\frac{3}{4}$ tablespoon sugar, $\frac{1}{4}$ teaspoonful vanilla extract and a sprinkle of salt and nutmeg. This combination took a little longer than eggs alone in the case of one man of a high acid, rapid-emptying type (fig. 11) but hardly as long in the case of a low acid individual (expt. 80).

The addition of eggs to milk distinctly alters the curdling effect of the gastric juice on such milk, the albumin preventing the formation of hard massive curds and giving rise to a light, flocculent precipitation of the casein.

The French toast used was made from 85 grams of bread with 2 eggs and fried in bacon fat. This toast required a little longer to digest than bread and butter alone but no longer than 2 hard boiled eggs (figs. 14 and 16) and must from this standpoint be considered as a satisfactory food.

Two eggs with bacon required in one case (fig. 14) only a little longer than fried eggs alone, while in another case (fig. 15) the bacon and eggs

left half an hour sooner. Individuals vary in their response to fatty foods but it appears that bacon and eggs are handled at least as readily as other foods high in fat and protein.

The frizzled beef and scrambled egg preparations used were made from 2 eggs with 25 grams chipped beef and a tablespoonful each of cream and butter. This chipped beef preparation took no longer to digest than scrambled eggs alone and gave rise to a more pronounced acid secretion (figs. 5 and 13). Meat-egg preparations would therefore appear to possess certain advantages over eggs alone.

Eggs are distinguished from meats by the lower acidities which they provoke and by their more rapid evacuation. As examples of this note charts 15, 16 and 21, illustrating the digestion of roast lamb and boiled tongue as compared with eggs. The results presented in this paper should also be compared with our findings on meats as presented in preceding papers (7). The average of the highest total acidities for all egg tests was about 80, and for free acidities 60. Meats on the other hand showed an average total acidity of 125. The average evacuation time for subjects of the rapid type on beef and beef products was 2 hours and 35 minutes as compared with 2 hours and 15 minutes for eggs and egg preparations. Subjects of the slow-emptying type required on the average 3 hours and 25 minutes for beef as compared with 3 hours and 5 minutes for eggs. Pork required a longer time than beef.

SUMMARY AND CONCLUSIONS

A series of over 90 experiments on 18 different subjects was carried out to determine the response of the normal human stomach to eggs prepared in various ways. Two eggs were used as the experimental meal except where otherwise specified. The fractional method of gastric analysis was employed. The evacuation times and highest acidities have been tabulated and curves plotted to show the comparative responses of certain subjects to different egg preparations.

The subjects were classified as belonging either to the rapid- or to the slow-emptying types. The average evacuation time for all egg preparations was for the first class 2 hours and 15 minutes and for the second class 3 hours and 5 minutes.

Eggs give rise to less stimulation of gastric secretion than meats and leave the stomach sooner. Beef, for example, showed an average emptying time of 2 hours and 35 minutes for the rapid- and 3 hours and 25 minutes for the slow-emptying type. The average of the highest

acidities developed in egg experiments was 80 as compared with 120 for beef. In general eggs show high combined acidities throughout the early period of digestion.

Raw egg white left the stomach much more rapidly than any other form of egg preparation. A moderate secretion of gastric juice was induced in subjects of a high acid type, but this did not become apparent until most of the egg white had left the stomach without being acted upon by the gastric juice. Egg white with 200 cc. of distilled water produced a more marked stimulation of acid secretion. Egg white with 200 cc. of orange juice led to a distinct gastric secretion and a rapid evacuation of the stomach.

Raw egg yolk required much longer to leave the stomach than egg white and higher acidities were developed. Whole raw eggs resemble egg yolk in their response whether unmixed or strained through cloth, showing the same delayed evacuation and greater acid stimulation as compared with egg white. Raw eggs produced somewhat less stimulation of acid secretion than boiled eggs and remained longer in the stomach.

Hard boiled eggs required on the average a few minutes longer for gastric digestion than soft boiled eggs but the acid response was similar in the two cases.

Scrambled eggs required a little longer to leave the stomach than boiled eggs. Fried eggs were handled as readily as soft boiled eggs or any other type of cooked egg. Eggs scrambled or fried with a large excess of fat remained in the stomach a little longer, the difference being most marked with the rapid-emptying type of individual. The belief that fried or moderately greasy foods give the stomach appreciably more trouble than others was not supported by our findings.

The response of the stomach to plain and Spanish omelets was found to be quite similar. Omelets remained in the stomach as long as scrambled eggs and longer than boiled eggs. Poached eggs, shirred eggs and soft cooked eggs were found to be among the more readily digested forms of eggs.

Eggs pickled in vinegar were digested in the same time as the hard boiled eggs from which they were prepared. Deviled eggs remain in the stomach a little longer than plain boiled eggs.

The eggs of the duck and turkey are handled by the stomach in the same way as hens' eggs, evacuation being somewhat delayed in the case of turkey eggs due apparently to their greater bulk.

The Chinese preserved egg called "pidan" gave rise to delayed and low acid responses in the stomach as well as delayed evacuation. This may have been due in part to the unappetizing character of these eggs.

Cold storage eggs, whether boiled or fried, could not be distinguished from fresh eggs as far as the response of the stomach was concerned. The same was true of the mixed frozen eggs of commerce, whether these were scrambled or used in the baking of cakes.

Eggs with milk, or egg-nog, leave the stomach a little more slowly than eggs alone, the egg albumin preventing the formation of indigestible curds in the stomach such as are likely to be formed with milk alone.

Eggs with bread or French toast remained in the stomach a little longer than bread and butter alone but not longer than hard boiled eggs.

Bacon and eggs were taken care of by the stomach almost as readily as fried eggs alone while possessing distinctly higher food value.

Frizzled beef with scrambled eggs were digested as quickly as scrambled eggs alone. Eggs and meat appear therefore to form a desirable combination from the standpoint of gastric digestion.

The authors wish to express their appreciation of the coöperation of the students of Jefferson Medical College, who kindly acted as subjects of these tests.

BIBLIOGRAPHY

(1) Beaumont: Physiology of digestion, 2nd Ed., Burlington, Vt., 1847.
(2) Pavlov: The work of the digestive glands, 2nd Ed., Philadelphia, 1910.
(3) Bateman: Journ. Biol. Chem., 1916, xxvi, 263.
(4) Penzoldt: Deutsch. Arch. klin. Med., 1893, xiv, 535.
(5) Jaworski and Gluzenski: Zeitschr. f. klin., Med., 1886.
(6) Bergeim, Rehfuss and Hawk: Journ. Biol. Chem., 1914, xix, 345.
(7) Fishback, Smith, Lichtenthaeler, Bergeim, Rehfuss and Hawk: This Journal, 1919, xlix, 174.
(8) Blunt and Wang: Journ. Biol. Chem., 1916, xxviii, 125; and private communication to the authors.

STUDIES ON THE BRAIN STEM

I. Regulation of Body Temperature in the Pigeon and its Relation to Certain Cerebral Lesions

F. T. ROGERS

From the Hull Laboratory of Physiology, University of Chicago

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INTRODUCTION

For more than a century the pigeon has been a favorite animal for physiological experiments involving the extirpation of various parts of the brain. Although the names of Rolando, Flourens, Vulpian, Munk, Ewald, Ferrier, etc., at once arise to mind in connection with such work, the interpretations of results are in many cases still in an unsatisfactory condition. One factor that in the past has not been properly appreciated is the one which is being emphasized by the new school of comparative neurologists, namely, that the anatomical relationships in the cerebral lobes of the bird brain are not the same as in the mammals. All published data on this subject at present are rather fragmentary and the morphology of the pigeon cerebrum is a thing of doubt and difficulty. Furthermore it is a notorious fact that few careful anatomical studies have been made in connection with physiological studies on the bird brain. (A few experiments have been made in this direction by McKendrick and by Edinger, with the Marchi method.) A better statement of physiological experiments on the pigeon brain still awaits the analytic work of the comparative neurologist on this common laboratory animal. The attempt is being made by the writer to correlate the physiological and anatomical results of brain lesions in pigeons in a more exact way than has been done hitherto.

Anatomical details are not considered in this paper aside from those of the great subdivisions of the brain. In order to illustrate more objectively the location of the lesions referred to, two figures are included of sagittal sections of the pigeon's brain (figs. A and B). There will be published elsewhere a detailed account, in so far as this is possible with present histological technique, of the parts of the brain that seem to be functional after operation.

Fig. A. Sagittal section of pigeon brain very near the median plane. Stained with iron hematoxylin.

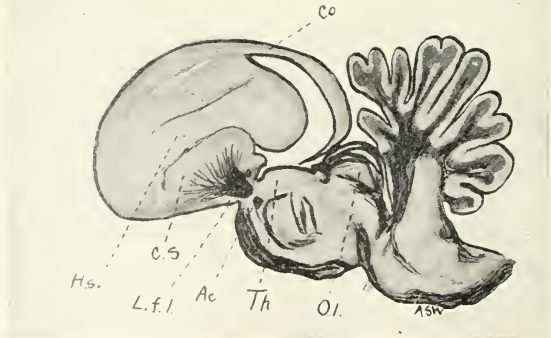

Fig. B. Lateral sagittal section of pigeon brain.

By the term decerebration is meant a section through the forebrain bundles between the striatum and the thalamus. *A.c.*, Anterior commissure; *Co.*, Cerebral cortex; *C.s.*, Corpus striatum (mesostriatum of Edinger); *DmTh.*, Dorsomedian nuclei of thalamus; *F.l.d.*, Medial longitudinal bundle; *H.s.*, Hyperstriatum (Edinger); *L.f.l.*, Lateral forebrain bundle; *M.f.b.*, Medial forebrain bundle; *O.ch.*, Optic chiasma; *O.l.*, Optic lobes; *Olf.b.*, Olfactory bulb; *Th.*, Thalamus.

In a previous paper on the hunger mechanism of the pigeon, the nature of decerebrate restlessness had to be considered. The writer there expressed the opinion that there seem to be variations of reflex excitability, *a*, in different decerebrate birds; *b*, in the same decerebrate bird at different times. This led the writer to make an extensive series of decerebrations to study the *variations* that followed in different animals when the attempt was specifically made to produce the same kind and extent of lesions in all cases. The results may be divided into three groups. The method of decerebration is described by the writer in this Journal (1).

Birds which lived less than two weeks after decerebration. In this group the writer has records of twenty-four birds. Examples of their behavior follow.

Bird 9. Lived 7 days. Nystagmus of head present, digestion seemed to be normal, feathers slightly fluffed. Great difficulty in maintaining equilibrium, stood on its claws with head and body drawn forward, or it fell backwards. Finally spread its wings and rested on its breast, tipped forward. Temperature subnormal, falling as low as 27°C. Autopsy showed cranial cavity filled with a big clot and slight traumatism of extreme anterior and lateral parts of thalamus.

Bird 12. General equilibratory disturbances, which *might* (?) be accounted for by a blood clot on cerebellum found at autopsy. Died second day after decerebration.

Bird 5. Feathers fluffed, no disturbances of equilibrium; listless in appearance, eyes always closed except when touched. No vomiting, no spontaneous movements. Lived ten days.

Bird 16. Slight equilibratory disturbances, feathers flat, occasional violent forced flying movements directed toward the right. Vomiting. Lived 6 days.

Almost every conceivable mixture of variations of this sort may occur —forced movements or immobility; feathers flat against the body or fluffed; nystagmus of head and distortion of neck; body temperature normal or subnormal; restless movements with crop empty and forced movements independent of the digestive conditions; birds become comatose and die.

Decerebration with no visible damage to thalamus. The second group comprises nineteen birds in which there was decerebration with no damage done to the thalamus detectable by naked eye observation. This is the group of so-called typical decerebrate birds; birds which show no forced movements, body temperature normal, restless walking movements when the crop is empty, and a sleepy attitude the remainder of the time. These birds lived for periods varying from three weeks to eight months.

Decerebration with visible damage to thalamus. The third group comprises eleven birds in which there is decerebration combined with visibly distinct damage to the thalamus. These birds are commonly supposed to differ from the classic decerebrate bird only in the absence of spontaneous movements. The birds of this group lived periods of time varying from two weeks to three months. More detailed description of this group will follow later.

Why these variations in physiological effects when the attempt was made to make the lesion identical in all cases? The interpretation given in the past has been that in different cases different reflex pathways have been injured; that in the thalamus is a subcortical center from which nerve pathways arise or terminate that induce certain modifications of body behavior. In how far this is true is a question that, as Tigerstedt says, "cannot for want of critical attention be definitely stated." In part the suggested explanation may be true. There must be some anatomical basis of the differences observed but equally certain is it that one big physiological factor has been wholly omitted in the few considerations of the subject available. As will be pointed out in this paper, decerebration with simultaneous thalamic involvement leads to a condition in which the animal loses the ability to maintain and regulate its body temperature. With these variations in body temperature there is a marked change in reflex excitability of the animal so that, in part if not wholly, the behavior of the animal is dependent on its body temperature. To elicit information dealing with this phase of the subject it therefore became necessary to determine the factors responsible for the variations and regulation of body temperature. This therefore forms the first paper and the application of this to reflex activities will be given in the following one.

TEMPERATURE VARIATIONS OF THE NORMAL BIRD

The body temperature of the normal pigeon varies usually from 40° to 42°C. as measured in the cloaca. Occasionally it may be a trifle less or a trifle more. According to the writer's tests the extreme limits may be considered as from 39.5° to 42.5°C. It is frequently observed that if the thermometer bulb be pushed beyond the cloaca into either the rectum or oviduct that the temperature of 43° to 43.5°C. may be recorded and withdrawal into the cloaca is followed by a fall to 42° or 42.5°. It therefore follows that in any careful comparative measurements the thermometer bulb must always be inserted into the cloaca a

constant distance for a constant time or always be subject to slight uncertainties. In the work here recorded because of this slight uncertainty the figures given at any time are always considered subject to a possible error of 0.5°C.

The variations in the normal bird have been described specifically by several workers. Thus Feré (2) found the normal temperature to vary from 41° to 42°, falling slightly when the animal is quiet, sleeping or incubating eggs, and rising slightly with excess muscular or nervous activity. Furthermore he considered that there is a slight variation according to sex and age, being higher in the male than in the female and higher in the young than in the older bird. Simpson and Galbraith studied the diurnal rhythm and found it similar to that of the mammals. Hilden and Stenbäck (3) have made a comparative study of the body temperature in various birds and confirm the statement of Simpson and Galbraith that, as a general rule, the range of diurnal variations is greater in smaller animals than in larger ones. They also state that by putting the birds in cages under artificial illumination the diurnal rhythm may be reversed and under such conditions the body temperature averages higher than in birds under normal conditions. The diurnal variations described may be readily confirmed and vary from 1° to 2°C.

Variations with external temperature. Exposing a normal bird to extremes of hot and cold, still compatible with life, produces little or no change in the body temperature. Thus sudden changes in the temperature of the bird's cage, varying from 4° to 38°C. for periods as long as twelve hours produced no changes greater than those which may occur in the limits of the diurnal rhythm (fig. 1).

The influence of starvation. Depriving the normal bird of food but not water for a period of five to seven days has little effect upon the body temperature. But during the second week of starvation the body temperature becomes subnormal, and after fourteen or fifteen days may be as low as 36°C. with the bird in a cage at the usual room temperature of 20° (fig. 2, A). During this stage of starvation the bird begins to lose its ability to maintain a constant body temperature and acts somewhat like a cold-blooded animal. Thus, at this time, exposure of the bird to cold is followed by a fall, and exposure to warmth is followed by a rise in body temperature. It is therefore possible by prolonged starvation of the pigeon to reduce it to a condition in which its body temperature is, in part, a function of that of the environment. In all such experiments care has been taken that the starving bird shall always have plenty of water. Feeding the bird promptly brings the

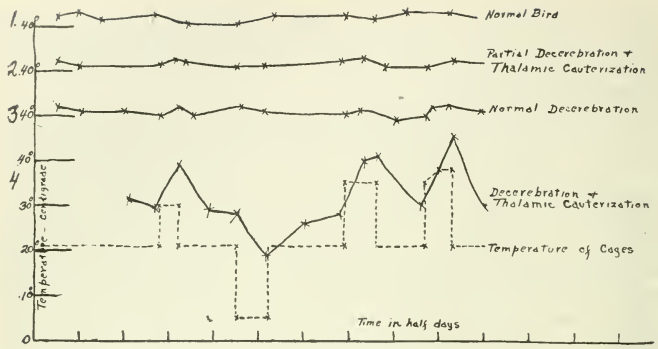

Fig. 1. Time in 12 hour intervals as abscissae; temperature in degrees Centigrade as ordinates. Temperature of cage in broken lines and of birds in continuous lines. Comparison of body temperature variations of four birds with the brain lesions indicated when all were simultaneously exposed to the atmospheric temperature variations of the cage indicated. *1*, Normal bird; *2*, bird with thalamic cauterization after partial decerebration; *3*, normal decerebration, thalamus intact; *4*, complete decerebration and thalamic cauterization.

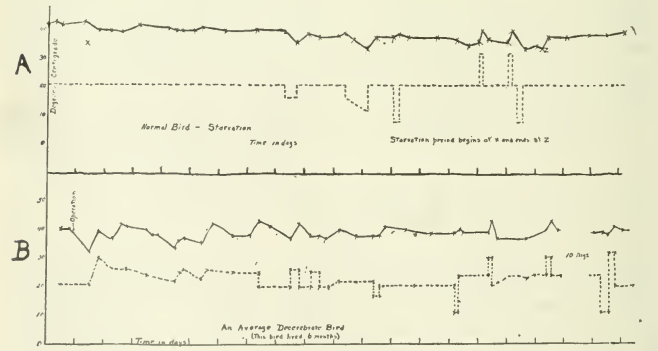

Fig. 2. *A*. Body temperature variations of a normal bird, deprived of food but given water. Time in days; temperature in degrees Centigrade. *B*. Temperature variations of a decerebrated pigeon in which particular pains were taken to make certain that no part of the cerebrum remained in the cranial cavity. Thalamus intact. Temperature of cage in broken lines; birds, in continuous lines.

body temperature back to normal if, of course, starvation is not pushed until death is imminent.

The influence of variations in water intake on the body temperature will be reported later.

TEMPERATURE VARIATIONS AFTER CEREBRAL LESIONS

Temperature studies on decerebrate birds have been carried out by Fredericq (4) and by Corin and Van Beneden (5). The former found that decerebration caused little or no disturbance of body temperature. The latter workers studied the diurnal variations and the carbon dioxide excretion as an index of heat production. They found a possible diurnal variation of 2.2°C., extreme limits of variations in normal birds of 39° to 43.6°, and that after decerebration the carbon dioxide production per kilo body weight is practically identical with that of the normal bird.

The writer's first results were rather confusing. But it finally seemed to appear that the results obtainable could be divided into four groups as follows:

a. Traumatism and pressure on the hemispheres.

b. Decerebration with careful preservation of the thalamus.

c. Decerebration with thalamic lesion.

d. Thalamic lesion with partial destruction of the cerebral hemispheres.

Traumatism and pressure on the hemispheres. In a series of four birds the hemispheres were exposed and roughly pierced in several places by forceps until the base of the cranial cavity was reached. At the same time the longitudinal and transverse sinuses were sectioned. The skin was then sutured over the bloody mass so as to compel the formation of a heavy clot over the hemispheres and cerebellum in addition to the traumatism. In spite of this rough treatment no marked alterations in body temperature occur, nor does the bird lose its ability to maintain a normal body temperature with wide variations in the temperature of the cage (fig. 3). Thus in the case figured although the bird was exposed to a temperature of 5° to 10°C. for a period of twelve hours, there was a fall in body temperature of 2°, a fall within the limits of normal variation.

Effects of decerebration. The results here reported are of those animals only which lived from one to five months after the brain lesion was made. The changes in body temperature after decerebration vary strikingly according to whether or not the thalamic nuclei are simultaneously

damaged. In a careful decerebration, avoiding excess hemorrhage and cutting through the base of the corpus striatum in such a way as to avoid direct traumatism of the diencephalon, it is found that the ability of the bird to maintain a normal body temperature in all variations of external environment is little or not at all affected (fig. 4 B). The bird maintains a normal temperature when exposed to cold and when exposed to heat may exhibit a slight rise, but one still within the limits of normal variation. Two cases have been found after this type of operation where there is an immediate temporary rise in body temperature. This, when it occurs, is striking (fig. 4 B) but no cases have been seen where this rise is distinctly greater than the upper limits of the normal variations. This is in harmony with the recent results of Moore (6) on the rabbit.

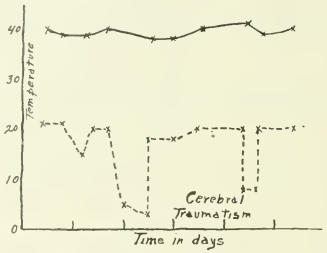

Fig. 3. Temperature variations of a bird after traumatism of the cerebral hemispheres. Temperature of the bird in continuous lines and of the cage in broken lines.

LESIONS OF THE THALAMUS

Decerebration with thalamic lesion. After a clean cut decerebration when the hemorrhage has been controlled, a clear view of the anterior and dorsal surfaces of the thalamus and of the third ventricle may be obtained. In a series of twelve birds a bilateral cauterization or traumatism of the dorsal and medial parts of the thalamus was done with a hot, sharp pointed probe immediately after decerebration. The cautery was applied after a clear view of the structures was obtained, using the habenula and third ventricles as landmarks. After this proceeding the birds commonly die within a few days unless care is taken to keep them warm. In other words, the prospects of such birds living is better in the summer than in the winter if the birds are kept at atmospheric temperature. If kept at a temperature of 25° to 30°C. they may be kept alive

indefinitely, very much as other decerebrate birds. After the operation described, the bird is reduced permanently to a cold-blooded condition (fig. 4A). Its body temperature seems to be largely a function of the temperature of the external atmosphere. Under such conditions its temperature can be arbitrarily set at any level and kept fairly constant by controlling the temperature of the surrounding cage. The temperature of the bird is not that of the cage, but averages 8° to 10° above it. By cooling the cage it is possible to throw the bird into a state of inac-

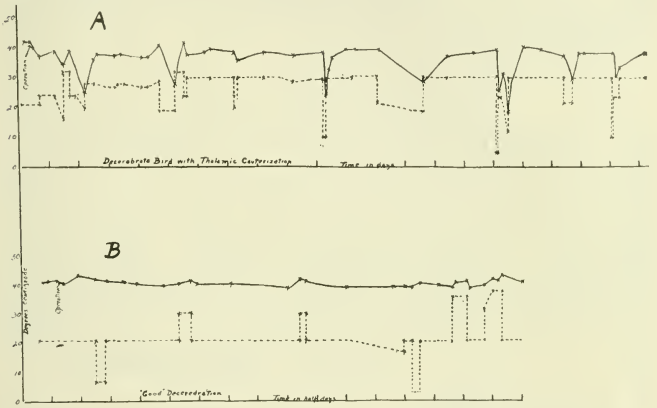

Fig. 4. *A*. Temperature variations of a bird with combined decerebration and thalamic cauterization when exposed to variations of atmospheric temperature indicated by the broken lines. Time in days and temperature in degrees Centigrade. *B*. Temperature variations of a good decerebrate preparation with no detectable trauma of the thalamus. Temperatures of birds in continuous lines and of cages in broken lines.

tivity or immobility that superficially resembles hibernation. By raising the temperature of the incubator the bird is thrown into a condition of hyperpyrexia. The temperature may be thus raised to 45° or 46°C. and death soon follows. The extreme limits to which a single bird's temperature has been pushed without death are 19.5° to 45.5°, a total variation of 16°C. The effects of these wide variations in body temperature on the behavior of the animal will be described in another report.

Thalamic lesion with partial decerebration. The preceding facts suggested of course that there must be some kind of mechanism in the brain stem of the bird that regulates its body temperature. When, however, further attempts at localization were made, surprising results followed. If the two occipital regions of the hemispheres are removed, taking care to leave intact the large superficial cortical artery which runs over the anterior two-thirds of the cerebral lobes, and then the dorso-medial portions of the thalamus are cauterized, it is found that there is no appreciable alteration of the body temperature or in the ability of the bird to maintain a constant body temperature at all temperatures of the environment (fig. 5).

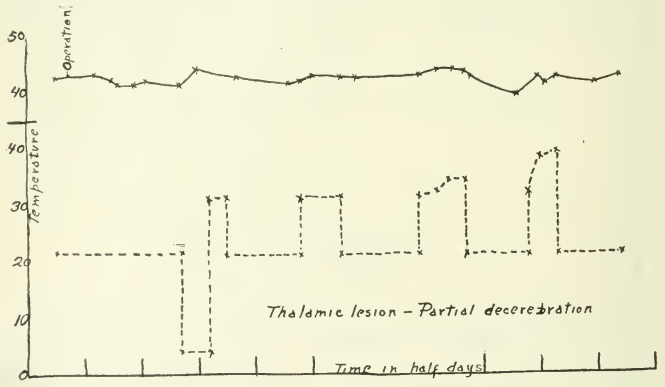

Fig. 5. Temperature variations of a bird with cauterization of dorso-median parts of the thalamus after removal of the posterior parts of the cerebral hemispheres. Temperature of bird in continuous lines and of the cage in broken lines.

The result therefore is that the maintenance and regulation of the body temperature may remain nearly normal—*a*, after extensive traumatism of the cerebral hemispheres complicated with subdural clots; *b*, after decerebration leaving the thalamus intact; *c*, after thalamic lesions leaving the anterior halves of the cerebral lobes in situ. On the other hand, a combination of decerebration and thalamic lesion reduces the bird to a poikilothermous condition. It therefore follows that in what is intended to be a simple decerebration, if there be temporary thalamic involvement, mixed results may follow as seen in the case of figure 2 B. This is characteristic of the average decerebrate preparation

where great care is taken to make certain that all the cerebrum has been removed. There is a period following operation when the temperature is inconstant and varies with that of the environment, but gradually this stage wears off and the animal regains the ability to maintain and regulate its normal temperature.

MINOR VARIATIONS IN THE DECEREBRATE BIRD

Although the decerebrate bird can maintain a normal temperature several differences between it and the normal bird are evident. In the decerebrate bird the range of diurnal variation may be greater than in the normal bird. Thus in a decerebrate bird three months after operation the body temperature during the course of the twenty-four hours varied from 39.5° to 42.2°, a variation of nearly 3°C.

The normal bird put in extreme cold may react by a rise in body temperature of about 1°C. I have never seen this in the decerebrate bird. A decerebrate bird exposed to sharp variations in the temperature of its cage may give corresponding variations in its body temperature which are rather sharp although still within the limits of the normal variation (fig. 1). This the normal bird does not do. (Possibly this may happen during sleep. It has not been tested.) If there be any variation it tends to be compensatory in character, falling slightly on exposure to heat or rising when exposed to cold.

Thus in a normal bird:

TIME	TEMPERATURE OF BIRD	TEMPERATURE OF CAGE
	degrees C.	*degrees C.*
10 a.m.	42.3	22
2 p.m.	42.8	22
6 p.m.	42.0	22
10 a.m.	42.5	22 and raised to 30°C.
2 p.m.	41.0	30 then lowered to 22°C.
6 p.m.	40.5	22
7 a.m.	40.5	22 then lowered to 5°C.
2 p.m.	42.2	5 then raised to 22°C.

The absence of this finer compensatory mechanism, quite variable indeed even in the normal bird, seems to be lacking in the decerebrate preparation. These finer reactions seem to be due to two simple facts. Sudden exposure of a normal bird to extreme cold leads to restless

struggling which may readily account for the slight rise in body temperature. On the other hand, exposure to heat (38° to 40°C.) leads to panting and forced breathing. In the decerebrate bird under the same conditions, cold does not directly stimulate restlessness nor does heat lead to panting. These are important factors the loss of which accounts for some of the irregularities described.

DISCUSSION

A further analysis of these and other possible factors such as alterations of blood distribution and changes in the feathers will be published later. Some of the effects described might be attributed to "shock." Shock, however, is a condition unknown in birds. Certainly, the corpus striatum may be removed without much change in body temperature regulation and equally certain is it that lesions of the thalamus which do not completely separate the hemispheres from the brain stem have little effect on the temperature regulation. It may be that in part the effects are due to altered circulatory conditions in the brain. The temperature alterations could not, however, be produced by pressure of blood clots alone on the hemispheres. There is the possibility that damage to the third ventricle has led to alterations in the pressure relations of the cerebro-spinal fluid and this may produce changes in the medullary centers which play a part in the regulation. Or it may be a combination of changes in both the external and internal cerebral fluid media.

SUMMARY

The normal pigeon exposed suddenly to temperatures of from −4° to 38°C. is able to maintain its body temperature constant within the limits of the diurnal variation. Within these limits, exposure to cold may lead to a slight increase and exposure to heat a slight decrease in the body temperature.

Prolonged starvation of the pigeon leads to a condition in which the body temperature becomes subnormal and rises or falls according to corresponding changes in the temperature of the external air. Feeding the bird when this stage has been reached quickly brings the body temperature back to normal.

Two compensating factors in the normal bird which tend to maintain the body temperature constant in extremes of heat or cold are: excess muscular activity and rapid, forced breathing or panting. Neither of these two factors appears in the pigeon after decerebration.

In the decerebrate pigeon with minimum damage to the thalamus, a normal body temperature is maintained in spite of variations of external temperature of from 5° to 38°C. Under such conditions the body temperature rises or falls accordingly, but only within limits of the normal diurnal variation.

Decerebration and thalamic cauterization reduce the animal permanently to a condition where the body temperature is in large part a function of the external atmospheric temperature. In such animals the body temperature may be lowered to 19°C. or raised to 46°C. by variations of atmospheric temperature of from 10° to 38°C. This renders possible the production in the pigeon of conditions resembling hyperpyrexia on the one hand, or "artificial hibernation" on the other.

Lesions of the dorso-median grey matter of the diencephalon which do not completely separate the fore-brain from the mid-brain, produce little effect on body temperature maintenance or its regulation.

BIBLIOGRAPHY

(1) Rogers: This Journal, 1916, xli, 555.
(2) Feré: Journ. de la Anat. et Physiol., 1899, xxxv, 808.
(3) Hilden and Steinbäck: Skand. Arch. f. Physiol., 1915, xxxiv, 382.
(4) Fredericq: Arch. de Biol., 1882, iii, 687.
(5) Corin and Van Beneden: Arch. de Biol., 1887, vii, 265.
(6) Moore: This Journal, 1918, xlvi, 253.

THE PHYSIOLOGICAL SIGNIFICANCE OF THE REACTION OF TISSUE CELLS TO VITAL BENZIDENE DYES

P. G. SHIPLEY

From the Osborn Zoölogical Laboratory, Yale University and the Anatomy Department of Johns Hopkins University[1]

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Although the results of vitally staining living animals with the acid benzidene dyes are well known, and the distribution of the dye in the body has been carefully studied under normal and pathological conditions, until very recently little attempt has been made to show experimentally how the color enters the cells in which it is found or in what state it exists in the cell cytoplasm. As yet, none of the theories which have grown up about the origin and significance of the stained granules with which the cytoplasm of the "pyrrhol cells" is studded has been generally accepted, and none can be said to have been proven.

Ehrlich (1) saw in the behavior of body cells toward injected dye solutions evidences of the existence of a specific chemoceptor, possessed by certain chromophil elements, which furnishes a bond between part of their cytoplasm and the stain. Goldman (2) concluded that the evidence accumulated in the course of his masterly series of experiments points to the demonstration by the dye of a secretory activity on the part of the pyrrhol cell. Tschaskin (3) believes that the benzidene dyes, isamine blue and trypan blue, color the mitochondria of the various trypanophil elements, and that the great dye masses seen in the clasmatocytes are the result of elaboration of a secretion granule by the chondriosomes of these cells: a secretion which is also stained by the vital azo dye-stuffs.

The most generally accepted theory in this country at present is that advanced by Evans and Schulemann (4) as the result of comparative experimentation with a large number of azo dyes. They believe that vital staining with dyes of this class is the result of phagocytosis by

[1] It is a pleasure to express here my gratitude to Prof. R. G. Harrison for permitting me to use the Osborne Zoölogical Laboratory and its equipment and for advice and assistance which have been of inestimable service.

special cells of ultramicroscopic dye particles, which, though apparently in molecular solution, exist in reality in a state of fine dispersion as an hydrosol. According to these authors any dyes which can be dispersed in particles sufficiently fine to pass readily through the blood-vessel walls are possible vital stains, and they have shown that the presence of multiple sulphonic acid radicles in the dye molecule is favorable to this condition. Dyes of this sort, when introduced into the circulation, pass out through the vessel walls into the tissues and invade them as a foreign body. As such, the dye is removed and segregated by a group of cells, morphologically different, but functionally united—a great scavenger tissue, the units of which Evans calls makrophages. Of course, neither the term "makrophages," nor the word "phagocytosis" (by which Evans means the collection and segregation of the vital dye), are used within the limits assigned these words by custom. The dye particles are probably not engulfed by the process of protoplasmic flowing which is the ordinary conception of phagocytosis, and many of the cells which are "trypanophil" are not phagocytes in the usual sense of the word. On the other hand, some of the cells which are most active in englobing bacteria and other coarse particles have under ordinary conditions no part in the segregation of vital dyes in the body of the living animal, e.g., the polymorphonuclear leucocytes.[2]

Hoffman (5), working in Aschoff's laboratory, has shown that if bits of embryonic liver were cultivated in the blood plasma of an adult animal, drawn immediately after an injection of trypan blue (plasma containing a large amount of dye in suspension) certain cells could be found in the new growth about the transplant which had in their cytoplasm larger quantities of the blue stain than did the majority of the new growing cells. These trypanophil elements were, he said, star-shaped, and the trypan blue existed in their cytoplasm as large masses or coarse granules, contrasting sharply with the fine blue granulations seen in the spindle-shaped "fibroblasts" which formed the mass of his newly grown tissue. Hoffman believed the coarsely granulated cells to be young phagocytic endothelial cells descended from those which line the liver capillaries (Kupfer cells).

[2] In this connection it is interesting to note the description by Downey of vitally stained granules in lymphocytes and granular leukocytes in the blood in doubly ligatured veins and about the site of injections of trypan blue. The author has seen vitally stained lymphocytiform cells in the circulating blood of frogs under pathological conditions.

Now the possibility of staining cells intra-vitam, which can be kept growing under conditions which facilitate the observation of single elements over long periods of time, offers a unique opportunity for studying the reaction of living cytoplasm to the dye, an opportunity which no examination of sections and film preparations from the tissues of vitally stained animals can give. It should be possible to observe an unstained cell in such a culture, to watch the first appearance of color in the cytoplasm and to follow the process of staining until the color has reached its maximum depth and intensity and the individual masses their greatest size and number in the cell body. It should be possible to observe the formation of the vacuoles which can sometimes be seen about the colored bodies and such an examination should throw light on how the dye enters the cell and answer the vexed question as to whether or not the colored masses in the cytoplasm of the pyrrhol cells, after vital staining, are merely aggregates of pure dye or whether the dye-stuff stains a preëxisting cell structure. Should the latter be the case, it ought to be possible to identify this body and to determine its physiological significance.

The technique of making cultures of cells in vitro has been so often described that it is unnecessary to go into it in detail here. These studies were made on the cells of chick embryos cultivated in plasma of healthy adults. The method of making the cultures was that devised by Harrison (6) and modified by Burrows (7), used almost exactly as the latter originally described it. The heart and liver were removed from chick embryos to Locke's solution with a needle and Noyes iridectomy scissors, and cut into bits under the binocular microscope. The fragments, with a small amount of the saline solution, were then transferred to a sterile cover slip and covered with a thin layer of plasma. The cover glass was sealed over the chamber of a deep hollow slide with a ring of vaseline and the culture so made was incubated at a temperature of 39°C.

The instruments, glassware, etc., used were previously sterilized, and sterile technique was observed throughout the operation. The plasma was handled in paraffined glassware and kept in an ice-salt freezing mixture. Great care must be used to prevent evaporation of fluids, since the consequent concentration interferes seriously with successful growth of the explanted tissue.

The first cultures studied were made of somatic cells of the chick embryo in the plasma of blood drawn from an adult hen one minute after intravenous administration of a 5 cc. dose of a 1 per cent solution

of trypan blue. When this blood was centrifuged, a beautiful deep blue plasma solution of the dye could be pipetted off from the corpuscles at the bottom of the centrifuge tube. It was soon found, however, to be more convenient to introduce the dye into the culture medium after the latent period and the initial rapid growth of the explanted tissue had passed and the new growth had become more stabile in its new surroundings. In succeeding cultures a solution of the dye in isotonic salt solution was added to the plasma clot in which the culture was growing after growth was well under way. This operation was done when the cultures were twenty-four to forty-eight hours old, and was carried out by injecting the dye solution into the plasma medium from a very slender curved pipette passed through the vaseline ring which sealed the cover glass to the hollow slide in the chamber of which the cultures were growing.

By the above described method, studies were made of the reaction of cells to the benzidene dyes, trypan blue, trypan red, Congo red, azo blue and benzopurpurin, to colloidal silver and manganese, to carbon granules of various sizes and other particulate foreign material. Cell cultures were supravitally stained with brilliant cresyl blue, neutral red and janus green and studies were made of cells stained with mixtures of two or more azo dyes and various combinations of benzidene vital staining and granule phagocytosis with supravital coloration. All the findings were controlled by observations on the living tissues of adult animals prepared for examination by appropriate methods.

In the young cells which grow in a thin membrane or in individuals which have separated from the tissue mass in such a culture and wandered off on the cover slip, the protoplasm is spread abroad in a very thin sheet, and cells in this condition were selected for examination, since in those growing deep in the clot along fibrin strands the cytoplasmic structures are crowded so closely together that it is difficult to make out detail in the mass.

The cytoplasm of these young cells contains beside the homogeneous, clear nucleus with its one or more grayish nucleoli, fat droplets, mitochondria and, in parenchyma cells from the liver, pigment and secretion granules. The central body, centriole, centrosomes, etc., cannot be seen as clearly as in the cells grown in salt solutions described by Lewis and Lewis (8) though their situation can usually be made out. Mitochondria are small, grayish, refractile bodies of varying shape and size and in plasma grown cells they are usually filaments, straight or curved rods or small granules. They have the power of changing from one to

another but they are only very slightly if at all motile in cells grown in plasma in contrast to the rapid, vigorous movements they make in the cytoplasm of cells planted in salt solution. They are selectively stained a beautiful blue green color by 1:10,000 solution of janus green, a dye which quickly indicates the mitochondrial nature of a doubtful granule.

Besides these bodies, cells in twenty-four hour plasma culture have another granulation which is sometimes impossible to distinguish in unstained cells from the granular forms of mitochondria. In these young cells there are very small granules which may or may not be seen to be surrounded by a small vacuole in the cytoplasm. They are very active at this stage and they move about in the cell freely, and very rapidly. They may dart along or across the cell, and may, after an interval of rest, return to their original position or they may go off in another direction. Frequently they travel along the length of the cell by short tacks from side to side. Movement is irregular and always jerky in character. Sometimes in the course of this movement, the vacuole enclosing the granule may be drawn out into a long thread which, when the granule comes to rest, reassumes its normal relation to it, or the thread may break and the vacuole round up without its granule, or the vacuole may disappear into the surrounding cytoplasm. I have never seen two vacuoles unite to form one.

Now if one examines an older culture, say a forty-eight hour or sixty-hour one, a change in some of the cells is at once evident. Cells may be found whose cytoplasm is packed with large grayish bodies on which pressure has forced the most varying and bizarre forms. They may be round or oval or angular or indented in every imaginable way. Sometimes the cells are full of them, sometimes a cell may contain only one or two. Sometimes in a single cell one finds all transition forms between the tiny motile granules seen in all young cells and the huge masses just described, and it is evident that the two forms are identical and that the large granules are the result of the growth of the small ones, though the large granules are non-motile and usually show no sign of a surrounding vacuole. These large granules are not contained in all cells. Many of the mesenchymal elements up to the death of the culture never contain any but the small type of granules. On the other hand, transition forms between the two types may occur.

The identity of these granules is further assured by the evidence of cultures stained supravitally with neutral red or cresyl blue. The motile granules and the large masses stain a brilliant red with the first named dye and a deep purple with the second. The vacuoles surround-

ing the small granules are colored rose-pink by cresyl blue and brick red by solutions of neutral red. Mitochondria of the active living cell are never stained by the above mentioned dyes but the mitochondria of a moribund or dead cell may be brilliantly colored by either. Even in supravitally stained preparations it is often not possible to see the vacuole about the large bodies; but some of these masses are found, at one side of which a narrow, stained, crescentic segment of the vacuole which surrounds the granule is visible. In other words, the vacuole is usually entirely filled by the swollen granule.

The reaction of these structures to supravital stains is sufficient to identify them with the cytoplasmic vacuoles and neutral red granules which Lewis and Lewis (8) have described in tissue culture cells and which they once interpreted as a degenerative change in the cytoplasm. In cells grown in plasma, however, the granule is the prominent feature of the picture given by supravital staining, instead of being a tiny dot swimming about in a huge vacuole as it is in cells grown in salt solutions. This difference in size relation between the vacuole and the segregation granule which it encloses results from the difference in the media in which the cells are growing. Living protoplasm in a glyco-saline medium is on a diet which, while sufficient to support life for a time, is extremely poor in particulate matter. In other words, the cells in saline media are living in molecular solutions. Plasma grown cells, however, are growing in a medium rich in colloids of various sorts. The significance of this environmental difference will appear later when the relation of foreign particles to the growth of the "neutral red" granule is made plain.

The so-called "neutral red granules" which the Lewises (8) have described have without a doubt the same physiological significance as the structure which they call the vacuole. Their neutral red granules are identical with the small type of neutral red granules which are found in plasma grown cells.[3]

If film preparations from the subcutaneous tissue of adult animals are examined, these same bodies may be found in all the connective tissue cells. They have the same appearance in unstained films and the reaction of the granule to neutral red and to cresyl blue is identical with that of the granules in culture grown cells. In adult tissues the large granules are found almost entirely in the cytoplasm of the clasmatocyte:

[3] In a later paper published in collaboration, Mrs. Lewis has established the identical nature of the vacuoles and neutral red granules which she and W. H. Lewis described previously in cells grown in vitro in saline solutions.

the fibroblast, on the other hand, being characterized by the possession of the small variety; but in the connective tissue of the adult animal, as in cultures of embryonic cells, it may be extremely difficult to draw a hard and fast line between the two cell groups, and transition forms between the two extremes may sometimes be found. It is very evident that these granulations are the granules which Maximow (9) and others have described in connective tissue cells after supravital staining with neutral red, and there is no doubt that the stainable masses in these culture grown cells represent the vacuoles and granules on which Renault (10) has based his theory of secretion by the "mode rhagiochrine"—the vacuoles and granules of segregation.

That is to say, we have to do here with a differentiation in vitro of indifferent mesenchyme cells which form resting wandering cells or clasmatocytes, and the differentiation is a process of elaboration of granules in the cytoplasm.

When the cells are stained by the vital azo dyes, trypan blue, trypan red, etc., the primary diffuse stain in cells is soon localized and, after twenty-four or forty-eight hours, the segregation granules contain all the color. The entire segregation granule may not be uniformly colored at once. Often the stain is first seen at one side of the granule where it forms a narrow crescent of color which broadens until it makes nearly the whole of the granular mass; or the dye may come into the granule equally from its entire periphery, in which case the edge of the granule is seen in optical section as a colored ring. The different stages of these processes of staining of the segregation granule by the dye, account for the crescent and ring shaped granules of dye which have been described in the clasmatocytes of the subcutaneous tissue of vitally stained animals.

All of the large granules in a single cell may not be colored at the same time. Sometimes a cell whose cytoplasm is merely a mass of granules of this sort will have only one or two which have been stained. The number of stained granules in a given cell, however, increases with the length of time the cell is exposed to the dye. The small type of granule takes the stain just as rapidly as the large. Colloidal metals like manganese and silver (collargol), when taken up by living cells, are segregrated and stored within segregation granules which they color.

The possession of segregation granules is not confined to connective tissue elements. They are present in large numbers in the cytoplasm of various parenchyma cells, e.g., of the liver; and exhibit in this situation the same staining reactions, thereby accounting for the blue granulations in the parenchyma cells of the livers of animals stained with

trypan blue. Most of these cells, however, are equipped only with small granulations of the fibroblast type.

The nature of the segregation granule and the reaction of the living cell to the benzidene dyes are best understood after a study of culture grown cells which have been fed with India ink. If carefully filtered India ink is mixed with the plasma used as a culture medium, the clot has a light yellowish brown color and appears homogeneous when examined with the dry objectives. Even with the aid of the oil immersion system, the largest granules of ink are scarcely visible as tiny brownish bodies, and the smallest are so minute as to be beyond the range of microscopic vision. In twelve hours, however, cells growing in such a clot have gathered large amounts of ink and stored it. Black masses, evidently aggregates of multitudes of small granules of carbon, can be seen in the mass of segregation granules and in vacuoles which, though they contain apparently only ink aggregates, give the same staining reactions as those about the ordinary neutral red granule.

The aggregation of submicroscopic carbon particles to form part of the segregation granule together with a study of the growth of the granular mass in the untreated cell leaves no other alternative for the interpretation of the significance of the segregation granule than as a mass of aggregated foreign material which the cell has gathered and stored and to which it is constantly adding albuminous and other submicrons derived from the surrounding tissue fluids where they exist in the colloidal (emulsoid or suspensoid) state.

Vital staining of cells with azo dyes is accomplished by the same process apparently by which the cell acquires and stores other substances in the colloid condition which are the material useful as food to the body as well as those which are mostly debris or actively injurious.

The granule which stains intra vitam in the cell body is then the equivalent of the protozoan food vacuole and since the amicrons of the semicolloidal dyes are stored in it, we have in benzidene vital staining an index and a demonstration of a feeding and storage reaction on the part of the cells of warm-blooded vertebrates.

The analogy between the "food vacuole" of the protozoa and the segregation vacuole and granule in the vertebrate cell is made very evident by staining amoebae and paramecium with solutions of trypan blue. After a varying interval the protozoan segregates the dye which has entered its cytoplasm, in preformed vacuoles which contain the chylomonas or other food which the protozoan has surrounded. The chylomonas may be seen unstained in or beside the aggregated mass of trypan

blue which grows larger as the animalcule remains in the dye solution. Moreover it is a matter of common knowledge that the vacuoles of the protozoan and the contained food mass are selectively colored intravitam by neutral red, and Loele (11) has called attention to the fact that both acid and basic dyes color the "oxydasekugeln" or nourishment vacuoles of paramecium. I may say here that the food vacuoles of amoebidae give the same reaction with brilliant cresyl blue 2B which characterizes the segregation granule and vacuole of the cells of warm-blooded vertebrates, and that a detailed study of these dye reactions in protozoa is now well under way.

Large granules of carbon and coarse granules of the high molecular dye-stuffs which gain entrance to the cytoplasm may become part of a preëxisting segregation granule in the substance of which they may be found embedded, or if they are of sufficiently large size, one or several may be found enclosed in an "inclusion" vacuole which has the same functional significance as a locus for the storage of dye-stuffs and other foreign material as the spaces about those granules of segregation which are visible in the untreated cell. The living cell deals in exactly the same way with bacteria, algae, fat droplets, red blood corpuscles, free pigment, etc., which it has engulfed in its cytoplasm, and it is possible to find in a cell, vitally stained with trypan blue, masses of the azo dye in the same "inclusion vacuole" which contains coarse particles phagocytized by the cell, whether those particles are some extraneous foreign granules like cinnabar or a cellular entity like a red blood corpuscle. Just so we may find pigment and the remains of red blood cells in the masses of trypan blue.

It is well known that basic dyes like neutral red will stain the so-called "inclusion vacuoles" about phagocytized material and will after a time color the inclusion itself, just as food masses and the fluid in the food vaculoes of protozoa are tinted by the same dye. How far fetched are the attempts which have been made to establish a functional differentiation of the neutral red staining granule of the cell's cytoplasm from the food masses and "inclusion" vacuoles on the basis of slight or imaginary differences in color tone after staining with neutral red will be at once evident when we remember that the shade of the neutral red stain may vary in the food vacuoles of the different protozoa and those of the higher metazoan forms whose gastro-intestinal epithelium still retains a recognized intracellular digestive function. In these lower forms the zoölogists recognize the tone differences after supravital staining as due to variations in the chemical composition of the digestive fluid in the

vacuole and the food mass (e.g., variations in amount of acid) but no one would venture to deny for these structures a fundamentally similar functional significance. Careful study of living cells in cultures and in the body of the adult animal and their behavior toward dyes and other organized particulate material shows conclusively that the "inclusion mass" and "segregation granule" are physiologically identical.

In short, all sorts of material which the cell allows to enter, or takes into, its cytoplasm, eventually finds its way into some of these vacuoles of segregation and becomes an integral part of the contained granule. Certainly this is true of particulate substances, and since the subcutaneous injection of distilled water is followed by the appearance of large fluid filled vacuoles in the connective tissue cells apparently identical with those which appear in cells grown in saline media, it seems likely that ingested fluids and solutions may meet with the same treatment in the cytoplasm. Again, some of the protozoa, for example paramecium, are able to concentrate or aggregate the relatively small molecules of phenolsulphonephthalein in their food vacuoles and to retain it for a considerable time under certain conditions.

The aggregation of ultramicroscopic granules of carbon into the neutral red body effectively disposes of the necessity of considering the possession of a dyeceptor by these bodies, to account for their coloration by pseudo-solutions of azo dyes. Even without the evidence for mechanical mixture which is offered by the incorporation of the inert carbon particle in the substance of the segregation granule, it is difficult to think of the gradual progressive process of granule staining by these dyes as the result of chemical combination of the dye with granular substance. Nor is it possible to credit these granules with the possession of a multitude of chemoreceptors capable of binding a range of substances from carbon to trypan blue whose molecules present the most diverse structure and among which there is not the least shade of chemical relation. The exact mechanism by which the cell takes the tiny particles in colloidal suspensions and pseudo-solutions into its cytoplasm is still uncertain; but it is not at all difficult to suppose that we are merely dealing with another manifestation of well-known physicochemical phenomena. The cytoplasm of the animal cell is almost universally recognized today as a colloid system and it is well known that such systems allow the entrance into and solution or dispersion in their dispersion means of substances whose particles have become adsorbed on the system's surface. Such an adsorption of amicrons of dye onto the surface of a cell bathed in a dye solution is only what is to

be expected in the light of the Gibb's law that substances in solution collect at interfaces since surface concentration tends to diminish free energy at such interfaces and under these conditions the adsorption of the disperse phase of a colloid sol is bound to follow.[4]

Under certain conditions this process may be actually seen to occur in tissue cultures. Collargol hydrosols are apparently toxic to the cells of chick embryos in culture and the silver gains access to the cytoplasm only with extreme difficulty. After a short time the bodies and processes of cells which have been treated with the silver colloid may be seen entirely covered and completely outlined by a thick coagulum of adsorbed silver granules.

Included in the cell's cytoplasm, the segregation of dye particles into the vacuole of segregation probably follows along the same lines, for we must remember that the protozoan "food vacuole" and the vacuole of segregation in the vertebrate cell are really no more and no less than fluid droplets, and that where their surfaces are in contact with the cytoplasm we have again an interface between two fluid systems. In vitally stained paramecium, one can sometimes see colored granules concentrated about the food vacuole before the color enters the fluid globule, in which the animal's food undergoes digestion. In this connection it is interesting to note the relation which seems to prevail between the motility of these food masses and that of the cytoplasm. In amoebae, whose cytoplasm is constantly and rapidly flowing in all directions, the food vacuole is passively moved about as the cytoplasm flows past. In the comparatively sluggish current of the protoplasm of the paramecium the food vacuole moves slowly over a route which exposes it at some time during its journey to the entire cell body. In the quiet cytoplasm of the cells of higher vertebrates, however, until movement is automatically mechanically checked by distention of the cytoplasm with masses of stored foreign material, the segregation granule is constantly rapidly and independently motile, and each one on its excursions covers a large part of endoplasm of the cell. It seems hardly necessary to point out the importance of the motility of the granule as an aid

[4] Evans has brought again to our attention a point in the morphology of the pyrrhol cells which was emphasized by Metchnikoff very early in his studies. If these cells are examined during life their periphery may be seen to be covered with tiny spine-like processes of the hyaline ectoplasm which are constantly and rapidly protruded and retracted—tiny pseudopodia. The constant changes in the surface of a cell whose ectoplasm is exhibiting this sort of movement and the resultant increase in the surface available for adsorbing particles must tremendously increase the absorbing capacity of the cell.

to the aggregation into its substance of particulate matter which has passed through the cellular ectoplasm, since by this means the granule wanders over a large portion of the cytoplasm collecting foreign substances as it goes, and, as it were, combs the cytoplasm for the food which protoplasmic currents bring to the vacuole in the amoeba.

Once in the vacuole the growth of the segregation granule becomes merely a matter of coagulation of amicrons to microns and the adsorption of dye granules on each other or on albuminous or other phases which are dispersed in the vacuolar fluid in the same way in which two or more small granules in a single vacuole may be seen to unite to form one. There are no doubt two factors which aid greatly, if indeed they are not entirely responsible for the aggregation of the component particles in the segregation granule—concentration of the colloid in the vacuole by constant accumulation of freshly absorbed particles and by reimbibition of water into the cytoplasm, concentration which alone is sufficient to cause aggregation of the disperse phase in a sol, and as Schulemann (12) has shown experimentally, the presence of electrolytes in the fluid in which the amicrons are dispersed. All of these colloidal dyes are more or less precipitated in the presence of electrolytes in solution.

While the above description is an attempt to picture the course of events which result in the vital staining of a cell by an acid azo dyestuff it holds also for the process by means of which the cell stores up colloidal proteins and other finely divided materials useful in the metabolic processes of the body. Moreover it is questionable whether staining with the "basic dyes" is always the entirely different process which Evans calls it and which at first glance one might believe it to be. Let us take, for example, the staining of the living cell by neutral red. This staining follows the same course in the cell as coloration by one of the benzidene dyes or metallic colloids. A diffuse, gradually increasing cytoplasmic stain is followed by the collection of the dye in the segregation granule and it is only the death of the cell which releases it to stain the nucleus, nucleoli and mitochondria. The same is true of cresyl blue. We have seen previously that even molecular solutions may be concentrated in the fluid surrounding the segregation granule and neutral red is probably not absolutely a molecular solution if its slow diffusion rate (13) may be taken as an indication, and furthermore neutral red may be precipitated from solution by salts. This dye probably hovers somewhere in that indistinct region between the colloid state and a state of true solution, and its sols probably represent

very tiny particles rather than dye molecules suspended in the dispersion means. Of course many dyes like janus green[5] beside entering the segregation vacuole color specific granules in the cytoplasm, and these phenomena, equally of course, are probably of an entirely different nature. But whether they result from a true chemical affinity or from some other cause, we have not yet been able to decide. Again the story of the colloid's existence in the cell is not finished with its aggregation with the food mass, but it often undegroes chemical changes from interaction with substances in the fluid about it, e.g., the metachromasia which follows aggregation of Congo rubin (Schulemann).

At all events the individual granules are certainly segregated in preformed vacuoles, where, by aggregation to like granules and other material, they form the neutral red staining body of the authors. In this way particles of the vital azo dyes are mixed with the segregation granule and color it. They can be said, therefore, to stain a previously existing structure, though the staining is not by a chemical union with a part of the granular molecule but a mixture with the granular substance.

It is very difficult to say with certainty whether or not new vacuoles are ever created in a cell to care for ingested dye-stuffs. That such a process occurs in caring for finely divided particulate matter is doubtful. It is possible that the segregation granule in some of the vacuoles may be

[5] I am not prepared to say what the factors are which govern the rate of absorption and segregation of dye-stuffs by the living cell or the quantitative and qualitative relation of the storage of ingested foreign material. Certainly the rapidity of absorption is not determined by the osmotic pressure of the solutions in contact with the cytoplasmic interface. Trypan red, a dye which is rapidly and readily diffusible is not stored by the cell in appreciable quantities until several hours after its exhibition, while brilliant cresyl blue, which hardly passes at all through semipermeable membranes in four and twenty hours, stains the segregation granule of exposed cells in a few minutes. Moreover, when cells are exposed to trypan blue sols metachromatic granular staining is rarely seen, although this dye is not a distinct entity. Two colors may be separated by diffusion experiments from its sols, one blue, which diffuses slowly, the other, a red, and rapidly diffusible dye. These two colors may represent chemically distinct substances; on the other hand, and this is much more probable, trypan blue may belong to that class of substances described by Michaelis which forms hydrosols in which the degree of dispersion is multiple (polydispersoid). Staining with weak solutions of neutral red is a comparatively slow process but intense coloration of the segregation granule follows almost instantly on the exhibition of a concentrated dye solution, but concentration of solutions of dyes of the benzidene series has no appreciable effect on the rapidity with which vital staining takes place. Consideration of the reason of these facts must suit for a more complete understanding of physico-chemical phenomena, especially those which have to do with adsorption.

made up almost entirely of the dye substance after prolonged feeding with pseudo-solutions of the benzidene colors but it is more probable that the dye is present only mixed with other ingested material. Nor is it well to ignore the possibility that some of the dye may be taken into the cell as an adsorption compound with albuminous or other particles which are always entering the cell from the body fluids. So many of these vacuoles exist in the cytoplasm of a normal cell that it is not necessary to assume that new ones are created to care for injected dye, and besides the large vacuoles filled with swollen granules of segregation, there is always a reserve of tiny vacuoles which are capable of swelling to care for a condition of overfeeding. We know, however, that when large foreign bodies are ingested, they are enclosed in a fresh vacuole formed during their engulfing. It has been already pointed out that such a vacuole is identical physiologically with the other segregation vacuoles normally found in the cells, and it has been shown above that the relationship is at once made evident by subsequent feeding with vital dyes the aggregates of whose amicrons may be seen segregated in the "inclusion vacuole" along with the englobed body whose ingestion brought it into being.

Combination of intra-vitam staining with an azo dye, for example trypan red, with supravital coloration of the mitochondria by means of a solution of janus green, demonstrates in a most striking manner, the distinct individuality of the two sorts of granules; and the same result may be obtained by staining growing cells simultaneously with janus green and neutral red. The mitochondria are not colored by the red dye, which stains the segregation granule, but are specifically stained with janus green. No relation has been observed between mitochondria and segregation granules and they are apparently distinct throughout the existence of the cell in spite of the assertions of Tschaskin (3), Levy (14) and Maximow (15) to the contrary.[6]

[6] I have seen, however, in active, vigorously growing cells, when first exposed to isamine and trypan blue, distinct staining of the mitochondria, which is not permanent. The dye soon leaves these tiny bodies to enter the segregation vacuole and the mitochondria are, in a short time, left colorless. This phenomenon must not be confused with the preagonal staining of mitochondria of moribund cells by solutions of benzidine dyes by which they are surrounded. In such cells the first evidence of impaired vitality is coloration of the mitochondria. This is followed by staining of the nucleoli and the precipitation and staining of a "chromatin network" in the nucleus. The tone gradually deepens and becomes homogenous throughout the cell body, which rounds up, shrinks and finally degenerates into a mass of granular debris. What the meaning of the primary mitochondrial

If a culture of cells grown in trypan blue is supravitally stained with neutral red, the blue granules take the red color also and become purple, then violet, then red, as the red dye becomes optically stronger than the blue. Staining of cells with mixtures of trypan blue and trypan red results in the presence in the cells of red granules and blue granules and granules stained violet or purple, as a result of a combination of different amounts of the two stains. It is not necessary to assume the possession of different chemoceptors for different dyes to explain this phenomenon. Since coloration of the segregation granule is due to the presence of dye granules in a mechanical mixture, the tint which the granule assumes after mixed color feeding depends only on the number of dye granules of each kind which happen to be poured into the same vacuole.

Since all sorts of materials which the cell ingests find their way into vacuoles of segregation, it is of course in these vacuoles that the intracellular digestion processes with which we are familiar must be carried on—the lysis of red blood corpuscles, bacteria, etc. The vacuole which originally probably contained only the segregated fluid which the cell had absorbed, apparently serves as a sort of cell stomach, an organulum in which the preparation of various materials capable of serving the cells in their amabolic processes takes place. We may think of the vacuole as a fluid globule into which the various secretions of the cytoplasm are poured, to act upon the mass of the segregation granule within, or upon specific substances which the cell may have swallowed and passed into the fluid globule which is the vacuole of segregation.[7] In these tiny stomachs, materials useful to the cell may be selected and separated from poisonous or useless matter, to be reabsorbed as food by the cytoplasm or passed on to other cells for nourishment or to build

staining may be I cannot say but an analogous process apparently goes on in some animal and plant cells under normal conditions since these granules may be found brilliantly colored by various natural pigments, such as anthrocyanin and chlorophil which has led some authors to use the term "chromochondria" to describe them. (Asvadourova N. Arch. d'Anat. Micr., T. 15, pp. 153-314). It is doubtless this sort of staining which has led Levy and others to believe that the segregation granule is the result of a mitochondrial metamorphosis.

[7] There can be little doubt, moreover, that just as many drugs and other substances (e.g., morphine) are, after absorption through the gastric or intestinal mucosa, reëxcreted into the gastro-intestinal tract, so waste products of cytoplasmic metabolism may find their way into these vacuoles of segregation as well as into the surrounding fluid of the tissue spaces, for it is hardly conceivable that the cell should instantly differentiate between one substance and another of like physical properties in solution.

secretion. In this connection it is interesting to note that reabsorption of material into the cytoplasm of these pyrrhol cells can be shown to occur experimentally. If the phagocytic cells are fed with emulsions of fat or oil stained with Sudan III, the droplets of fat which are taken into their cytoplasm gradually grow paler and at length may lose their color entirely. If this process is allowed to continue uninterrupted, finally all the stain may be removed from the foreign oil and is found coloring the normal fat in the body of the injected animal.[8] This course of events is followed even though the injected oil is an indestructible compound like the petroleum oils of the naphthene series. How far the destruction of fat-laden phagocytes may aid in producing the final effect cannot be told but the growing pallor of the droplets enclosed in the cytoplasm of healthy cells is a certain indication of reabsorption of the dye from the engulfed oil into the cytoplasm of the cell itself, just as is the staining of the animal's own fat sure proof that the dye is given off from the phagocytes to the animal's body. The waste is after a time cast out again as débris into the surrounding tissue fluid or freed in toto together with food products by the destruction of the short-lived and fragile cells which contain them.

Therefore, inasmuch as the vital benzidene dyes are deposited in these organs, the colored masses in the clasmatocytes, etc., after injections of these colored pseudo-solutions, in a way, are also an index of secretory activity on the part of the stained cell as Goldmann and students of the French school have suggested.

The power to carry on these processes is in some degree common to all cells since even the nerve cell contains vitally stainable granules, but certain elements—the Kupfer cell, the clasmatocyte, etc.—are especially active in this direction, and it is probable that we must consider these as a primitive cell type nearly related to unicellular organism. The degree to which the body cells which are not under ordinary conditions included in the class to which the clasmatocytes belong are called upon to perform these phagocytic functions is variable and is controlled by factors of which as yet we have a very imperfect comprehension. Cells of the same type in different animals will manifest very different reactions toward materials in the colloid state introduced into the animal's circulation. For example, Wislocki has found that the liver cells of dog-fish will store amounts of trypan blue as large as those found in the cytoplasm of the Kupfer cells, while the liver cells in Cyprinidae after the same treatment contain no traces of the dye whatsoever.

[8] Shipley and Cunningham, and Cunningham and Wislocki unpublished.

Again under conditions which vary from the normal any cell may be called upon to exercise a latent potentiality for ingestion, storage and digestion, as the lining cells of the blood vessel which normally refuse trypan blue or take it only in small amounts, will, following embolism, revert, functionally speaking, to a primitive type and engulf large quantities of dye-stuff (16).

We are most familiar with the pathological physiology of the "pyrrhol cells"—their ability to destroy bacteria and other invaders which they have ingested, their relation to malignant neoplasm and parasitic cysts. Their importance as collectors of extraneous matter and the part they play in the organism's defensive reaction against foreign bodies is well recognized but we are still much in the dark as to their rôle under normal conditions in the everyday life of the body. In reactions to pathological stimuli, we are dealing only with an exaggerated manifestation of a normal physiological digestive reaction which has been only slightly modified to meet an emergency (formation of giant cells, etc.). To dismiss these cells as scavengers is to do them an injustice, for however important this function may be, their service to the body is a far greater one. Most of the material which the blood and lymph streams carry to the body passes through their hands and is stored and it may well be prepared by them for the more highly specialized cell which is to be the ultimate consumer. They are able to carry on in the body true intracellular digestion perhaps by means of a ferment (the makrocytase of Metchnikoff) analogous to the amoebodiastase which Mouton (17) isolated from cultures of amoebae. Recent studies have shown that these cells are capable of carrying on complicated chemical reactions such as the liberation of iron from hemoglobin and they are extremely active in the ingestion and storage of foreign fats, pigment, etc., which come to the tissue via the blood stream. The localization of specialized cells of this class in large numbers along the course of the blood vessels gives them easy access to incoming food-laden fluids and their close relationship to the vascular channels facilitates the disposal of waste which they have winnowed out and liberated from their cytoplasm, or which escapes together with stored food after the destruction of their bodies. Material which has escaped these adventitial cells is seized upon and ingested by the great pyrrhol cells which wander about in the tissue spaces of the body or by specialized "nurse cells" like the makrophages of the testis, in various body organs. Cells of this class receive from the tissue fluids all sorts of "raw" food material which they probably pass, changed and purified by their secretions, to cells less able to perform digestive and selective functions.

BIBLIOGRAPHY

(1) Ehrlich: Brit. Med. Journ., 1913.
(2) Goldmann: Beitr. z. Klin. Chir., 1909, lxiv, 192.
(3) Tschaskin: Fol. Haem., 1912, xiv, H. 3.
(4) Evans and Schulemann: Science, N. S., 1914, xxxix, no. 1.
(5) Hoffman: Fol. Haem., 1914, xviii, H. 2, 137.
(6) Harrison: Journ. Exper. Zoöl., 1910, ix.
(7) Burrows: Journ. Exper. Zoöl., 1911, x, no. 1.
(8) Lewis and Lewis: Amer. Journ. Anat., 1915, xvii, no. 3.
(9) Maximow: Arch. f. Mikr. Anat., 1906, lxvii.
(10) Renault: Arch. d. Anat. Micr., 1907, ix, Fasc. iii–iv.
(11) Loele: Fol. Haem., 1913, xiv, H. 3, 308.
(12) Schulemann: Zeitschr. f. Exper. Path. u. Therap., 1915, xvii, H. 3.
(13) Teague and Buxton: Zeitschr. f. Physik. Chem., 1907, lx, 469.
(14) Levy: R. Accad. d. Lincei, 1916, xxv, S. 5 a.
(15) Maximow: Arch. Russes. d. Anat. d. Hist. et d'Embryol., 1916, i, 1.
(16) Batchelor: Proc. Amer. Assoc. Anat., 1914, Anat. Rec., 1914, viii, 139.
(17) Mouton: Compt. rend. Acad. d. Sci., 1901, cxxxiii, 204.

STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION[1]

I. The Effect of Unilateral Excision of the Adrenal, Section of the Splanchnic Nerve and Section of the Renal Nerves on the Secretion of the Kidney

E. K. MARSHALL, JR. and A. C. KOLLS

From the Pharmacological Laboratory, Johns Hopkins University, Baltimore

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One of the authors, Marshall, in collaboration with Davis, found that the complete removal of the adrenals in cats leads to an impairment of kidney function. This is signalized by a rise in the urea content of the blood to about twice its normal value, and a much diminished rate of excretion of urea and creatinine in the adrenalectomized animals compared to the normal, when the kidneys are subjected to increased work by the injection of a mixture of urea, creatinine and sodium chloride. Moreover, these changes may occur with a normal blood pressure and at a time when the cats are in excellent physical condition. It was suggested that these results indicated the secretion of some substance by the adrenals which is necessary for the maintenance of normal renal activity (1). The recent work of Addis and his co-workers (2) strengthens this suggestion. These authors find that the subcutaneous injection of adrenalin in proper dosage increases the "urea excreting activity of the rabbit's kidney." The observations of Marshall and Davis on cats were confirmed for rabbits by these investigators in that they found that "the removal of both suprarenal glands is followed by a depression of the urea excreting activity of the kidney, which is greater than that which follows similar operations in which the suprarenals are not removed." Some time previous to the appearance of the work discussed above, Cow (3) published an article on the regulation of the functional

[1] The data given in this series of papers were presented in abstract before the American Society for Pharmacology and Eperimental Therapeutics, New York, December, 1916. See Proceedings, Journ. Pharm. Exper. Therap., ix, 346, 1917, and before the same society in Baltimore, April, 1919.

activity of the kidneys by means of the adrenals. He claimed both histologically and physiologically to have demonstrated a direct vascular connection between the adrenals and the renal capsule in cats, by which adrenalin could be transported to the kidney directly without first passing through the general circulation. This caused a decrease in the secretion of urine, and hence the adrenals acted as inhibitors of renal secretion so far as the elimination of water was concerned.

These different conceptions of the action of the adrenals on the kidneys are not necessarily opposed to one another, for it might be conceived that they could act at the same time. That is, the products of adrenal secretion accelerate the elimination of urea, but inhibit that of water. In view of this it appeared interesting to us to test out the effect of unilateral removal of the adrenal upon the kidney of that side.

The results obtained indicated at once a definite effect upon the secretion of the kidney on the operated side as compared with the kidney on the normal side. If the adrenals acted as inhibitors for water and accelerators for urea, and if such a control as Cow postulated were functioning, one would expect an increase in the excretion of water and a decrease in the excretion of urea. However, an increase in the excretion of both water and urea was noted on the operated side.

It has been known for many years that section of the splanchnic nerve causes an increased flow of urine. Claude Bernard (4) showed that division of this nerve on one side in the dog increased the secretion of urine on that side. This has been confirmed by Eckhard (5), Knoll (6), Klecki (7), Vogt (8), Grek (9), Rhode and Ellinger (10), and Jungmann and Meyer (11), but is denied by Schwarz (12) and Peyrani (13) who claim a diminished secretion of urine for this procedure.

Since adrenalectomy is always associated with unavoidable injury to the splanchnic which lies immediately behind the gland, the possibility of nerve injury as an explanation for the urinary findings after adrenalectomy seemed very logical. To fortify this new point we chose also to section the nerves which surround the renal artery. This latter procedure does not interfere with the nerve supply of the adrenals and should demonstrate whether one can interpret the findings after adrenalectomy as due wholly to injury to the nerve supply of the kidney.

Another method used in connection with the search for a possible vascular relation between the adrenal and the kidney was the ligation of the adrenal vein at its junction with the vena cava. By this means it was hoped that the vascular connections which Cow describes as coursing from the gland to the capsule of the kidney would show some

influence on the function of the kidney on the operated side, because after ligation of the main exit for the venous blood, the collateral circulation through these vessels would come more into evidence.

METHODS

Dogs were used in all the experiments. As the previous work had been carried out on cats, these animals were first used, but the difficulties encountered in collecting the urine separately from each kidney and in obtaining a sufficient amount for analytical procedures caused them to be discarded. Furthermore, it is probable that the dog's kidney resembles the human more nearly than the cat's. The various operative procedures, excision of the adrenal, section of the splanchnic nerve and section of the renal nerves, were done both immediately before observations were made and also some days or weeks previous. In the latter case, the operations were performed aseptically under ether anesthesia. In the case of excision of the adrenal, the ventral route was chosen. The splanchnic nerve was sectioned just above the adrenal gland, the major splanchnic being always cut, while frequently other strands were also severed. In sectioning the renal nerves the following method was used. The peritoneum was incised and the renal artery and vein exposed. The artery was then freed of all visible nerve strands. This method does not completely denervate the kidney, but is probably freer of objection than painting the artery with phenol. Before making the observations on the secretion of the kidneys, the animals were anesthetized with paraldehyde, 1.7 cc. per kilo by stomach being given. Approximately 20 cc. of water per kilo were given with the paraldehyde. In some cases, where experiments were prolonged, it was found necessary to deepen the anesthesia and a saturated solution of paraldehyde in 0.8 per cent saline was given intravenously. The animals were kept warm by means of an electric hot pad. This procedure produced a very satisfactory anesthesia, and one in which the kidney was apparently but little affected. We were well aware of the danger in using anesthetics in studying renal secretion (14), but believe paraldehyde is freer from objection than the commoner anesthetics which are used. The secretion of urine was usually good even before a diuretic was administered, and the elimination of phenolsulphonephthalein was the same as is shown by unanesthetized animals (15), (16). The ureters were exposed through incisions in the flanks and cannulated with small glass cannulae. All observations were made with the animals in the prone position. This method described by

Quinby and Fitz (17) is more satisfactory than exposing the ureters through the abdomen and making observations in the supine position.

The urine was collected in small graduated cylinders over periods which were usually one hour in duration. The first period was usually one without administering any diuretic, although occasionally 0.8 per cent sodium chloride was injected to start the flow. At the beginning of the second period, diuresis was produced with 10 per cent sodium chloride intravenously which was given in doses of 2 to 4 cc. per kilo. In a few of the earlier experiments, 300 mgms. of diuretin were injected. In some of the experiments the third period consisted in injecting lactose (300 mgms. per kilo) and measuring the amounts eliminated. Phenolsulphonephthalein was given usually at the end of the experiment in the usual dose of 6 mgms. intravenously (15).

The specimens were carefully measured, the specific gravity determined with a small picnometer and analyses made for chlorides, urea and creatinine. Chlorides were determined by the method of McLean and Van Slyke (18), urea by the urease method described by one of us (19), and creatinine by Folin's colorimetric method (20). Phenolsulphonephthalein was determined with a Dubosq colorimeter. Lactose was estimated by the polariscope. At the conclusion of the experiment the kidneys were examined grossly and sections preserved in formaldehyde for histological study. In these experiments urea, creatinine and chlorides were determined in the urine at first because these substances had been previously examined by Marshall and Davis (1) in their work with cats. Later in the course of the investigations other urinary constituents were also determined. The chlorides figures are expressed for the sake of convenience as sodium chloride, although we are well aware that a portion of the chloride of the urine is present in combination with potassium. However, when diuresis was produced by hypertonic sodium chloride, the chlorides excreted were probably mainly in the form of sodium chloride.

RESULTS

Results of ligation of the adrenal vein. The following experiments indicate that ligation of the lumbar vein draining the adrenal has no effect on the secretion of the kidney. If the mechanism postulated by Cow is correct and functions normally, we would expect more epinephrin to be discharged into the kidney on the operated side, and hence expect a smaller flow of urine from that side. In the following summaries of typical experiments under this heading and subsequent ones, the first

column indicates the duration of the periods of collection, the R and L represent the urine from the right and left ureters respectively, and the various urinary constituents are expressed as percentages and also as the total amount in milligrams eliminated during the period.

Experiment 1, Dog MK 8. Female, weight 9.9 kilos, February 9, 1916. On January 29, 1916, operation under ether ligating lumbar vein draining right adrenal.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
10.07–11.07	R	4.5	1.075	3.40	153.0	0.210	9.4	0.04	1.8
	L	4.7	1.075	3.39	159.3	0.182	8.5	0.04	1.9
11.07–12.07	R	23.2	1.032	1.00	232.0	0.033	7.6	1.17	271.0
	L	24.6	1.031	0.97	238.6	0.037	9.1	1.17	286.0
12.07– 1.07	R	17.0	1.040	1.08	183.6	0.038	6.5	1.22	207.0
	L	19.5	1.039	1.03	200.8	0.040	7.8	1.21	235.0

At 1.07, sulphonephthalein was injected and in the next hour the right eliminated 19.0 cc. with 38.5 per cent, and the left 18.5 cc. with 37.2 per cent of the amount injected.

Experiment 2. Dog MK 9. Male, weight 7 kilos, February 11, 1916. On February 5, 1916, lumbar vein draining left adrenal ligated.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
10.50–11.50	R	1.8	1.059	3.64	65.5	0.184	3.3	0.78	14.0
	L	2.4	1.056	3.69	88.6	0.177	4.2	0.71	17.0
11.50–12.50	R	19.3	1.022	1.06	204.0	0.025	4.8	1.47	283.0
	L	20.0	1.020	0.96	198.0	0.024	4.8	1.45	290.0

At 12.50, sulphonephthalein was injected and the right secreted 40 per cent and the left, 42 per cent of the amount injected.

Results of excision of the adrenal. The changes in the secretion of the kidney following removal of the adrenal on one side as compared to the secretion of the kidney on the opposite side may be summarized as follows: The kidney on the operated side secretes more urine usually of a lower specific gravity and lower percentage of urea, constantly of a

lower percentage of creatinine and phthalein and an increased percentage of chlorides. The specific gravity and concentration of urea may be higher on the side with the most urine, as in experiments 3 and 5. During diuresis from injection of hypertonic sodium chloride, the difference in the elimination of water by the two kidneys is augmented, and the specific gravity and percentage of urea, creatinine and phthalein are less on the side with the greater amount of fluid, while the chlorides are still generally increased in percentage. The total quantities of chlorides are greatly augmented, of urea less, and of creatinine and phthalein approximately about the same or only slightly increased on the operated side. The one experiment in which lactose was injected indicates that lactose resembles creatinine and phthalein. These changes are the same whether the observations are made immediately after removal of the adrenal or some weeks later.

Experiment 3. Dog MK 14. Male, weight 10.5 kilos, March 17, 1916. Left adrenal removed one hour and a half before experiment was begun.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.06–12.46	R	1.8	1.038	1.68	30.2	0.271	4.9		
	L	2.2	1.056	3.60	79.2	0.228	5.0		
12.46– 1.46	R	4.0	1.052	1.86	74.4	0.164	6.5	0.43	17.2
	L	22.0	1.027	1.06	233.2	0.031	6.8	1.13	248.6

At 1.46 sulphonephthalein injected and in next hour, right eliminated 7.0 cc. containing 36.8 per cent, and left, 24.0 cc., containing 39.1 per cent of amount injected.

Experiment 4. Dog MK 17. Male, weight 9.4 kilos, March 22, 1916. Left adrenal removed about one hour and a half before commencing experiment; 10 per cent saline given before first period.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
1.00–1.45	R	6.5	1.025	0.82	53.3	0.076	4.9	1.31	85.1
	L	32.5	1.020	0.62	201.5	0.021	6.8	1.61	523.2
1.45–2.30	R	9.2	1.028	1.26	115.9	0.078	7.2	1.65	151.8
	L	27.0	1.021	0.81	218.7	0 027	7.3	1.77	477.9

At 2.30, sulphonephthalein injected and in next hour, right eliminated 15.5 cc. with 41.0 per cent, and left, 36.5 cc. with 44.3 per cent of amount injected.

Experiment 5. Dog MK 15. Male, weight 13.5 kilos, March 25, 1916. Left adrenal removed March 18, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.00–1.00	R	2.6	1.059	2.04	53.0	0.326	8.5	0.14	3.6
	L	5.2	1.057	3.90	203.0	0.176	9.1	0.44	22.9
1.00–2.00	R	7.0	1.040	2.29	160.3	0.111	7.8	0.87	60.9
	L	20.5	1.026	1.23	252.2	0.041	8.4	1.21	248.0

At 2.00, 4 grams lactose in about 20 cc. water injected intravenously. In the next hour, right eliminated 13.7 cc., containing 1.20 grams, and left, 23.0 cc., containing 1.24 grams. In next hour, sulphonephthalein was excreted 44.3 per cent on the right, and 44.0 per cent on the left.

Experiment 6. Dog MK 21. Male, weight 11.2 kilos, March 28, 1916. Right adrenal removed on November 5, 1915.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
3.00–4.00	R	10.1	1.045	3.87	390.9	0.079	7.8	0.80	80.8
	L	3.9	1.054	5.70	222.3	0.185	7.2	0.16	6.2
4.00–5.00	R	62.5	1.020	0.99	618.8	0.012	7.5	1.43	893.8
	L	17.0	1.028	2.17	368.9	0.040	6.8	1.33	226.1

At 5.00 sulphonephthalein injected, right excreted 33.0 cc. with 47.0 per cent, and left, 7.0 cc. with 41.0 per cent of amount injected.

Results of section of the splanchnic. The changes after unilateral section of the splanchnic nerve are seen from the data in the following experiments to be identical with those following unilateral excision of the adrenal. The kidney on the side of the section eliminates more urine of a lower (or higher) specific gravity, and there is decreased (or increased) percentage of urea, decreased percentage of creatinine and phthalein, and increased percentage of chlorides. During sodium chloride diuresis, the urine is greater in amount and always of a lower specific gravity, there is a decreased percentage of urea, creatinine and phthalein, and an increased percentage of chlorides. The absolute amount of chlorides eliminated is greater on the operated side, of urea greater but in a less degree than chlorides, and of creatinine and phthalein only slightly greater or the same. The few experiments with lactose indicate that it resembles creatinine and phthalein in its excretion.

Experiment 7. Dog MK 23. Female, weight 5.70 kilos, April 6, 1916. Left splanchnic nerve major and minor sectioned two hours before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.22–1.25	R	2.0	1.050	2.04	40.8	0.250	5.0	0.44	8.8
	L	4.6	1.037	2.94	135.2	0.114	5.2	0.98	45.0
1.25–2.25	R	18.0	1.021	1.22	219.6	0.028	5.0	1.31	235.8
	L	34.0	1.015	0.68	231.2	0.014	4.9	1.33	452.2

At 2.25, sulphonephthalein was injected, and in the next hour, right eliminated 21.0 cc. with 38.5 per cent, and left, 58.0 cc. with 39.5 per cent of amount injected.

Experiment 8. Dog MK 22. Male, weight 7.4 kilos, April 20, 1916. Left splanchnic sectioned April 1, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*
12.45–1.50	R	1.7	1.034	6.06	103.0	0.364	6.2
	L	3.2	1.037	6.99	223.7	0.210	6.7
1.50–2.50	R	9.5	1.024	2.26	214.7	0.064	6.1
	L	27.5	1.016	1.00	275.0	0.022	6.1

At 2.50, 2.2 grams lactose injected intravenously. During the next hour 1.05 grams in 13.8 cc. urine were eliminated by the right kidney, and 0.92 gram in 27.6 cc. urine by the left; 3.50–4.50, right eliminated 41.5 and left, 45.0 per cent of injected phenolsulphonephthalein.

Experiment 9. Dog MK 32. Female, weight 8.0 kilos, July 18, 1916. Left splanchnic sectioned on May 19, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
1.35–2.35	R	2.05	1.055	3.10	63.6	0.218	4.5		
	L	2.50	1.053	2.42	60.5	0.214	5.4		
2.35–3.35	R	11.5	1.024	1.10	126.5	0.050	5.8	1.21	139.2
	L	21.0	1.019	0.73	153.3	0.029	6.1	1.33	270.3
3.35–4.35	R	13.8		0.84	115.9	0.042	5.8	1.17	161.5
	L	21.6		0.65	140.4	0.029	6.2	1.28	276.5

At 3.35, 2.4 grams lactose were given intravenously; in the period 3.35–4.35, 716 mgm. were eliminated by right kidney and 850 mgm. by the left. At 4.35, sulphonephthalein was injected and during the next hour, right eliminated 35 per cent, and left, 37 per cent of amount injected.

Experiment 10. Dog MK 27. Male, weight 8.6 kilos, November 17, 1916. Left splanchnic sectioned and piece removed on April 26, 1916.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.13–12.13	R	2.8	5.82	163.0	0.217	6.0	0.58	16.2
	L	4.0	4.47	178.8	0.143	5.7	0.91	36.4
12.13– 1.13	R	10.8	2.62	283.0	0.058	6.3		
	L	23.0	1.48	340.4	0.027	6.1		

At 1.13, sulphonephthalein given and in next hour right eliminated 9.3 cc. with 33.0 per cent, and left, 27.5 cc. with 34.0 per cent of amount injected.

Results of section of the renal nerves. The changes incident to sectioning the renal nerves on one side are, as far as can be judged, by the experiments carried out, identical with those of sectioning the splanchnic or removing the adrenal gland. The following summaries of experiments indicate this.

Experiment 11. Dog MK 33. Female, weight 7.0 kilos, June 7, 1916. Renal nerves on left side sectioned one-half hour before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.45–12.45	R	3.25	1.053	1.26	41.0	0.069	2.2	Trace	
	L	3.80	1.054	1.20	45.6	0.066	2.5	Trace	
12.45– 1.45	R	5.0	1.048	1.47	73.5	0.067	3.3	0.80	40.0
	L	8.0	1.041	1.17	99.5	0.040	3.4	1.03	87.6

At 1.45, 2.1 grams lactose injected intravenously. In next hour, right eliminated 4.3 cc. containing 670 mgm., and left, 8.0 cc. containing 736 mgm. At 2.45, sulphonephthalein was injected, right eliminated 5.5 cc. with 47.6 per cent, and left, 12.2 cc. with 46.2 per cent of amount injected.

Experiment 12. Dog MK 34. Male, weight 4.8 kilos, June 13, 1916. Renal nerves on left side sectioned one hour before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
11.15–12.45	R	2.35	1.079	3.84	90.2	0.167	3.9	0.04	0.9
	L	3.85	1.072	3.09	119.0	0.108	4.2	0.18	6.9
12.45– 1.45	R	5.2	1.036	2.22	115.4	0.043	2.2	1.40	72.8
	L	25.0	1.022	0.74	185.0	0.011	2.8	1.35	337.5
1.45– 2.45	R	5.8		0.72	41.8	0.038	2.2	1.26	73.1
	L	26.0		0.47	122.2	0.009	2.3	1.39	361.4

In the third period, 1.44 grams of lactose were injected intravenously. At 2.45, sulphonephthalein was given, and in next hour right secreted 2.3 cc. containing 34.3 per cent, and left, 11.6 cc. containing 35.8 per cent of amount injected.

Experiment 13. Dog MK 35. Male, weight 6.2 kilos, June 19, 1916. Renal nerves on left side sectioned June 14, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
10.55–11.45	R	5.15	1.031	1.95	100.4	0.115	5.9	0.86	44.3
	L	11.00	1.024	2.01	221.1	0.053	5.8	1.01	111.1
12.40– 1.40	R	8.3		1.67	138.6			1.21	100.4
	L	25.5		0.59	150.5			1.45	369.8

Experiment 14. Dog MK 84. Male, 4.5 kilos, January 29, 1917. Left renal nerves sectioned on January 3, 1917.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	per cent	mgm.	per cent	mgm.	per cent	mgm.
11.45–12.45	R	1.0	3.69	36.9	0.160	1.60		
	L	1.8	3.75	57.5	0.091	1.65		
12.45– 1.45	R	2.0	1.76	35.2	0.095	1.90	0.34	6.8
	L	4.5	1.43	64.5	0.042	1.90	0.62	27.9

At 1.45, sulphonephthalein was injected and in next hour, right eliminated 2.5 cc. with 29.3 per cent, and left, 6.2 cc. with 29.7 per cent of amount injected.

DISCUSSION

As stated above, the effect upon the secretion of the kidney after unilateral extirpation of the adrenal consists in the secretion of a greater volume of urine of lower specific gravity, a higher percentage and absolute amount of chlorides, a decreased percentage but greater absolute amount of urea, and a decreased percentage but approximately the same amounts of creatinine and phenolsulphonephthalein. We are not concerned here with the question as to whether the creatinine and sulphonephthalein are secreted in exactly the same amounts by the two kidneys. The figures indicate sometimes a slight increase, sometimes a slight decrease, and sometimes no change on the side with the greater flow. The important point is that no unmistakable and constant change occurs in the secretion of creatinine and phenolsulphonephthalein as is seen with water, urea and chlorides.

These characteristic changes can be produced equally well by section of the splanchnic nerve or by section of the renal nerves. In fact, the effects of these three procedures cannot be distinguished from one another by examining the urines secreted by each kidney. In removing the adrenal gland, injury to the fibers of the splanchnic nerve is unavoidable. It would appear, therefore, unnecessary to postulate any direct connection of the adrenal with the kidney of the same side to explain the phenomena observed; that these phenomena can be reproduced by nerve sectioning where the nerve supply of the adrenal gland is undisturbed is shown by the experiments on cutting the renal nerves.

The relation between the adrenals and the kidney as postulated by Cow (3) does not appear to function normally in the dog. This is further shown by the experiments on ligating the lumbar vein of one gland. No change in the urine on that side occurs. However, the inter-relations of the adrenals and kidneys as shown by Marshall and Davis (1) and signalized by a decreased functional capacity of the kidneys after the total ablation of the glands, and that indicated by Addis and his co-workers (2) by showing an effect of adrenalin on the urea excreting function of the kidneys are in no wise affected or invalidated by these experiments. In these cases, the effects can be produced through the general circulation, and not by local connections of the adrenal and kidney.

Regarding the mechanism of the production of the changes in the secretion of the kidney after splanchnotomy, the usual explanation given is an effect on the blood flow through the kidney, although some

investigators are inclined to attribute it to a specific secretory effect of the nerve. In the following paper, we are inclined to believe that we have accumulated positive evidence that the first of these explanations is sufficient.

The behavior of the different urinary constituents in relation to the different volumes of urine secreted by each kidney in these experiments is similar, in some respects, to that observed in similar experiments and also in experiments of a somewhat different nature. Knoll (6) examined the urine in dogs after cutting one splanchnic, and found the urine of the operated side of a lower specific gravity, lower percentage of solids and urea (determined by Liebig's method), while the total elimination of solids and urea was greater. Grek (9) noted an increase in the absolute as well as percentage amount of chlorides after unilateral splanchnotomy in the dog. This was confirmed by Jungmann and Meyer (11). In a short preliminary note Rhode and Ellinger (10) state that after unilateral section of the splanchnic or renal nerves, the urine of the operated side contains a smaller percentage of solids (as determined by specific gravity and freezing point) but a greater absolute amount. The acidity to phenolphthalein was less, but the total amount of acid eliminated was greater. These changes were observed in animals subjected to operations weeks or months previously. No details are given in their preliminary note and the detailed communication promised has not come to our notice.

The increased elimination of urine by the kidney on the operated side in these experiments can be considered in the nature of a diuresis as compared to the secretion of the organ of the normal side. Certain changes in the constituents of the urine are characteristic of diuretic urine in general. V. Schröeder (21), studying caffeine diuresis in the rabbit, found the total solids and nitrogen of the urine increased but not to the same extent as the water. Thompson (22) noted the same for nitrogen and urea during sodium chloride diuresis. Katsuyama (23) found caffeine diuresis in the rabbit accompanied by an increase in the alkali chlorides, but to a greater extent in the case of the sodium than the potassium. Later (24), he noted the same for urea and diuretin diureses. Loewi's (25) studies on diuretics, mainly caffeine and sodium nitrate injected into dogs, indicated during diuresis an increase in the chlorides, urea (total nitrogen), sugar in hyperglycemia, and injected phosphates, but no change in the elimination of the sugar from phlorhizin diabetes or the phosphates formed in metabolism. However, Bock (26) working with rabbits found that injection of solutions of

glucose, sodium chloride and sodium sulphate, and purine derivatives produced a diuresis with increase of the phosphates, while a diuresis by the administration of water by mouth caused no increase in the phosphate elimination. Baetzner (27), on the other hand, has failed to confirm Bock's observations that water diuresis causes no increase in the elimination of phosphates in rabbits, but found a very marked increase. Rowntree and Geraghty (15) studied the influence of various diuretics on the elimination of phenolsulphonephthalein in cats. They came to the conclusion that

> under the conditions of our experiments it was found that those diuretics which are known to exert some stimulating influence on the activity of the secreting cells, or those diuretics in connection with which evidence is at hand indicating a stimulating action on the secreting cells (caffeine, urea, dextrose, phlorhizin, calomel), slightly increase the phthalein output, whereas those diuretics which act entirely by changes in osmotic tension or by changes in blood-pressure, etc. (hypertonic sodium chloride solution, potassium nitrate and digitalis), apparently have little or no effect on its excretion.

To sum up, then concerning the effect of diuresis on the elimination of urinary constituents, the total solids, total nitrogen, urea and chlorides are increased while the phosphates are probably increased to a lesser extent. The percentage of chlorides may even rise in the diuretic urine, while the other constituents are decreased in percentage at least during the height of the diuresis. The phenolsulphonephthalein excretion is not influenced by diuresis produced by sodium chloride. Cushny (28), in his recent monograph, calls particular attention to the difference in behavior of substances during diuresis. According to him, all the substances of the urine are increased in amount during diuresis. The "no-threshold" substances—urea, phosphates, sulphates—are invariably reduced in percentage but slightly increased in absolute amount, while the "threshold" substances—sodium chloride—are often reduced in percentage, but may actually rise in some circumstances.

The changes which we have observed, therefore, are similar to those characteristic of an increased flow of urine, the percentage of chlorides rising while that of urea, creatinine and sulphonephthalein falls. The total amount of chlorides and urea is markedly increased, while the creatinine and phthalein are approximately unchanged. The behavior of creatinine in diuresis has not been studied, but it is known to be more or less independent of the volume of urine excreted.[2] Similar changes

[2] Our experiments reported in this paper would tend to show that creatinine may be independent of diuresis. However, they were not performed with this object. More careful experiments are being carried out on this point.

in the behavior of various urinary constituents have been observed where the secretion of one kidney was lessened by opposing an obstruction or resistance to the flow. It was shown that water and chloride decrease greatly in amount on the side with the lesser flow, while urea, phosphates, sulphates and indigo-carmine are much less decreased. Moreover, the last named constituents are present in higher percentage while the chloride may be present in higher percentage on the side with the smaller secretion (28). The behavior of creatinine and phthalein under these conditions has not been examined.

The changes in the urine after section of the splanchnic, extirpation of the adrenal or section of the renal nerves appear to persist for months after the operation. This was also noted for the section of the splanchnic and renal nerves by Rhode and Ellinger (10). On the other hand Quinby (29), in experiments on removing the dog's kidney and re-implanting it, found the changes in the urine of the operated side to disappear after a period of 10 to 14 days. In one experiment on the section of the renal nerves, observations made 20 days later indicated that the secretion of each kidney was identical, but this experiment is opposed by many others, particularly on section of the splanchnic or removal of the adrenal. Further work is necessary to explain the apparent discrepancy with Quinby's work, but it must be remembered that the procedures employed are not identical.

The changes in the various constituents of the urine after these experimental procedures, which are similar in many ways to the changes induced by other experimental methods of causing an increased or decreased flow of urine from one kidney, and their bearing on the theories of urinary secretion, will be discussed in a subsequent communication.

SUMMARY

The changes produced in the secretion of one kidney by unilateral removal of the adrenal can be duplicated by sectioning the splanchnic nerve on one side, and also by section of the nerves on the renal artery and vein. It is not necessary to postulate any direct functional vascular connections between the adrenal and the kidney, as has been claimed by Cow, to explain these changes. Unilateral removal of the adrenal appears to affect the kidney of the same side only in so far as the nerves going to the kidney have been injured. This work, however, in no way invalidates the conclusion that complete removal of the adrenals depresses the function of the kidneys.

After the experimental procedures employed in this work,—unilateral removal of the adrenal, section of the splanchnic or section of the renal

nerves—the kidney on the operated side secretes in general a more dilute urine containing a greater percentage of chlorides but a smaller percentage of urea, creatinine, lactose and phenolsulphonephthalein. This is always the feature during diuresis produced by sodium chloride, but during a normal flow the urea percentage may be higher on the side with the greater amount of urine. The total amount of water, chlorides and urea is greater on the operated side, while but little or no change is noticed in the total amount of creatinine and phthalein eliminated on the two sides. The similarity of these changes to those occurring during diuresis and in the lessened flow of urine produced by partial obstruction of the ureter has been discussed.

BIBLIOGRAPHY

(1) Marshall and Davis: Journ. Pharm. Exper. Therap., 1916, viii, 525.
(2) Addis, Barnett and Shevky: This Journal, 1918, xlvi, 39.
Addis, Foster and Barnett: This Journal, 1918, xlvi, 84.
Addis, Shevky and Bevier: This Journal, 1918, xlvi, 129.
(3) Cow: Journ. Physiol., 1914, xlviii, 443.
(4) Bernard: Lecons sur les proprietes physiologique des liquides de l'organisme, 1859.
(5) Eckhard: Beitr. z. Anat. u. Physiol., 1869, iv, 153.
(6) Knoll: Beitr. z. Anat. u. Physiol., 1872, vi, 41.
(7) Klecki: Arch. f. Exper. Path. u. Pharm., 1897, xxxix, 173.
(8) Vogt: Arch. f. Anat. u. Physiol., (Physiol. Abt.) 1898, 399.
(9) Grek: Arch. f. Exper. Path. u. Pharm., 1912, lxviii, 305.
(10) Rhode and Ellinger: Zentralbl. f. Physiol., 1913, xxvii, 12.
(11) Jungmann and Meyer: Arch. f. Exper. Path. u. Pharm., 1913, 73, 49.
(12) Schwarz: Arch. f. Exper. Path. u. Pharm., 1900, xliii, 1.
(13) Peyrani: Biol. Centralbl., 1881–2, i, 599.
(14) See especially: Frey: Arch. f. d. gesammt. Physiol., 1907, cxx, 66; Thompson: Arch. f. Anat. u. Physiol., (Physiol. Abt.), 1894, 117.
(15) Rowntree and Geraghty: Arch. Int. Med., 1912, ix, 284.
(16) Keith and Pulford: Arch. Int. Med., 1917, xx, 853.
(17) Quinby and Fitz: Arch. Int. Med., 1915, xv, 303.
(18) McLean and Van Slyke: Journ. Biol. Chem., 1915, xxi, 361.
(19) Marshall: Journ. Biol. Chem., 1913, xiv, 283.
(20) Folin: Journ. Biol. Chem., 1914, xvii, 469.
(21) V. Schröder: Arch. f. Exper. Path. u. Pharm., 1886, xxii, 39.
(22) Thompson: Journ. Physiol., 1900, xxv, 487.
(23) Katsuyama: Zeitschr. f. Physiol. Chem., 1899, xxviii, 587.
(24) Katsuyama: Zeitschr. f. Physiol. Chem., 1901, xxxii, 235.
(25) Loewi: Arch. f. Exper. Path. u. Pharm., 1902, lxviii, 410.
(26) Bock: Arch. f. Exper. Path. u. Pharm., 1908, lviii, 227.
(27) Baetzner: Arch. f. Exper. Path. u. Pharm., 1913, lxxii, 309.
(28) Cushny: The secretion of the urine, London, 1917, 88.
(29) Quinby: Journ. Exper. Med., 1916, xxiii, 535.

STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

II. A Comparison of the Changes Caused by Unilateral Splanchnotomy With Those Caused by Unilateral Compression of the Renal Artery

E. K. MARSHALL, JR. and A. C. KOLLS

From the Pharmcaological Laboratory, Johns Hopkins University

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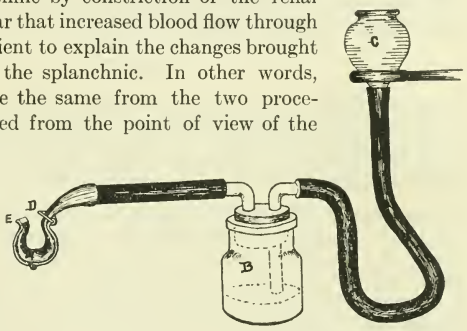
In the preceding paper, certain changes occurring in the urine after section of the splanchnic nerve on one side have been described. Many investigators are inclined to attribute any effects of the nerves upon the secretion of the kidney as due to vasomotor influences (1). The question, however, of true secretory action of the splanchnic or vagus upon the renal cells is an old one, but several recent investigations have been concerned with this problem. Asher and Pearce (2) claimed to have demonstrated secretory fibers in the vagus, but later Pearce and Carter (3) failed to confirm the earlier work, finding no change in the oxygen consumption of the kidney when secretory action was supposed to take place. Jungmann and Meyer (4) found an increase in the chlorides both in percentage and absolute amount following puncture of the floor of the fourth ventricle and that this was abolished after section of the splanchnic. Rhode and Ellinger (5) found that the changes after unilateral section of the renal nerves persisted for weeks or months after the operation. These changes they state to consist in the secretion of a larger volume of urine, of a lower percentage content in solids, and acidity to phenolphthalein, but a greater total amount of solids and acids. These last two investigators conclude that the splanchnic exerts a specific inhibitory-secretory action on the renal cells, which cannot be explained by vasomotor changes. Jost (6) has also advanced evidence that stimulation of the splanchnic can cause a diminution of the urine flow aside from the vasomotor effect. The evidence of these investigators, however, is not conclusive and does not appear to be generally accepted (1). Numerous investigations have shown that an animal can survive with a kidney which is completely deprived of its

nerves, and Lohenhoffer and Quinby have shown that a transplanted or re-implanted kidney suffices for life and also shows a perfectly normal function (7). In the last year, Addis and his co-workers (8) have again advanced the idea of specific nervous control of the kidney to explain their results of the increase in the rate of urea excretion caused by adrenalin and the decrease caused by pituitrin. The changes, which we have described in the preceding paper of this series as resulting from the section of the splanchnic nerve, have been shown to resemble the change occurring in the urine in general when the secretion is markedly increased or decreased. The similarity to forms of diuresis and in partial obstruction of the ureter has been pointed out by Cushny (9), and has been discussed in our previous communication. It would appear then on *a priori* grounds that a specific secretory action of the splanchnic nerve need not be invoked to explain these changes.

The splanchnic nerve is known to contain the vasomotor fibers for the renal vessels, and its section would produce a vasodilatation in the kidney with a corresponding increased amount of blood flowing through the organ. In fact, Burton-Opitz (10) has demonstrated by the use of his "stromuhr" that stimulation of the splanchnic causes a diminished flow of blood through the kidney while section of the same nerve causes an increased blood flow through the organ. Moreover, the splanchnic nerve of each side supplies only the renal vessels of that side (11). Can the changes in the secretion of the kidney after section of the splanchnic be attributed entirely to the increased blood flow through the organ? The blood flow through the kidney can be conveniently diminished by partial constriction of the renal artery. A comparison of the changes produced by section of the splanchnic with those produced by decreasing blood flow suggested itself as an aid in determining the cause of these changes. The changes following partial constriction of the renal artery in rabbits have been examined by Yagi and Kuroda (12). They found with diminished blood flow, after producing diuresis by injection of salines, a decreased amount of urine, chlorides, urea and sulphates. When a mixture of sodium chloride and sodium sulphate was injected as a diuretic, the reduction in chlorides was much greater than the fluid on the constricted side, that is, the percentage of chlorides was greater on the side of greater flow. When a mixture of urea and sodium chloride was used to produce diuresis, the water was more affected than chlorides. In both cases the urea was less affected than the water or chlorides, and the sulphates still less so. Thus, we see that the changes produced here are similar to those caused by section of the splanchnic

when the kidneys of the sides secreting the greater quantity of urine are compared. However, the elimination of creatinine or phenolsulphonephthalein was not examined by these authors, their work was carried out on rabbits, and the secretion was only compared under the influence of a mixture of diuretics. Since our previous work on the section of the splanchnic had been carried out on dogs, and the changes in the elimination of chlorides, urea, creatinine and phenolsulphonephthalein extensively studied both during normal secretion and in diuresis produced by sodium chloride, we considered it important to re-investigate the effect of partial occlusion of the renal artery. Should it be possible to produce changes of an opposite nature to those obtained by sectioning the splanchnic by constriction of the renal artery, it would appear that increased blood flow through the kidney was sufficient to explain the changes brought about by section of the splanchnic. In other words, the changes might be the same from the two procedures when considered from the point of view of the secretion of different amounts of urine, in one case one kidney eliminating more urine by virtue of the splanchnic being sectioned and in the other the organ on the normal side secreting more on account of decreased blood flow on the other side. If the changes occasioned by nerve section could be annulled by pressure on the artery, the value of the argument would be increased.



PRESSURE CUFF

Fig. 1

The methods employed were those described in the previous communication (13). The renal artery was exposed by an incision through the flank, and care was taken to disturb the nerves as little as possible, but the results show that some nerves were generally injured. We wished to adjust the pressure carefully so as to be able to study the effect of pressure on the renal artery after section of the splanchnic nerve on the same side. The apparatus used for exerting the pressure was a small rubber cuff, enclosed in an aluminium band. The cuff could be

filled with water and pressure exerted by raising a column of mercury. This apparatus we have called the "pressure cuff." It is illustrated in figure 1. *A* is a flattened tube of thin rubber dam which is covered with thin silk gauze or bolting silk and attached to a piece of rubber tubing. *B* is a small glass bottle, and *C* a thistle tube. *A* is filled with water and surrounded by the hinged aluminium band, then slipped about the artery and fastened by slipping the wire clasp *D* over the small projecting piece of metal, *E*. The tube connected to *A* is then passed through a small incision made conveniently near the kidney, and passed to the outside where *B* which has previously been filled with water and *C* are attached. *C* is then filled with mercury. Various degrees of pressure can be exerted on the renal artery by raising or lowering the mercury column by elevating *C*.

RESULTS

The first experiment described is one in which an attempt was made to neutralize the changes induced by section of the splanchnic nerve by diminishing the blood flow with pressure on the renal artery. The following summary presents the essential details and tabulated results.

Experiment 1. Dog MK 81. Weight 7.1 kilos. December 22, 1916. Left splanchnic sectioned. Ureters cannulated. Left renal artery exposed through flank and "pressure cuff" placed around it. Diuresis produced by injection of 10 per cent sodium chloride. Urine collected for one-half hour periods without pressure on renal artery, then pressure adjusted so that each kidney secreted approximately the same amounts of urine. At 4.38 pressure had been satisfactorily adjusted; at 5.41 pressure was completely removed from left renal artery.

	TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
			cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
I	3.25–3.55	R	13.0	0.55	72.0	0.012	1.60	1.20	156.2
		L	26.5	0.34	90.1	0.006	1.65	1.23	325.9
II	3.55–4.25	R	8.6	0.49	42.5	0.017	1.50	1.18	101.3
		L	30.5	0.28	85.4	0.004	1.47	1.29	393.5
III	4.38–5.08	R	6.75	0.87	59.2	0.020	1.47	1.00	67.5
		L	7.75	0.67	51.9	0.017	1.35	1.16	90.0
IV	5.10–5.40	R	8.00	0.88	70.5	0.020	1.63	0.81	65.0
		L	11.00	0.53	58.5	0.012	1.38	1.13	125.0
V	5.43–6.13	R	5.7	0.75	42.7	0.026	1.52	0.57	32.5
		L	22.2	0.38	76.5	0.007	1.57	1.01	225.0

In the first two periods the urine from the operated side exhibits all the changes described as characteristic of splanchnic section. In the third period, the pressure adjusted has been just about enough to cause the two kidneys to secrete about equal amounts of urine, and the figures are not far from those obtained from an experiment on a normal animal. The correspondence to the normal is not exact but much better than one might expect when we realize that diminishing the blood flow by constricting the renal artery is not at all identical with nature's method of increasing it by vasodilatation in the various branches of the renal artery. In period IV, the pressure is not as effective or has decreased and the correspondence with the normal is not as close. That the kidney has not been injured by this slight pressure on the artery is probable when we see in period V, where the pressure is removed, a quick return to the condition of periods I and II or at the commencement of the experiment.

The following summaries are typical of other experiments in which the blood flow through the kidney was diminished by pressure on the renal artery.

Experiment 2. Dog MK 98. Weight 7.80 kilos. February 5, 1917. "Pressure cuff" placed on left renal artery, and ureters cannulated. Diuresis produced by injection of 10 per cent sodium chloride and physiological saline. After operation, animal was allowed to recover for about one hour, then collection of urine from each kidney commenced. At 1.18 pressure applied to left renal artery, and adjusted so that the left kidney was secreting about one-half as fast as the right. At 2.22 pressure lessened slightly. At 3.19 pressure removed.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.15–12.45	R	3.0	1.67	50.2	0.033	0.99	1.16	35.0
	L	3.0	1.51	45.2	0.032	0.96	1.24	37.4
12.48– 1.18	R	12.1	0.87	106.2	0.011	1.38	1.41	170.7
	L	11.9	0.78	93.6	0.011	1.25	1.45	173.4
1.52– 2.22	R	12.7	0.51	65.3	0.007	0.99	1.56	200.7
	L	7.4	0.61	44.8	0.012	0.92	1.27	93.0
2.48– 3.18	R	10.5	0.58	60.9	0.009	1.02	1.24	130.8
	L	7.9	0.65	51.8	0.012	0.99	1.34	106.2
3.27– 3.57	R	6.8	0.55	37.8	0.011	0.75	1.12	77.7
	L	6.2	0.57	35.7	0.011	0.68	1.25	77.7

Experiment 3. Dog MK 102. Weight 7.80 kilos. February 13, 1917. "Pressure cuff" placed on left renal artery, and ureters cannulated. At 1.37 slight pressure on left renal artery. At 2.40–3.30 pressure very much increased on left renal artery, and adjusted so that the right kidney was secreting about ten times

as much as the left. At 4.08 pressure completely removed. At 11.23–12.00, intravenous infusion of 50 cc. of a 3 per cent sodium chloride, 3 per cent urea and 0.3 per cent creatinine solution. At 3.37, infusion of 15 cc. more of above mixture.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.23–12.53	R	11.4	1.28	145.3	0.162	18.5	0.93	106.7
	L	14.0	1.07	150.8	0.127	17.8	0.94	132.0
1.37– 2.07	R	17.8	0.84	150.1	0.092	16.4	1.08	193.7
	L	15.3	0.86	131.9	0.101	15.5	1.26	193.2
2.07– 2.37	R	10.0	1.01	100.8	0.108	10.8	1.16	116.0
	L	10.0	1.02	101.7	0.105	10.5	1.20	120.0
3.37– 4.07	R	12.5	1.32	164.7	0.086	10.8	1.28	160.0
	L	1.5	2.92	43.8	0.740	11.1	1.73	26.0
4.14– 4.44	R	7.8	0.45	35.3	0.072	5.6	1.39	108.7
	L	11.6	0.80	93.5	0.047	5.4	1.25	145.0

Experiment 4. Dog MK 99. Weight 6.4 kilos. February 7, 1917. At 11.15, "pressure cuff" placed on left renal artery. Ureters cannulated. Between 1.30 and 2.30, pressure adjusted on left artery. At 3.35 pressure considerably increased. At 4.10 pressure removed.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.20–12.50	R	4.25	2.33	99.0	0.047	2.01	1.80	76.5
	L	5.70	1.80	102.6	0.035	2.00	1.66	94.4
12.50– 1.20	R	8.0	0.81	64.4	0.018	1.46	1.65	132.0
	L	12.5	0.63	79.7	0.012	1.56	1.60	200.4
2.30– 3.00	R	19.2	0.49	94.2	0.008	1.59	1.54	296.8
	L	9.4	0.64	60.0	0.015	1.38	1.57	148.0
3.05– 3.35	R	18.5	0.50	93.0	0.008	1.51	1.55	287.2
	L	12.6	0.66	83.4	0.011	1.42	1.60	200.8
3.40– 4.10	R	20.2	0.44	88.5	0.007	1.44	1.59	322.8
	L	8.2	0.76	62.4	0.016	1.34	1.48	122.0
4.55– 5.25	R	14.2	0.53	76.5	0.011	1.55	1.76	250.5
	L	13.3	0.57	77.0	0.011	1.47	1.74	232.5

DISCUSSION

The changes caused in the secretion of the kidney by diminishing the blood flow by compression of the renal artery are exactly in the opposite direction to those caused by section of the splanchnic nerve. Water is diminished, chlorides are frequently diminished in percentage as well as absolute amount, while urea and creatinine are increased in percentage but diminished in absolute amount. The creatinine is excreted in nearly the same quantities by the two kidneys, although in general the one with the diminished blood flows tends to excrete slightly less. It might be mentioned again that decreasing the blood flow by constricting the renal artery may not be exactly comparable to decreasing it by a mild vasoconstriction in the body, and moreover, we have found that if the blood flow is decreased to a great extent, all substances excreted fall markedly in absolute amount on the affected side. A certain blood flow is apparently necessary to furnish enough oxygen for nutrition of the renal cells.

The following table which is collected from experiments reported in the preceding article and those given in this paper indicate the identity of the changes when the blood flow is decreased and when the splanchnic is sectioned. In the case of the experiments in which nerves are severed,

Comparison of changes after section of splanchnic and after compression of renal artery

PROCEDURE		WATER	CHLORIDE	UREA	CREATININE	DOG NUMBER
Compression on renal artery	R	171	217	146	107	MK 98
	L	100	100	100	100	
Section of splanchnic	R	100	100	100	100	MK 32
	L	182	200	121	105	
Compression on renal artery	R	147	143	111	105	MK 99
	L	100	100	100	100	
Section of splanchnic	R	100	100	100	100	MK 32
	L	156	171	121	107	
Compression on renal artery	R	250	263	143	107	MK 99
	L	100	100	100	100	
Removal of left adrenal	R	100	100	100	100	MK 15
	L	293	407	157	107	

the secretion of the kidney of the normal side is represented as 100, and that of the other compared to this. In expressing the results of experiments on diminished blood flow, the secretion of the kidney with the lesser flow is taken as 100 and the one on the normal side compared to this. Data are selected not at random, but to obtain as close a correspondence as possible for it is our desire to show only that the changes characteristic of section of the splanchnic can be reproduced by a procedure which affects the blood flow.

It appears then that there is little doubt that the changes which have been described as characteristic of sectioning the splanchnic or renal nerves can be entirely reproduced by compressing the renal artery. This, of course, would cause a diminished pressure in the kidney as well as a diminished blood flow and smaller amount of oxygen to be utilized. However, increasing the blood flow would also cause an increased pressure and an increased amount of oxygen. Richards and Plant (14), in their extremely important experiments on the perfusion of the kidney by a method free from the objections coincident to so many perfusion experiments, found that urine flow depended more on the rate of blood flow than blood pressure in the kidney. It is conceivable that the effects of increased and decreased blood flow depend entirely on the carrying of more or less oxygen to the kidney, but this is unlikely as long as the amount of oxygen is sufficient for the needs of the cells. Barcroft and Straub (15) found that blood flow might frequently change without any change in the oxygen consumption. It seems reasonable then to ascribe the effects of compression of the renal artery (provided it is not carried to too great an extreme, where decreased oxygen supply undoubtedly enters as a factor) to diminished blood flow. The changes, therefore, caused by sectioning the splanchnic or renal nerves can be explained by the increased blood flow known to occur under these conditions.

The fact that the changes characteristic of splanchnotomy persist for weeks and months after the operation has been advanced as an argument that mere vasodilatation cannot explain the changes. The vessels by this time would be supposed to have regained their tone. But as Bayliss (16) has pointed out during the observations, vasoconstrictor impulses, partly reflex, are being sent to the vessels of the kidney on the uninjured side, while they are absent on the side with sectioned nerves. Again, our experiments with nicotine would tend to support this as the differences in secretion of the two kidneys, even after the splanchnic has been sectioned for months, can be caused to disappear

after administration of nicotine. This is discussed more fully in a subsequent communication of this series.

It appears, therefore, that the burden of proof still rests with those who would assign a specific secretory-inhibitory action to the splanchnic nerve aside from the changes which it causes by being the chief vasomotor nerve to the kidney. As long as the changes produced can be explained entirely as vasomotor phenomena, it is unnecessary to invoke a specific secretory action for the nerve.

SUMMARY

The changes caused in the secretion of the kidneys after section of the splanchnic nerve on one side are in all respects examined similar to those caused by changes in blood flow through the kidney. The effect of section of the splanchnic is an increased flow through the kidney which is responsible for the changes in secretion. It appears unnecessary at present to assign a specific secretory function to the nerve to explain these changes.

BIBLIOGRAPHY

(1) Cushny: The secretion of the urine, London, 1917, 100.
Bayliss: Principles of general physiology, London, 1915, 358.
(2) Asher and Pearce: Zeitschr. f. Biol., 1914, lxiii, 83.
(3) Pearce and Carter: This Journal, 1915, xxxviii, 350.
(4) Jungmann and Meyer: Arch. f. exper. Path. u. Pharm., 1913, lxxiii, 49.
(5) Rhode and Ellinger: Zentralbl. f. Physiol., 1913, xxvii, 12.
(6) Jost: Zeitschr. f. Biol., 1914, lxiv, 441.
(7) For literature, see Quinby: Journ. Exper. Med., 1916, xxiii, 535.
(8) Addis, Shevky and Bevier: This Journal, 1918, xlvi, 129.
(9) Cushny: The secretion of the urine, London, 1917, 102.
(10) Burton-Opitz and Lucas: Arch. f. d. gesammt. Physiol., 1909, cxxvii, 143.
(11) Burton-Opitz and Lucas: Arch. f. d. gesammt. Physiol., 1908, cxxv, 221.
Burton-Opitz: This Journal, 1916, xl, 437.
(12) Yagi and Kuroda: Journ. Physiol., 1915, xlix, 162.
(13) Marshall and Kolls: This Journal, 1919, xlix, 302.
(14) Richards and Plant: Journ. Pharm. Exper. Therap., 1915, vii, 485.
(15) Barcroft and Straub: Journ. Physiol., 1910, xli, 145.
(16) Bayliss: Principles of general physiology, London, 1915, 358.

STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

III. The Effect of Nicotine on the Secretion of the two Kidneys after Unilateral Section of the Splanchnic Nerve

E. K. MARSHALL, JR. and A. C. KOLLS

From the Pharmacological Laboratory, Johns Hopkins University, Baltimore

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It is well known that nicotine, after a brief period of stimulation, causes a paralysis of sympathetic ganglia. It appeared probable that the injection of nicotine into an animal, in which unilateral section of the splanchnic had been performed, might amount to the section of the nerve on the other side. The effects, then, of unilateral splanchnotomy should be abolished by the injection of nicotine as far as the difference in the secretion of the two kidneys is concerned.

The methods employed were similar to those described previously. Blood pressure, urine flow and kidney volume were recorded in some of the experiments. A modified type of the Roy oncometer was used.

The following protocol (exper. 1) is illustrative of the general type of procedure employed. The effect of section of the splanchnic in producing a relative increase in the amount of urine on the operated side, as well as the effect of nicotine injected intravenously, of promptly causing the difference in fluid secretion of the two kidneys to disappear is clearly shown. Before section of the splanchnic nerve, the ratio of the amount of urine secreted by each kidney is nearly unity; after section of the nerve, the left kidney secretes about three times as much as the right; the intravenous injection of nicotine causes the two kidneys to again return to the normal ratio and secrete the same amounts of urine.

One of the most characteristic changes in the composition of the urine after section of the splanchnic nerve is a relative, as well as an absolute increase in chlorides. After nicotine this discrepancy in the amounts of chloride eliminated by the two kidneys disappears. Before section

of the splanchnic (exper. 1), each kidney eliminated the same amount of chloride; after section, the left eliminated much the greater amount; the injection of nicotine caused each kidney again to eliminate equal quantities of chloride.

Experiment 1. Dog MK 30. Male, weight 4.5 kilos. May 5, 1916.

1.15 p.m. Given 7.6 cc. of paraldehyde and 90 cc. water by stomach tube.

2.25–3.00 p.m. Operation through midline abdominal incision, carefully dissecting free the left splanchnic, placing a loose ligature about it and passing this ligature through an incision in the flank. Animal placed on abdomen, and ureters cannulated. Saphenous vein cannulated for injection.

3.07 p.m. Injection of 9 cc. of 10 per cent sodium chloride.

3.20 p.m. Injection of 20 cc. of 3 per cent sodium chloride.

3.20–3.35 p.m. Urine, right 0.60 cc.; left 0.62 cc.

3.35–3.50 p.m. Urine, right 1.80 cc.; left 2.05 cc.

3.50–4.05 p.m. Urine, right 1.96 cc.; left 1.75 cc.

4.10 p.m. Left splanchnic cut; 4 cc. of 10 per cent sodium chloride injected.

4.12–4.27 p.m. Urine, right 0.65 cc.; left 1.89 cc.

4.27–4.42 p.m. Urine, right 0.55 cc.; left 1.69 cc.

4.42 p.m. Injection of 9 cc. of 10 per cent sodium chloride.

4.42–4.57 p.m. Urine, right 1.90 cc.; left 5.20 cc.

4.58 p.m. Twenty milligrams nicotine tartrate in 2 cc. water injected intravenously. Anuria until 5.02.

5.02–5.17 p.m. Urine, right 0.59 cc.; left 0.75 cc.

5.17–5.32 p.m. Urine, right 1.51 cc.; left 1.59 cc.

5.23 p.m. Injection of 10 cc. 3 per cent sodium chloride.

5.32–5.47 p.m. Urine, right 1.90 cc.; left 2.40 cc.

5.47–6.02 p.m. Urine, right, 1.77 cc.; left 2.63 cc.

6.02–6.17 p.m. Urine, right 2.8 cc.; left 4.40 cc.

		PERIOD											
		1	2	3		4	5	6		7	8	9	10	11
Urine, *cc.*...	Right	0.60	1.80	1.96	Section of left splanchnic	0.65	0.55	1.90	Injection of nicotine	0.59	1.51	1.90	1.77	2.82
	Left	0.62	2.05	1.75		0.89	1.69	5.20		0.75	1.59	2.40	2.63	4.40
Ratio.....	Left / Right	1.03	1.14	0.90		2.91	3.07	2.74		1.27	1.05	1.26	1.48	1.56
Sodium chloride, *mgm.*	Right	18		22		14		30		35	32			
	Left	21		19		56		82		33	38			

The following protocols are condensed and the results expressed in the form of tables. Experiment 2 is a normal control animal in which urine volumes from each kidney were measured as well as the amounts

of chloride eliminated. Nicotine was then injected exactly as in the operated animals and the effects noted. The data show that the amounts of urine excreted by each kidney, as well as the quantities of chloride eliminated in the time intervals employed are very nearly equal, and any differences that are encountered are negligible compared with the differences obtained by sectioning the splanchnic or the injection of nicotine. The injection of nicotine in the normal animal causes, as one would expect, no change in the ratio of the amounts of urine or sodium chloride eliminated by the two kidneys.

Experiment 2. Dog MK 39. Female, weight 6.1 kilos. June 23, 1916. Normal control. At various intervals during the experiment diuresis produced by injection of 10 per cent sodium chloride. Between periods 7 and 8, 20 mgm. nicotine tartrate in 4 cc. water injected intravenously. Time of periods, 15 minutes each.

		PERIOD												
		1	2	3	4	5	6	7	Injection of nicotine	8	9	10	11	12	13
Urine, cc.	Right	0.79	0.90	1.95	3.30	1.7	4.3	3.7		0.76	1.25	3.50	3.50	3.55	1.20
	Left	0.78	1.05	2.15	3.72	1.9	4.3	3.7		0.80	1.25	4.20	3.50	3.30	0.80
Ratio..	Left / Right	0.98	1.16	1.10	1.13	1.12	1.00	1.00		1.05	1.00	1.20	1.00	0.93	0.67
Sodium chloride, mgm.	Right		13.8		50.0	26.0	70.0	60.0		27.0		51.0	50.0		
	Left		13.9		56.0	28.0	69.0	61.0		28.0		59.0	47.0		

Experiment 3 shows the effect of nicotine on the relative increase of urine produced by removal of the adrenal gland on one side. It has been shown by the authors that this can be explained entirely by unavoidable injury to the splanchnic fibers going to the kidney. The effect produced is the same as when the splanchnic is sectioned above the adrenal.

Experiment 4 shows that the action of nicotine takes place even if the adrenal (or splanchnic) has been removed some time previously. The adrenal was removed 4 months before the observations were made.

Experiment 3. Dog MK 42. Male, weight 9.2 kilos. June 30, 1916. Left adrenal removed just before making observations. Diuresis produced at various intervals by injection of 10 per cent sodium chloride. Between periods 5 and 6,

27 mgm. nicotine tartrate injected; between periods 16 and 17, 18 mgm. nicotine tartrate injected. Length of each period, 15 minutes.

		PERIOD								
		1	2	3	4	5	6	7	8	9
Urine, *cc*	Right	0.40	0.65	0.50	1.25	0.95	2.80	2.10	3.75	3.55
	Left	0.70	2.40	1.55	4.20	3.50	3.25	1.60	2.55	2.80
Ratio	Left / Right	1.75	3.70	3.10	3.36	3.70	1.16	0.76	0.68	0.79
Sodium chloride, *mgm*	Right					29.5		70.2		
	Left					133.2		32.4		

		PERIOD								
		10	11	12	13	14	15	16	17	18
Urine, *cc*	Right	3.96	2.35	2.52	1.50	1.46	1.25	1.50	1.15	1.60
	Left	3.88	2.40	3.35	2.15	2.76	2.20	3.00	1.20	1.80
Ratio	Left / Right	0.98	1.02	1.33	1.43	1.91	1.80	2.00	1.04	1.13
Sodium chloride, *mgm*	Right		17.0		11.8		6.9			
	Left		14.2		28.5		26.0			

Experiment 4. Dog MK 19. Female, weight 10.7 kilos. July 19, 1916. Left adrenal removed aseptically under ether anesthesia March 23, 1916. Dog allowed to recover. Diuresis produced at intervals during experiment by injection of 10 per cent sodium chloride. Between periods 4 and 5, 30 mgm. nicotine tartrate in 6 cc. water injected.

		PERIOD								
		1	2	3	4	Injection of nicotine	5	6	7	8	9
Urine, *cc*	Right	5.0	2.1	3.4	2.7		16.4	9.7	12.6	12.4	15.5
	Left	20.2	10.2	17.9	5.1		16.4	11.7	12.7	11.6	14.6
Ratio	Left / Right	4.04	4.86	2.1	1.90		1.00	1.20	1.00	0.92	0.94

Blood pressure tracings were taken in practically all the experiments just before, during and after the administration of nicotine, as well as a graphic record of the flow of urine from each kidney. The blood pressure tracing was continued at intervals during the course of the experi-

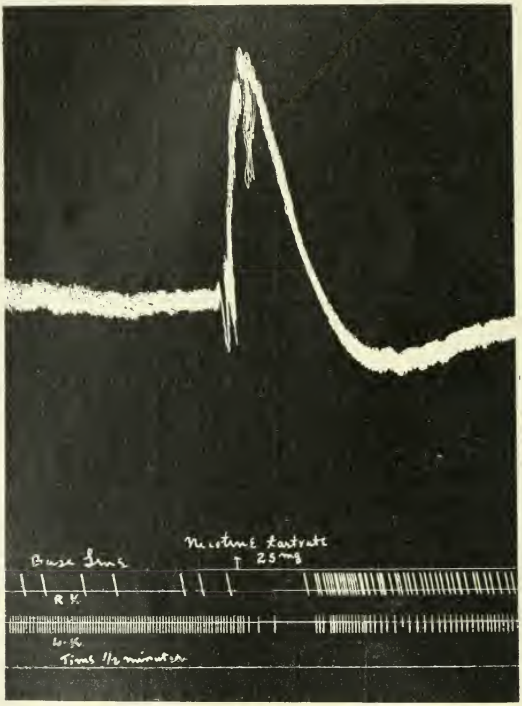

Fig. 1

ment. Figure 1 shows graphically the influence of nicotine in causing the kidneys which have been secreting different amounts to approximate one another. The rise of pressure due to the general vasoconstriction in which the kidney participates causes a temporary anuria, but the rise only being very transient, the anuria quickly disappears. As noted

from the records of urine flow, the effect of nicotine on the secretion of the kidneys is very rapid, generally the two kidneys secrete the same number of drops of urine per unit of time a few minutes after the injection of nicotine. Sometimes, and in fact generally, it was noted that after the transient anuria from nicotine, the intact kidney (right) always commenced secreting slightly before the other. In some of the protocols which have been presented it is also noted that frequently after nicotine the intact kidney secretes slightly more than the other. Of course, one might argue that the function of this kidney was slightly greater than the other, a thing which is frequently noted in comparing the two kidneys of a normal dog. But the phenomenon that after nicotine administration the right kidney has a tendency to secrete somewhat more rapidly than the left occurs so regularly that it cannot be a coincidence. Although further experimental work appears necessary to decide the cause for this phenomenon, the following explanation may be advanced. Nicotine acts upon the vasomotor centers in the same way, as "puncture diuresis." As the left kidney is almost entirely isolated from central nerve control, and the conducting mechanism from the center to the right kidney is intact, the effect is greater upon the right than the left and at this stage of nicotine action we have a greater flow from the kidney of the unoperated side. The "puncture diuresis" is generally admitted to be due to impulses emitted from the medulla oblongata passing to the kidney through the splanchnic nerves. These impulses may consist of either stimulation of the vasodilator center or depression of the vasoconstrictor, or a combination of both (1). In the course of the action of nicotine we have both stimulation and depression, so conditions would be favorable for this mode of action.

After the rise of pressure from nicotine has subsided there is generally a slight fall which lasts for some time. This, however, did not regularly occur, even when the kidneys were secreting at an equal rate. Occasionally the pressure fell very rapidly after the rise due to the administration of nicotine until death occurred. The marked respiratory effect of nicotine was always noted, and occasionally respirations ceased and artificial respiration had to be resorted to for a short time. The toxic effects as noted on the different animals seem to depend a great deal on individual variation, some animals succumbing very shortly after the administration of a dose of nicotine far smaller than that which other animals received without any serious symptoms of a circulatory or respiratory effect being noted.

The most reasonable explanation of the effect of nicotine in causing the two kidneys to secrete practically equal amounts of urine after unilateral section of the splanchnic nerve is to be sought in its well-known paralytic action on all ganglia cells.

The response of the kidney to splanchnic stimulation was tested before and after the administration of nicotine by means of the oncometer. Several experiments of this type were carried out both with and without

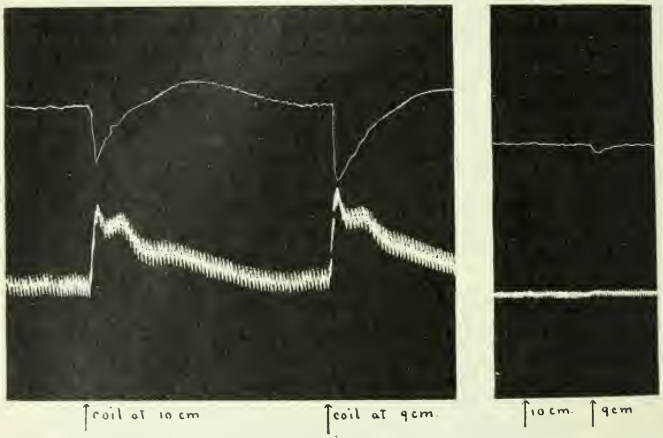

Fig. 2. Dog MK 44. Female, weight 4.5 kilos. Blood pressure tracing from the carotid. Left kidney freed carefully from the surrounding tissue and placed in oncometer. Left splanchnic carefully dissected out just above the adrenal, and placed in a shielded electrode. Splanchnic stimulated with induction coil for 5 seconds. After satisfactory normal records were obtained, 12.5 mgm. nicotine tartrate in 2.5 cc. water were injected into the saphenous vein. Tracing A shows effect of stimulating splanchnic (coil at 10 cm. and 9 cm.) before administration of nicotine. Tracing B indicates that practically no effect is obtained 20 minutes after administration of nicotine (coil 10 cm. and 9 cm.).

sectioning the nerve before stimulation. In all the experiments of this type, a definite contraction of the kidney volume was obtained with the stimulation before nicotine was given, but after the administration of the drug only a very slight or no response was obtained to the same stimulus. Figure 2 represents a typical tracing from one of these experiments.

We can conclude, therefore, that nicotine in the dosage given paralyzes the ganglia of the fibers of the splanchnic nerve supplying the renal vessels. The dosage which has been employed to produce the effects described has varied between 2.8 mgm. and 4.4 mgm. per kilo of nicotine tartrate or 0.9 to 1.5 mgm. of nicotine; 0.2 mgm. per kilo was found to produce no effect, while 0.25 mgm. per kilo produced a transient effect for 5 minutes.

The length of time which the paralysis of the renal ganglia has lasted has varied very widely with the same dosage in different animals. Thus, in experiment 1, the paralysis has begun to wear off after 30 minutes with a dosage of nicotine of 1.5 mgm. per kilo, while in experiment 3, the paralysis lasts for one and one-half hours with a dosage of 1.0 mgm. of nicotone per kilo. In other experiments it was frequently found that with about this dosage of nicotine there was no apparent decrease in the paralysis after several hours. The length of time which the paralytic action of the drug on the renal ganglia continues depends apparently on the individual susceptibility of the animal, and resembles in this respect somewhat the toxic action of nicotine.

The fact that nicotine exerts the same action on an animal in which the splanchnic nerve has been sectioned some months previously strengthens the suggestion made in the second communication of this series to account for the fact that the changes characteristic of unilateral splanchnotomy persist for months. Although the vessels of the kidneys on the operated side may have regained their tone by this time, during the observations vasoconstrictor impulses are being transmitted to the normal kidney, while the organ on the side with the sectioned nerve is free of them. Nicotine not only abolishes these impulses but also paralyzes the ganglia on both sides. A few experiments, not included here, have shown that after section of the renal nerves on one side, nicotine will neutralize the effects of this. This confirms in a physiological way the conclusions of Renner (2), on histological grounds, that ganglia cells exist as far as the renal hilus.

SUMMARY

The marked difference in the amounts of urine secreted by the two kidneys after unilateral section of the splanchnic nerve is caused to disappear by the administration of nicotine intravenously in dosage of 1 to 1.5 mgm. per kilo. The relative and absolute increase of chlorides characteristic of splanchnic section is also abolished by this procedure.

This effect of nicotine occurs whether the nerve is sectioned just before the observations or some months previously. The cause is to be found in the paralysis of the sympathetic ganglia cells by nicotine.

BIBLIOGRAPHY

(1) Cushny: The secretion of the urine, London, 1917, 103.
(2) Renner: Deutsch. Arch. f. klin. Med., 1913, cx, 101.

STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

IV. Unilateral Ligation of One Branch of One Renal Artery and Unilateral Splanchnotomy

E. K. MARSHALL, JR. and A. C. KOLLS

From the Pharmacological Laboratory, Johns Hopkins University, Baltimore

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In the first two communications of this series, it was shown that certain changes in the elimination of water, chloride, urea, creatinine and phenolsulphonephthalein were characteristic of the section of the splanchnic. It was further shown that these changes could be explained entirely as due to an increased blood flow through the kidney. In these experiments one kidney was compared with the other, the two organs being subject to the same extra-renal influences—changes of blood pressure, changes of blood composition, the influence of the anesthetic, if any, etc. Any differences in the secretion of the two kidneys can, therefore, safely be ascribed to a difference in blood flow. We can say, then, that the secretion of water and chlorides is very markedly affected by changes of blood flow, the secretion of urea is definitely affected but to a less extent than water or chlorides, while the secretion of creatinine and phenolsulphonephthalein is very little if at all affected by this procedure. It is well known that the creatinine output in any given individual on a constant diet is quite constant from day to day and does not vary with the volume of urine. Phenolsulphonephthalein has also been shown to be more or less independent of the volume of urine (1). In fact, the originators of this test of renal function state that phthalein is eliminated in a different way from salt and water.

It is possible to reduce the amount of active renal tissue by ligating one branch of the renal artery (2). Of course, in the reduction of the amount of renal tissue by this method, the blood supply is also reduced, that is, the portion of a kidney has per unit of weight the same blood supply as the original kidney. However, it occurred to us as possible to increase the blood supply of the portion of a kidney to something like the blood supply of the original kidney by sectioning the splanchnic nerve as well as ligating the posterior branch of the renal artery. Here,

then, we would have a kidney about one-half the size of the normal one but with a blood supply increased and probably somewhat approximating that of the normal kidney. It appeared interesting to examine the elimination of substances under these conditions compared to their elimination by the intact kidney on the other side.

The methods adopted were exactly similar to those described in the preceding papers. The posterior branch of the renal artery was exposed by a midline incision through the abdomen, and ligated. The splanchnic was sectioned at the same time just above the adrenal gland. The following summaries are typical of the experiments.

Experiment 1. Posterior branch of left renal artery tied. Dog MK 49. Male. Weight 5.3 kilos. At 10.15 posterior branch of left renal artery ligated; 10.30 ureters cannulated. At 11.30 intravenous injection of 50 cc., 0.8 per cent saline. At 12.30, 10 cc. of 10 per cent sodium chloride given intravenously. At 1.30, 1.6 grams lactose given intravenously.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	mgm.	mgm.	mgm.
11.00–12.30	R	2.75	88.3	4.2	
	L	1.65	50.5	2.1	
12.30– 1.30	R	19.5	165.8	2.9	197.0
	L	10.6	76.4	1.5	111.2
1.30– 2.30	R	24.5	164.2	3.9	235.2
	L	14.0	78.4	2.0	145.6

At 2.30, 6 mgm. of phenolsulphonephthalein were injected intravenously. In the next hour the right kidney secreted 18.0 cc. urine containing 41.5 per cent, and the left, 9.3 cc. urine containing 21.2 per cent of the injected dye.

Experiment 2. Posterior branch of left renal artery ligated; left splanchnic sectioned. Dog MK 114. Weight 9.9 kilos. March 14, 1917. Left splanchnic sectioned just above the adrenal gland. Posterior branch of left renal artery ligated. Ureters cannulated. At 11.55 given 15 cc. of 10 per cent sodium chloride solution.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	mgm.	mgm.	mgm.
12.00–2.00	R	13.1	321.0	8.00	215.0
	L	12.2	194.5	4.35	187.5
2.00–4.00	R	13.8	385.5	7.15	
	L	10.2	214.5	4.00	

Experiment 3. Posterior branch of left renal artery ligated; left splanchnic sectioned. Dog MK 115. Weight 10.1 kilos. April 5, 1917. Left splanchnic sectioned just above adrenal gland. Posterior branch of left renal artery ligated. Ureters cannulated. At 12.10, 12.35, 1.05 and 2.35, 10 cc. of 10 per cent sodium chloride solution injected.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
10.35–12.35	R	17.0	180.0	10.08	102.4
	L	20.8	156.9	5.96	196.6
12.35–2.35	R	24.9	351.0	12.24	414.0
	L	30.1	264.8	6.50	427.4

Experiment 4. Posterior branch of right renal artery ligated; right splanchnic sectioned. Dog MK 100. Weight 6.10 kilos. February 9, 1917. At 10.20 posterior branch of right renal artery ligated. At 11.45 right splanchnic nerve sectioned in thorax just above the diaphragm. Ureters cannulated. At 1.05, 12 cc. of 10 per cent sodium chloride solution were injected. At 2.05, 1 cc. (6 mgm.) of phenolsulphonephthalein given intravenously.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
12.05–1.05	R	4.8	31.5	1.50	
	L	5.0	52.2	3.00	
1.05–2.05	R	19.5	46.8	1.28	200.0
	L	17.4	100.2	2.64	184.0

During the next hour, 6.4 cc. of urine were secreted by the right kidney containing 16 per cent of the injected phthalein, while 2.6 cc. were obtained from the left, with 34.5 per cent.

It is seen that ligation of the posterior branch of one renal artery in the dog is followed by a reduction of the water, urea, chlorides, creatinine and phenolsulphonephthalein to about one-half of that eliminated by the other kidney (exper. 1). However, if the blood supply of the portion of a kidney is increased by section of the splanchnic, water and chlorides are eliminated equally well or in fact sometimes in greater amount than by the entire kidney with a normal (or less than other) blood flow. The creatinine and sulphonephthalein are not appreciably increased by the increased blood flow, while the elimination of urea may be increased considerably but not to the marked extent of water

and chlorides, it still remaining less than the amount eliminated by the normal kidney.

These results are exactly what would be expected by our previous work on the effects of blood flow. They serve, however, to clearly emphasize that when two kidneys are dividing the work of renal excretion between them and the extra-renal factors are the same for both, the relative amounts of water and chlorides which will be eliminated by each depends more upon the blood flow than the size of the kidney, while the relative secretion of creatinine and phenolsulphonephthalein depends mainly on the amount of active renal tissue and to a much less if any extent upon a blood flow within certain limits. If the blood flow is very much decreased, creatinine and substances resembling it are markedly affected, but here other factors (e.g., insufficient oxygen) are brought into play. Richards and Plant (3) found in the perfused kidney that a certain minimum blood flow was necessary for any secretion of urine.

SUMMARY

When the posterior branch of one renal artery is ligated, and the secretion of such a kidney compared to the other, it is found that roughly about one-half as much water, chlorides, urea, creatinine and phenolsulphonephthalein are eliminated. When the blood flow of such a kidney is increased by section of the splanchnic, the portion of a kidney may eliminate more water and chlorides than the other intact kidney, while creatinine and phenolsulphonephthalein will still be reduced about as much as before. Urea stands in an intermediate position.

BIBLIOGRAPHY

(1) Rowntree and Geraghty: Arch. Int. Med., 1912, ix, 284.
(2) Brödel: Johns Hopkins Hosp. Bull., 1901, xii, 10.
MacNider: Journ. Med. Res., 1911, xxiv, 425.
(3) Richards and Plant: Journ. Pharm. Exper. Therap., 1915, vii, 485.

STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

V. Chloride and Sulphate Diuresis after Unilateral Splanchnotomy

E. K. MARSHALL, JR. and A. C. KOLLS

From the Department of Pharmacology, Johns Hopkins University, Baltimore

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During the course of the investigation which has been described in the preceding papers, an attempt was made to measure the relative elimination of sulphates by the two kidneys after unilateral section of the splanchnic. In order to obtain a larger amount of sulphates for determination, diuresis was produced by an intravenous injection of sodium sulphate instead of the sodium chloride solution usually employed. It was found that the difference in urine secretion by the two kidneys tended to disappear during sulphate diuresis instead of being magnified as it was during chloride diuresis.

That sodium sulphate and sodium chloride act in a different manner in producing diuresis is well known. It is also recognized that in the sulphate diuresis changes must occur in the kidney itself, while in chloride diuresis changes in the blood are probably mainly responsible (1). The difference between sulphate and chloride in producing diuresis is well shown by the experiments of Barcroft and Straub (2), in which they found that sulphate diuresis was accompanied by an increase in the oxygen consumption of the kidney and that chloride was not. Also, Knowlton (3) found that the addition of gelatine to a hypertonic sodium chloride solution diminished diuresis when it was injected intravenously, while under the same conditions gelatine had little effect upon sodium sulphate diuresis.

The experiments which we have carried out are reported because they furnish another method of demonstrating the difference between chloride and sulphate in producing diuresis.

The methods used were those already described as employed in these investigations. The following experiments which are shown graphi-

cally in the accompanying charts are selected from a number which have been performed.

Experiment 1. Dog M6. Male, weight 9.2 kilos. Anesthetized with paraldehyde. Left splanchnic sectioned and ureters cannulated. Urine collected from each kidney in 15 minute periods. At points indicated, 20 cc. of 10 per cent sodium chloride and 15 cc. of 10 per cent sodium sulphate solutions given intravenously. The results are shown in figure 1.

Experiment 2. Dog M1. Male, weight 8.0 kilos. Anesthetized with paraldehyde. Left splanchnic sectioned and ureters cannulated. Urine collected from each kidney for 10 minute periods. At points indicated, 10 cc. of 10 per cent sodium chloride, and 5 cc. of 10 per cent sodium sulphate injected intravenously. (Fig. 2.)

Since it has been previously shown that the changes after section of the splanchnic nerve can be explained by the increased blood flow through the kidney, experiments were carried out to determine if the difference between sulphate and chloride diuresis could be demonstrated under conditions where blood flow was decreased in one kidney. Cushny (4) found that when pressure was exerted on one renal artery of a rabbit so as to keep the volume of that kidney constant (as measured by the oncometer), injection of 3 per cent sodium chloride caused a marked diuresis in the normal kidney but none in the one with compressed artery. He interpreted this as proving that saline diuresis was due to changes in the circulation of the kidney, but has recently stated that the experiment proves nothing. The fact that the oncometer does not measure blood flow during diuresis and that the pressure in the renal artery and capillaries was reduced, invalidates the conclusions drawn (5). The changes following the reduction of diuresis by compression of the renal artery in rabbits were studied by Yagi and Kuroda (6). They used, however, mixtures of sodium chloride and sodium sulphate or sodium chloride and urea to produce diuresis. With the amount of pressure and the mixtures of diuretics used, there was always a marked difference in the amounts of urine from the two kidneys.

The "pressure cuff" previously described (7) was used for compressing the renal artery on one side. In accordance with our expectations from the results obtained with sulphate and chloride injections after increasing the blood flow of one kidney by sectioning the splanchnic nerve, the limitation of diuresis is much greater with chloride diuresis than sulphate by compressing the renal artery. The following experiment is typical of several performed in this way.

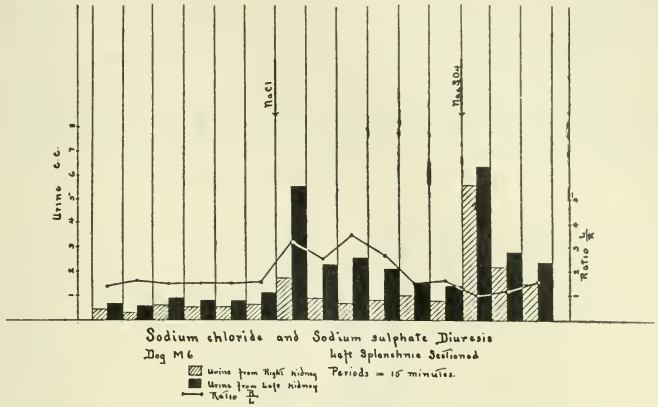
Urine c.c.
NaCl
Na2SO4
Ratio L/R
Sodium chloride and Sodium sulphate Diuresis
Dog M6
Left Splanchnic Sectioned
Periods = 15 minutes.
Urine from Right Kidney
Urine from Left Kidney
Ratio L/R

Fig. 1

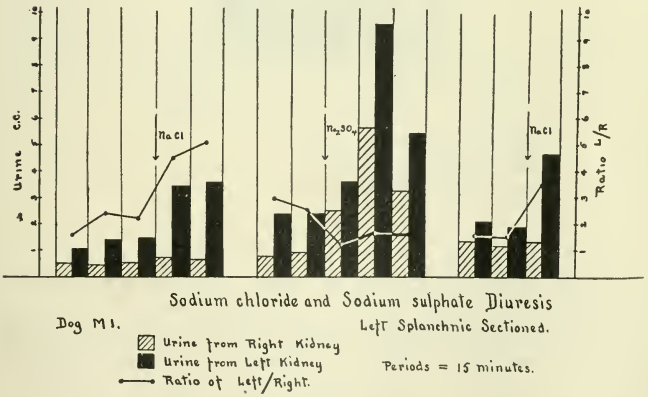
Urine c.c.
NaCl
Na2SO4
NaCl
Ratio L/R
Sodium chloride and Sodium sulphate Diuresis
Dog M1.
Left Splanchnic Sectioned.
Urine from Right Kidney
Urine from Left Kidney
Ratio of Left/Right.
Periods = 15 minutes.

Fig. 2

Experiment 3. Dog MK 116. Female, weight 5.5 kilos. Anesthetized with paraldehyde. Compression on left renal artery. Urine collected from each kidney in half-hour periods. At point indicated, 20 cc. of 10 per cent sodium sulphate and 20 cc. of 10 per cent sodium chloride injected intravenously. (Fig. 3.)

As possible explanations of the differences observed with sulphate and chloride in producing diuresis after sectioning one splanchnic, a paralysis of some portion of the sympathetic nerve elements as produced by nicotine (8), or a more or less maximal dilatation of the vessels of

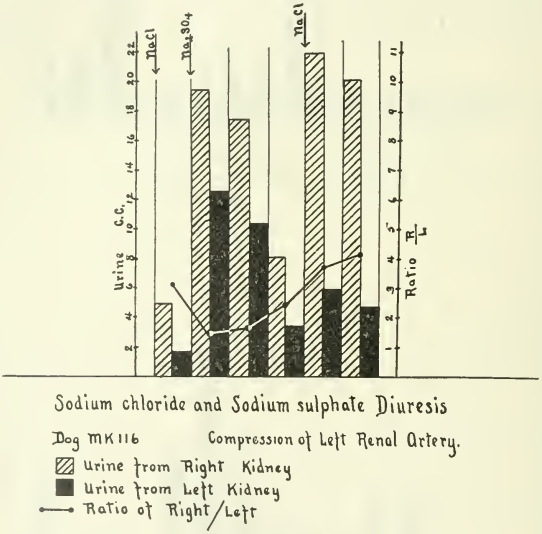

Fig. 3

each kidney by the injected sulphate might at first suggest themselves. That neither of these explanations is correct is shown by the experiments contrasting sulphate and chloride diuresis after careful compression of one renal artery. Moreover, neither of the above actions is known to be attributed to the sulphates. As stated before, however, there is a great deal of evidence showing that sulphates and chlorides behave very differently in excretion, and act in a different manner in producing diuresis. Whatever explanation finally explains the differ-

ences in action of sulphate and chloride in producing diuresis will explain our findings. That the sulphate diuresis occurs mainly from changes in the kidney itself as first suggested by Magnus (9), appears reasonable from the data at hand, but whether a stimulation of the renal cells or inhibition of re-absorption in the tubules is considered responsible depends on the theory of urine secretion that is accepted.

SUMMARY

After section of the splanchnic nerve on one side, sodium chloride produces a greater diuresis on the operated side than the normal one. Sodium sulphate, on the other hand, produces an almost equally good diuresis from each side. Compression of one renal artery limits the diuresis from sodium chloride much more than it does from sodium sulphate.

BIBLIOGRAPHY

(1) Cushny: The secretion of the urine, London, 1917, 146.
(2) Barcroft and Straub: Journ. Physiol., 1910, xli, 145.
(3) Knowlton: Journ. Physiol., 1911, xliii, 219.
(4) Cushny: Journ. Physiol., 1902, xxviii, 431.
(5) Cushny: The secretion of the urine, London, 1917, 125.
(6) Yagi and Kuroda: Journ. Physiol., 1915, xlix, 162.
(7) Marshall and Kolls: This Journal, 1919, xlix, 323.
(8) Marshall and Kolls: This Journal, 1919, xlix, 326.
(9) Magnus: Arch. f. Exper. Path. u. Pharm., 1900, xliv, 396.

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STUDIES IN SECONDARY TRAUMATIC SHOCK

III. Circulatory Failure Due to Adrenalin

JOSEPH ERLANGER and HERBERT S. GASSER

From the Physiological Laboratory of Washington University, St. Louis

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Early in 1917 the English Medical Research Committee, reporting for Bainbridge and Trevan (1), announced that shock can be produced in animals by the continuous injection of adrenalin for 20 minutes or more, at such a rate as to keep the arterial pressure up to that attained by moderate stimulation of sensory nerves. They found that during the injection the arterial pressure rose to a high level, that the systemic venous pressure either was not altered or fell, and that the portal pressure rose and remained at a high level, indicating an obstruction to the flow of blood through the liver. In the course of our investigation of shock we have repeated certain phases of the experiments of Bainbridge and Trevan and as a result have gained considerable information bearing upon the so-called shock-producing action of adrenalin, and upon the action of massive doses of adrenalin in general.

In the course of this investigation information was desired with regard to the effect of interposing a high resistance to the passage of the blood through the liver at the point where the normal peripheral resistance of this organ presumably resides. This led to a study of the effect upon the circulation of injecting into the portal vein toward the liver a suspension of lycopodium spores. This series of experiments forms the basis of the second part of this paper.

THE CIRCULATION AFTER THE CONTINUOUS INJECTION OF MASSIVE DOSES OF ADRENALIN

Methods

The adrenalin, usually consisting of from 6 to 11 cc. of the 1:1000 solution freshly obtained from Parke, Davis and Company, was injected, usually into the femoral vein, in the course of 21 to 29 minutes, an aliquot part entering the circulation rapidly at the beginning of each half-minute. After the first two or three injections the arterial pressure no longer falls in the intervals;—the effect becomes continuous. In a few experiments the injection was made into a mesenteric vein. We were unable to distinguish any clear difference in the response of the organism to the administration of the adrenalin by these different paths. A record was made of the pressure in the carotid artery, and the peripheral resistance was measured by the inflow method (2), (3). In some of the experiments readings of the jugular and portal venous pressures were also made from time to time, by the method previously described. It must be borne in mind in connection with these experiments that the inflow method gives but faint clue to the action of the vasomotor center as long as the adrenalin is exerting its effects peripherally. Dogs alone were used; they were anesthetized with ether. The criteria of shock herein employed have been those described in the first paper of this series (3).

Results

The action of these doses of adrenalin has been so variable that a clear conception of it can be conveyed only by describing and discussing a number of the experiments. An attempt at generalization before such a detailed treatment would surely fail to carry conviction.

Experiment 41 (fig. 1). The first injection, lasting 21 minutes, consisted of about 6 cc. of 1:1000 adrenalin. It raised the arterial pressure from 120 mm. Hg. and held it at about 168 mm. Hg. With the termination of the injection the arterial pressure fell abruptly to 74 mm. Hg., and then to 62, before it recovered slowly to 92 mm. Hg.

The inflow rate (femoral area) was not followed during the injection period, but immediately thereafter it was almost nil. It quickly increased, however, to attain more than twice its initial rate within 5 minutes, and then decreased somewhat more deliberately, finally becoming constant at about half the initial rate. It would seem, therefore, that the adrenalin at first practically stops the blood flow in the femoral area, but that, with the termination of the injection, there develops, almost immediately, a tremendous dilatation. When this has passed

off and the peripheral action of the adrenalin presumably has come to an end, the center seems to be normally active; indeed it is more than normally active, presumably under the stimulus of an abnormally low arterial pressure.

A second injection of 6 cc. adrenalin in the course of 20 minutes raised the arterial pressure to 164 mm. Hg. and caused the heart to become very irregular, *c*. With the completion of the injection the arterial pressure declined somewhat more slowly than after the first injection, but it finally got as low as 40 mm. Hg. The heart here, *d*, seemed to be failing; but about 20 minutes later, at *e*, the heart's action improved and the arterial pressure began to rise.

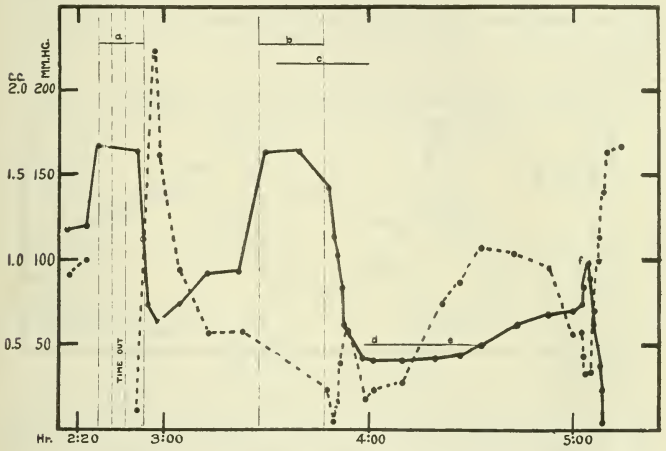

Fig. 1. Experiment 41. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -. *a*, injecting 6.0 cc. adrenalin, 1: 1000; *b*, injecting 6.0 cc. adrenalin, 1: 1000; *c*, heart irregular; *d*, heart seems to be failing; *e*, heart improving; *f*, trachea clamped until animal dies.

This injection, also, practically stopped the femoral inflow. And similarly, the inflow rate, after the termination of the injection, started almost at once to increase; but it soon decreased again to begin a slower ascent. The parts of the inflow curve between the termination of the second injection and about *5:00* may be regarded as picturing *a*, the recovery of the vasoconstrictor mechanism from the peripheral action of the adrenalin (1st ascent); *b*, the stimulation of the center by the low arterial pressure (1st descent); *c*, the giving way of the center under the influence of the low arterial pressure of 40 mm. Hg. (2nd ascent); and finally, *d*, the recovery of the center as the arterial pressure rises (2nd descent), the center here being stimulated by the low arterial pressure to maintain a high grade of constriction. As the animal seemed to be recovering from the second dose the experiment was terminated by clamping the trachea, at *f*. The preliminary as-

phyxial constriction followed by complete failure of the constrictor center is readily discernible in the chart.

In this experiment, therefore, the injection of adrenlin, 1: 1000, at the rate of about 0.04 cc. per second failed to produce shock. The circulation was, however, distinctly damaged by each of the injections, as evidenced by the low arterial pressure and the high tone of the center that finally developed. The experiment gives no clue to the cause of the disturbance, except to indicate that a part of the effect of the second dose probably was exerted through the heart.

Experiment 42 (fig. 2). The first injection (5 cc. of 1: 1000 adrenalin in 22 minutes, dog weighing 6 kilo) raises the arterial pressue from 107 to 140 mm. Hg. After the completion of the injection the arterial pressure falls in 12 minutes to 67 mm. Hg. and then rises more slowly to about 90 mm. Hg. where, practically, it remains.

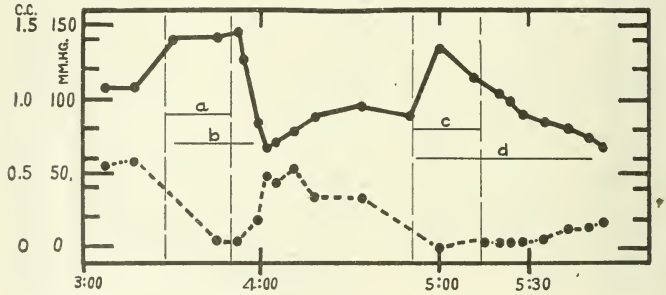

Fig. 2. Experiment 42. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, - - ●- - ●- -. *a*, injecting 5.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, injecting 7.5 cc. adrenalin, 1: 1000; *d*, heart irregular.

During and immediately after the injection, the inflow (femoral) is practically nil, but in 12 minutes it has returned almost to the initial level. Subsequently, however, the inflow rate falls somewhat. As in the preceding experiment, therefore, when the conditions become constant after the injection, the vasomotor tone is high, yet the arterial pressure is somewhat depressed. Evidently the circulation has been hurt; even under the influence of a vasomotor constriction the arterial pressure is subnormal.

The second dose consisted of 7.5 cc. of 1: 1000 adrenalin; it was given in 23 minutes. Although it constricts the arteries even more markedly than did the first dose, yet the arterial pressure does not rise so high; and despite continuation of the constriction, the arterial pressure falls during the terminal part of the injection as well as subsequently. At the time the experiment was terminated the inflow rate was increasing, but still was well below normal, and the arterial pressure was falling. The heart became irregular during the injection and remained so almost to the end. It is doubtful if the fall in pressure can be attributed to this effect of the adrenalin upon the heart, for when the heart eventually became regu-

lar the blood pressure did not improve. Presumably the process would have gone on to death if the experiment could have been continued longer.

In this experiment the cause of the low arterial pressure is not definitely determinable; it probably is not due to the effect upon the heart, and certainly not to peripheral dilatation. By exclusion, therefore, a diminution in blood volume seems to be the disturbing factor.

Experiment 43 (fig. 3). In this experiment the jugular and portal venous pressures were followed in addition to the arterial pressure and the femoral inflow. The dog weighed 5.8 kilo. The injection of 8 cc. of a 1: 1000 solution of adrenalin in the course of 28 minutes at first raised the arterial pressure from 100 to 140 mm. Hg., but the pressure soon fell to 122 and after some 21 minutes began to fall again and at such a rate that, by the completion of the injection, the arterial pressure was back to its initial level, and still was falling. It had fallen to 90 mm. Hg. when, without any apparent cause or warning, respiration stopped, *c*. Ar-

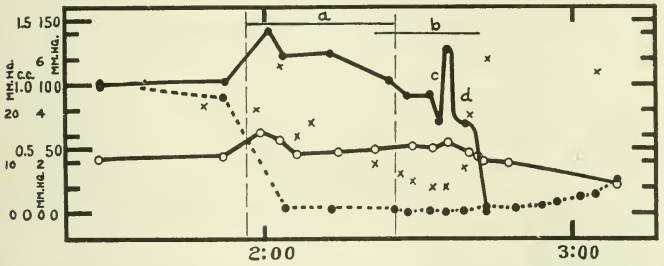

Fig. 3. Experiment 43. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○—; jugular pressure, x x x. *a*, injecting 8.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, respiration stopped, artificial respiration started; *d*, heart stopped.

tificial respiration, without ether, was immediately inaugurated. The arterial pressure abruptly fell 20 mm. Hg. but rose again, presumably under the influence of the artificial respiration and possibly also of a change in the character of the heart beat, to 127 mm. Hg. This pressure was maintained only as long as the changed heart beat lasted,—about 2 minutes; then the arterial pressure fell back to, indeed, somewhat below the level from which the sudden rise had started. Shortly thereafter, however, the heart very suddenly and unexpectedly stopped beating. We are of the opinion that in this case the sudden cessation of respiration and also the sudden stoppage of the heart were due to the direct and separate action of the adrenalin upon the medullary center and upon the heart. Whether this action was exerted directly upon the organs in question or indirectly through their blood vessels is a question that must be left open. This statement is made in full knowledge of the almost universal belief that adrenalin has but little action upon the blood vessels of these organs. We will have occasion to refer to this topic again.

The femoral inflow was practically stopped by the injection. It did not increase materially until the animal died, and it was still far below normal even 25 minutes after death. The fact that the inflow accelerated perceptibly at the moment the animal died may be taken to mean that during life the peripheral action of the adrenalin had been reinforced somewhat through central action. It is obvious, however, that in experiments of this character it matters little what the center may be doing,—for the vessels are maximally constricted by the direct action of the adrenalin.

The injection raised the portal pressure promptly from 10.5 to 15.5 mm. Hg., but in the course of 9 minutes it dropped back practically to the original level. It then slowly rose to 13 mm. Hg., fluctuated slightly at the time respiration stopped, and then fell after death, though no more rapidly than before.

Although these observations on the portal pressure cannot be interpreted in the absence of information with regard to the simultaneous behavior of the splanchnic, at least, if not also of the hepatic, resistance, they are none the less of some value in that they indicate that a high portal pressure is not the cause of the circulatory disturbance. The height to which the portal pressure is raised here is not sufficient, presumably, to cause a dangerous retention of blood in the portal area.

The jugular pressure, excepting one reading made shortly after beginning to give the adrenalin, falls progressively during and subsequently to the administration of the adrenalin. It mounts again, and quite rapidly, at the time respiration fails. This was probably caused by the pressure of the artificial respiration, and in part by the failure of the heart. It should be noted that the inception of the cardiac irregularity, *a*, is without effect upon the jugular pressure. This fact may be taken to mean that the arrhythmia did not reduce the effectiveness of the heart as a pump.

In this experiment, therefore, the fall in arterial pressure occurring between the termination of the injection and failure of the heart cannot be attributed to loss in arterial tone, for the arteries are markedly constricted (unless other areas do not behave as does the femoral area, and of this more later); and probably not to failure of the heart, for then the jugular pressure would have risen, as it did when the heart actually failed at the end; nor to accumulation of blood in the portal area, for the portal pressure is not high enough to hold out of the general circulation enough blood to dangerously reduce the volume of blood in circulation; nor to the accumulation of blood in the systemic veins, for the jugular pressure is not increased. We are then forced to ascribe the fall in arterial pressure to a reduction in the volume of blood returned to the heart.

Experiment 44 (fig. 4) gives a more prolonged picture of the after-effects of adrenalin injection. The same observations were made as in the preceding experiment. The injection of about 8 cc. of 1: 1000 adrenalin in the course of 21 minutes into a dog weighing 7.5 kilo, but exceedingly fat, raised the arterial pressure from 84–95 to 154 mm. Hg. If we neglect some variations that seem to be of no present significance, the arterial pressure then fell with a constantly diminishing rate until the end of the experiment approached. The animal died presumably through eventual, though gradual, failure of the respiration. With the failure of respiration and the consequent failure of the heart, the arterial pressure of course, fell more rapidly again.

The femoral inflow was practically stopped by the adrenalin. The peripheral vessels began to dilate appreciably only some 40 to 50 minutes after terminating the injection, and only an hour before respiration failed. At the moment of death the inflow rate was still far below the initial level. In this experiment, again, we see the effect of final failure of the center upon the tone of the peripheral vessels; the dilatation occurring at this time proves that the constriction is only partly due to the peripheral action of the adrenalin. The low arterial pressure presumably so stimulates the center as to cause it to maintain a higher grade of constriction than the peripheral action of the adrenalin alone is capable of maintaining. It can also be seen that the arteries are not completely dilated even 1 hour 20 minutes after death. In large part this slow port-mortem inflow rate is due to something transpiring in the main artery; for the inflow rate into the unclipped artery is only a bit faster than the inflow rate into the clipped artery. The first impression one gains upon encountering this phenomenon is that a clot has formed in the main artery. But this can not be the case, for with death there is always

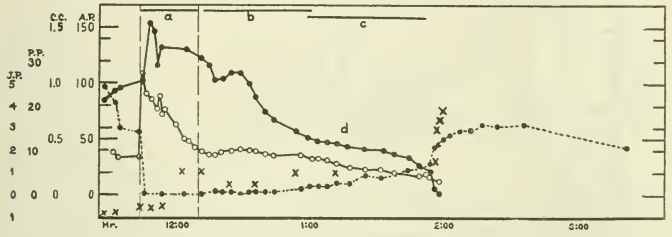

Fig. 4. Experiment 44. Effect of injecting adrenalin. Arterial pressure, —●—●—; portal pressure, —○—○—; femoral inflow, - - ●- - ●- - ; jugular pressure, x x x. *a*, injecting 8.0 cc. adrenalin, 1:1000; *b*, heart very irregular; *c*, respiration diminishing.

an acceleration similar to the one here seen. This would be impossible if the slowing were due to the presence of a clot.

The adrenalin promptly raises the portal pressure from 8.0 to 9.5 to the astounding height of 27.0 mm. Hg.; but the pressure immediately begins to fall and by the end of the injection period it has returned within the initial range where, to all intents and purposes, it remains for about an hour. But finally, and despite the gradual dilatation of the leg (and also, presumably, of the intestinal) arteries, the portal pressure falls well below its initial level.

The jugular pressure rises during the injection period from about −0.5 to 1.0 mm. Hg. where it remains with inconsequential fluctuations until the heart practically ceases to beat, when it rises to 3.5 mm. Hg. There is no obvious change in venous pressure in association with the beginning and the termination of the period of cardiac irregularity such as might be expected if the irregularity were associated with a change in the efficiency of the heart.

In this experiment, to summarize, the arterial pressure falls despite a strong peripheral constriction and despite a portal pressure, which, excepting only the

period of injection is normal or below normal. To the eye the mesenteric veins, in this case, seemed to be small. There is a slight rise in jugular pressure as a result of the adrenalin injection. This might be considered as the expression of an accumulation of blood in the systemic veins, due either to diminution in the efficiency of the heart or to a diminution in the capacity of the arteries. The heart was irregular for a time, but the record is without evidence that this irregularity interfered with the work of the heart. An insufficient supply of blood to the heart seems, therefore, to be the only factor remaining to account for the failure of the circulation. This animal was definitely in shock during the last 50 minutes of the experiment.

Experiment 46 (fig. 5). In this experiment the intestinal inflow was followed instead of the femoral inflow. The dog weighed 7.9 kilo. The first dose of adrenalin was 9 cc. of 1 : 1000 in the course of 28 minutes. It raised the arterial pres-

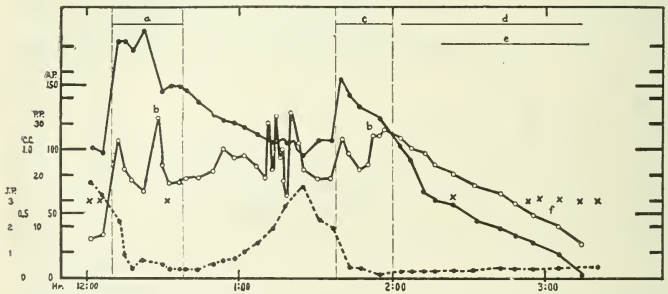

Fig. 5. Experiment 46. Effect of injecting adrenalin. Arterial pressure, —●—●—; portal pressure, —○—○—; intestinal inflow, - - ●- - ●- -; jugular pressure, x x x. *a*, injecting 9.0 cc. adrenalin, 1 : 1000; *b*, brief pause to refill syringe; *c*, injecting 11.0 cc. adrenalin, 1 : 1000; *d*, heart irregular; *e*, respiration slowing; *f*, starting artificial respiration.

sure from 100 to 180, indeed, to 190 mm. Hg. Before the completion of the injection the arterial pressure began to fall and, barring certain fluctuations which do not concern us here, it declined gradually to its initial level, when the second dose was administered.

The intesinal inflow rate was markedly decreased during the injection, but it did not fall quite to zero. It increased subsequently and became normal in the course of 47 minutes. But then, corresponding with a definite rise in the arterial pressure, the peripheral resistance again increased.

The portal pressure was markedly increased by the injection and it remained high, though irregular, subsequently. The jugular pressure did not fluctuate more than 0.5 mm. Hg. during the entire course of the experiment, not even when the heart was irregular and the respiratory rate slow.

At 1:10 a series of interesting, though perhaps irrelevant waves in arterial and portal pressure was registered. In the original record it can be seen that the

pressure in the splenic (portal) vein rises very abruptly at the instant the arterial pressure begins to fall. It is to be presumed, therefore, that the cause of this phase of each wave is a splanchnic dilatation.

The circulatory conditions were not quite normal when the second injection of adrenalin was begun. The arterial pressure, to be sure, was about normal, but this normal level was maintained apparently through over-activity of the vasomotor center; and the portal pressure (18 mm. Hg.) was considerably above the initial level. Inasmuch as the intestinal arterioles were somewhat constricted at this time, it follows that the high portal pressure must be attributed to a continued high portal resistance. The conditions with respect to the portal pressure following the first injection evidently were very different from those heretofore observed: they were favorable to the retention of blood in the abdominal viscera. Yet the circulation showed no definite evidences of failing.

The second injection of adrenalin (11 cc. of 1:1000) now raised the arterial pressure to 152 mm. Hg.: but then, despite the continuance of the injection, the pressure steadily fell and the heart stopped about 1½ hours after terminating the injection. The intestinal inflow dropped practically to zero and showed very little tendency to increase even after the animal died. By the test described in another place (3) it was found, shortly after the completion of the second injection, that the slowing of the inflow rate was due to constriction of the main inflow artery. The portal pressure rose irregularly during the injection period and then fell steadily, crossing the pre-injection level after about 20 minutes had elapsed. The fall in the portal pressure undoubtedly was due to the coincident fall in the arterial pressure.

The possibility that the fall in the arterial pressure in experiments of this character is due to dilatation in the splanchnic area while the somatic (femoral) area is constricting, finds no support in the results of this experiment. Furthermore, the failure of the jugular pressure to change materially seems to exclude an accumulation of blood in the systemic veins through functional incapacity of the heart as a factor in the failure of the circulation. This is the first experiment in which the portal pressure has remained at a high level during most of the injection and post-injection periods. Therefore, in this case the possibility that the failure of the circulation is due to accumulation of blood in the portal area cannot be excluded, though, as has been said, the portal pressure after the first injection remained high and yet the circulation was satisfactorily maintained. It is recorded in the notes that the mesenteric veins were distended.

Experiment 48 (*fig. 6*). The intestinal inflow was followed in this experiment also. The animal weighed not over 7 kilo and received a large dose of 1:1000 adrenalin (about 11 cc. in the course of 21 minutes). The arterial pressure was elevated at once from 100 to 170 mm. Hg.; it then immediately began to fall. With the exception of two irregularities this fall, upon the whole, was steady, and in the course of 2½ hours carried the arterial pressure down to 80 mm. Hg. Of the two irregularities in the pressure fall, the first was a fall and rise in association with a very decided temporary slowing of the respiration, *c*. We believe these two events were causally related and are indicative of some transitory central change. It will be noted that at this time, judging by the inflow rate, the adrenalin action was at it height. The second change consisted of an abrupt rise in the arterial pressure which undoubtedly is to be attributed to the disappearance of the heart

irregularity, *b*, which had persisted almost from the very beginning of the injection.

The inflow rate decreased during the injection, reaching zero with the first estimation after the cessation of the injection. It then gradually increased, but even 2 hours later it was still decidedly below normal. The portal pressure at first rose from 5 to 18 mm. Hg., fell somewhat, and then rose to attain a second crest about 70 minutes after beginning the injection. It then fell more or less steadily. The form of this part of the portal curve obviously is influenced by the arterial pressure. The fall in arterial pressure is not, however, the sole cause of the fall in portal pressure; for while the arterial pressure falls from 107 to 80 mm., a ratio of 1.3 to 1, the portal pressue falls from 16 to 5 mm., a ratio of 3.2 to 1. In other words, the portal pressure falls faster relatively than the arterial pressure, and this more rapid fall in portal pressure occurs despite a progressive dimi-

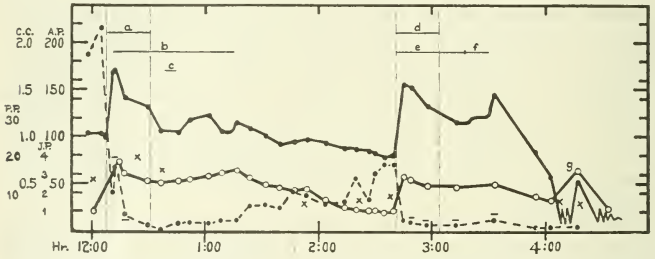

Fig. 6. Experiment 48. Effect of injecting adrenalin. Arterial pressure —●—●—; portal pressure, —○—○—; jugular pressure, x x x; intestinal inflow, - - ●- - ●-- (for the readings marked by the sign (—) the inflow pressure was on a much higher than the usual level). *a*, injecting 11.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, respiration slow; *d*, injecting 11.0 cc. adrenalin, 1: 1000; *e*, heart irregular; *f*, change in type of heart irregularity; heart regular subsequently; *g*, artificial respiration; the heart at this time was beating periodically.

nution in the splanchnic resistance which ordinarily would tend to raise the portal pressure. To be in a position to interpret these changes in the portal pressure it would be necessary to know also the state of the portal resistance in the liver. Since this information is not at hand a decision with regard to the cause of the more rapid fall in the portal pressure cannot be reached.

The continuous fall in arterial pressure probably is not due to increasing incapacity of the heart. The jugular pressure to be sure is raised somewhat (1 mm.) during the administration of the adrenalin, but later it falls below the initial level; and though the heart beat under the influence of the adrenalin becomes irregular, this irregularity, *b*, does not persist.

A second dose of adrenalin, similar to the first, was now given. At this time the circulation had by no means returned to its initial state. The arterial pressure, despite a relatively high peripheral resistance, was at least 20 mm. Hg. below normal. Whether conditions would in time have become normal is a ques-

tion that cannot be answered. The portal pressure, though, had returned to its initial level. As a result of the injection the arterial pressure rose to 156 mm. Hg. and the heart became irregular. The arterial pressure then fell steadily until, 40 minutes later, a change in the character of the heart irregularity, *f*, apparently caused the pressure to rise slightly; another and greater rise occurred 10 minutes later when the heart beat became regular. The fall in arterial pressure was then resumed, a decrease from the level of 144 to 57 mm. Hg. taking place in the course of 30 minutes. The heart then exhibited a peculiar type of irregularity, the nature of which does not concern us here.

The intestinal inflow rate was cut down by the injection almost to zero, and had not begun to increase even 90 minutes later, when the heart became irregular.

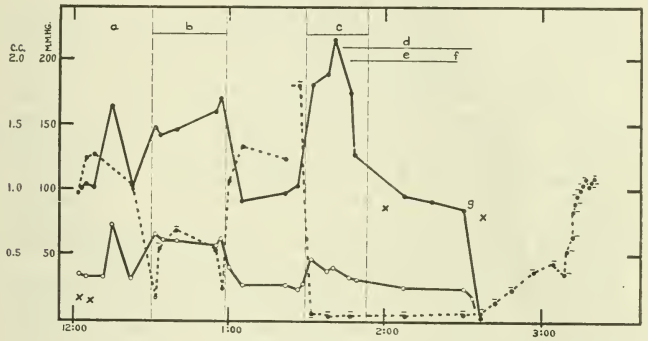

Fig. 7. Experiment 49. Effect of injecting adrenalin, small and large doses. Arterial pressure, — ●— ●—; portal pressure, — ○— ○—; jugular pressure, x x x; intestinal inflow, - - ●- - ●- - (for the readings marked by the sign (—) the inflow pressure was on a much higher than the usual level). *a*, adrenalin injection started and discontinued; *b*, injecting 11.0 cc. adrenalin, 1:10,000; *c*, injecting 11.0 cc. adrenalin, 1:1000; *d*, heart irregular; *e*, respiration markedly slowed, stopped at *f*; *g*, artificial respiration started.

The portal pressure was permanently elevated by the injection; it showed some slight fluctuations which were always of the same sign as the large fluctuations in the arterial pressure. Here, therefore, the arterial pressure falls despite a high peripheral resistance and despite the absence of any evidence of cardiac failure. The portal pressure, however, remained high during most of the experiment. Therefore, the possibility that the fall in arterial pressure is due to the accumulation of blood in the splanchnic veins cannot in this case be excluded.

Experiment 49 (*fig. 7*). This experiment was performed primarily for the purpose of comparing the effects of small with the effects of the large doses of adrenalin given in previous experiments. The first attempt to inject 1:10,000 adrenalin, *a*, had to be discontinued on account of an accident. Then, after the circulation had returned to its previous state, 11 cc. of 1:10,000 adrenalin were given

over a period of 28 minutes, *b*. During the injection period the arterial and portal pressures, as usual, were raised, the intestinal inflow rate slowed. Though this is also the result commonly obtained with the larger doses there are, nevertheless, certain outstanding differences. These are,—the steady rise in arterial pressure, the absence of any tendency on the part of the portal pressure to fall after the preliminary rise, and the incompleteness of the peripheral constriction. In the period subsequent to the injection, the striking differences are,—the immediate relaxation of the arterioles beyond their initial size, with a return to the normal inflow rate in the course of 20 minutes; the immediate descent of the arterial pressure to a subnormal level with a relatively rapid recovery to the normal; and the almost immediate return of the portal pressure to the normal level. The circulation became normal in every respect.

The second injection consisted of 11 cc. of 1: 1000 adrenalin; it was given in the course of 24 minutes, *c;* this dose, therefore, was about ten times as large as the first dose. The arterial pressure rose to the unusual height of 216 mm. Hg., but, as usual, began to decline before the injection was completed, and at about the time, *d*, the heart became permanently irregular. Slightly later, *e*, the respirations became very slow and this slowing increased until the breathing stopped altogether, *f*. The heart stopped after the respiration, and despite the inauguration of artificial respiration, *g*, before the heart had actually stopped. By the time the respiration stopped the arterial pressure had fallen to 84 mm. Hg.

The inflow rate fell practically to zero when the injection of adrenalin began, aud it remained there until the animal died. Then some relaxation occurred, indicating that the previous constriction, though mostly due to the peripheral action of the adrenalin, had also been maintained in part by central action.

The portal pressure was instantly increased by the adrenalin but immediately began to decline, while showing some fluctuations in correspondence with those of the arterial pressure. It was subnormal during a large part of the period. The jugular pressure was markedly increased; for this it is possible that both the altered respiration and heart action were responsible.

It can scarcely be maintained that this animal was brought into a state of shock; but it is of significance that at the end the arterial pressure was somewhat subnormal despite an intense arterial constriction. Death was due to some action of the adrenalin, direct or indirect, upon the respiratory center.

Summary and discussion of experimental data

Now, with a variety of results before us, an attempt will be made to ascertain the usual reactions to the injection of adrenalin and the significance of those reactions in relation to the shock problem.

1. Action on the arterial pressure. The arterial pressure *during the injection* period invariably is high, and higher during a first than during a second injection. When the dose is relatively small the arterial pressure usually is consistently high (figs. 2, *a* and 7, *b*); but when the doses are large the pressure usually begins to fall before the end of this period (figs. 4, *a*; 5, *a*; 6, *a*). One gains the impression that the larger the dose

the earlier and the more profound is this fall in pressure apt to be. It always is more marked after a second than after the first dose. The decline during the injection period, though, usually falls short of carrying the arterial pressure back to its initial level. These differences in the effect of dosage on the arterial pressure may depend in part upon differences in the action of the adrenalin upon the heart; but that the fall in pressure is not wholly dependent upon the behavior of the heart is indicated by the fact that it may begin before the irregularity (fig. 4) and may not occur when the heart becomes irregular (fig. 2, *b*). A second large dose never raises the arterial pressure as high as did the first dose, provided it also was large.

After the administration of a small (fig. 7, *b*) or of a moderate dose (fig. 1, *a*) the arterial pressure usually falls rather abruptly to a subinitial level, and then mounts slowly, eventually reaching normal, after small doses (fig. 7, *b*), but usually not regaining the normal level after moderate doses (figs. 1, *a*; 2, *a*). A second dose that produces approximately the same immediate reaction, leaves the arterial pressure still lower (fig. 1, *c*). After a large first dose, barring accidental occurrences, the fall in arterial pressure begun during the injection period continues without any break to mark the conclusion of the injection (figs. 4, 5, 6), it may be, until the animal dies (fig. 4); though this is more commonly seen after a second large dose (figs. 2, 5, 6). A decline of this character that does not kill, tends to terminate below the initial level (figs. 5, *a*; 6, *a*). So far as can be judged by the arterial pressure, therefore, large doses of adrenalin do long-lasting damage to the circulation, if they do not actually carry the pressure down to a dangerously low level.

2. Action on the peripheral resistance. Without exception the response to adrenalin during the injection period has been a marked peripheral constriction causing, in the case of the larger doses, practically a complete cessation of inflow (figs. 2, 3, 4, 5, 6, 7).

After terminating the injection of a small dose (fig. 7, *b*) the resistance quite promptly decreases to, or slightly below, normal; after a moderate dose (figs. 1, *b*; 2, *a*) the relaxation that occurs is apt to be slower and short of complete return to normal; while after large doses, and often after moderate doses given a second time, the relaxation is very slow and usually very far from complete even after two hours or more have elapsed (figs. 4; 5, *c*; 6, *d*). This long-lasting constriction, however, usually is not wholly due to direct action of the adrenalin upon the blood vessels; a part of the tone is central in origin as is evidenced by the acceleration of dilatation that usually occurs at the moment

the animal dies (figs. 3, 4, 7). The splanchnic and the femoral areas are alike in their response to these doses of adrenalin. In one experiment (fig. 1, *a*) the constriction of the period of injection gave way very rapidly to an extreme dilatation, followed at once by a gradually developing constriction which probably was central (compensatory) in origin.

The literature on the action of adrenalin on the blood vessels has recently been reviewed by Hartman (4). There the statement occurs that "dilatation in the blood vessels of skeletal muscles is the usual response to adrenalin in animals." Dilatation is also said to occur in the intestinal vessels, especially after large doses (5), (6). Our findings do not agree with these statements. To be sure, the method we have employed in following the peripheral resistance furnishes discontinuous information; and if very brief periods of dilatation occur during the continuous injection of adrenalin there is the possibility that our method might fail to detect them. The fact, however, that we have never encountered dilatation *during* the injection of adrenalin certainly renders this possibility a very remote one, indeed. In the post-injection period, as has been said, we have encountered striking and momentary dilatation, but only once (fig. 1, *a*); and a very slight transitory dilatation, also, only once (fig. 7, *b*). With these exceptions, the constriction produced by both moderate and large doses has always outlasted the injection period, in some of the instances as long as 2 hours.

Hoskins, Gunning and Berry (7) found that adrenalin in "all" doses constricts the skin vessels but not those of the muscle. Later, however, Gunning found (8) that massive doses of adrenalin slow the blood flow through muscle. This correction suggests that all of the discrepancies depend merely upon dosage and method of administration. However this may be, it is certain that the constriction we have observed is so marked that it cannot, in the case of the femoral readings, be accounted for on the basis of a constriction affecting the skin vessels alone.

Gruber (9) fails to obtain dilatation of the blood vessels of muscle shortly after section of the nerve; neither, apparently, does he, under such circumstances, obtain constriction. Even, therefore, if it were admitted for purposes of argument that we get constriction because the nerves have been injured (and such experiments as 41 [fig. 1, 2nd injection] conclusively show that constriction is obtained when the nerves unquestionably are active), the reduction in inflow rate that we obtain is far too great to be referable to constriction of the skin vessels alone.

But whatever the cause of the discrepancy, we can assert *a*, that our method of determining the effect of the adrenalin upon the peripheral resistance is free of the ambiguities that attach to the outflow and plethysmograph methods employed by other investigators; *b*, that our method demonstrates that in the dosages we have employed constriction of both the somatic and the splanchnic areas is the main if not the only effect of the continuous injection of adrenalin; and *c*, that this constriction often persists for hours after the dose has been administered, partly, perhaps, as a central compensatory response to the low arterial pressure, or to a tendency to a low arterial pressure.

Our method of following the vasomotor tone does not help to dissociate the factors, central and peripheral, upon which the constriction depends. There is, of course, no doubt but that during the injection of the adrenalin any part the center may be playing in maintaining the constriction must be insignificant in comparison with that effected by local action. That the center, however, is a factor in maintaining the constriction subsequent to the injection is indicated by the acceleration of the recession of the constriction that often occurs at the moment the animal dies. But even with death the dilatation is relatively slight. This failure of the injection rate to return to, and then to pass beyond, the initial value probably is a manifestation of the phenomenon that has already been described as occurring at the termination of long experiments in which no adrenalin whatever has been employed (3), and which we felt might be related to the phenomenon of post-mortem contraction described by MacWilliam (10). In the present experiments, however, it is possible that the long-lasting constriction is due to the direct action of the adrenalin upon the walls of the large arteries (11).

3. The systemic venous pressure. Our method of measuring the venous pressure gives, to all intents and purposes, the pressure in the right auricle. Pressure changes here are of course slight at best, and are affected not alone by the force from behind and by the capacity of the heart to empty itself, but also by variations in the effectiveness of the respiratory pump. It is not surprising, therefore, that the literature on this subject is contradictory. Thus Bainbridge and Trevan (12), for example, find that the pressure in the vena cava is not altered by the injection of adrenalin; whereas Schmid (13), Elliott (11) and Edmunds (15) say it is elevated.

Our results in this respect have been quite inconstant. In one experiment (fig. 5), the jugular pressure changed but little; in two (figs. 3, 6) it fell; and in two instances (figs. 4, 7) it rose. In both of the last,

though, there were two possible and perhaps unusual causes for the rise. One cause was a change in the character of the respirations: in one case (fig. 4) this change was slight and the venous pressure was elevated but little; in the other (fig. 7), the respiratory change was very marked and the rise in venous pressure also was marked. The other possible cause of the rise in venous pressure is related to the effect the adrenalin had on the portal pressure. For these two experiments were exceptional in that after the injection the portal pressure (see below) quickly resumed its normal level. The marked constriction of the arterioles caused by the adrenalin and the relative emptiness of the portal veins that a normal pressure in them bespeaks, together might have had the effect of distending the systemic veins. But whatever the cause of the inconstancy of the jugular pressure may be, it is obvious that it permits us to conclude only that the heart as a rule is capable of moving on the blood that is brought to it.

Action on the heart. In many of the experiments there were indications of deleterious action of the adrenalin upon the heart, the heart developing a very marked irregularity either while the adrenalin was being injected or subsequently. This irregularity was not of the kind that is produced by vagal action. The arterial pressure at these times occasionally (figs. 1, *d, e;* and 6, *b*) gave evidence that the heart was not as efficient as when it was beating regularly; at other times, however, the inception or the disappearance of the irregularity was without obvious effect upon the arterial pressure. Furthermore, with but one possible exception (fig. 7, 2nd dose), the jugular pressure failed to exhibit with the inception and disappearance of this irregularity, the alterations that are taken to indicate diminishing or increasing capacity of the heart for work. No effort has been made to ascertain the nature of the irregularity. But whatever it may be, it seems justifiable to conclude that failure of the heart is not an important factor in the *gradual* failure of the circulation after the administration of adrenalin. The *sudden* stoppage of the heart that occasionally has occurred while the arterial pressure still was sufficient (figs. 3 and 7) may have been caused by some direct action of the adrenalin upon the heart. But this is a question that does not concern us here.

Action on respiration. The adrenalin in almost every instance has had a marked effect upon the respiration. This has consisted *a,* of transitory slowing (fig. 6, *c*); *b,* of progressive slowing of the respiratory rate (figs. 4, *c;* 5, *e*), sometimes leading to surprisingly low rates (6 per minute in fig. 7); or *c,* of stoppage of respiration, sometimes sudden (fig.

3, *c*), sometimes preceded by slowing (fig. 5, *f*). The administration of artificial respiration in the latter instances at most has delayed death only a few minutes (figs. 3, 5). Therefore, lack of pulmonary ventilation can scarcely be regarded as the cause of death or of the breakdown of the circulation. It is not the purpose of this paper to discuss the cause of failure of the respiratory center.

The portal circulation. Except, perhaps, its action on the peripheral resistance, the most interesting effects of adrenalin are those it exerts upon the portal circulation. The first effect of the injection of adrenalin invariably is to raise the portal pressure, it may be as high as 28 (fig. 5) or 30 (fig. 6) mm. Hg., though usually to not over 15 mm. Hg. The portal pressure then usually falls practically to the initial level *a*, either during the injection (figs. 3 and 7, 2nd dose), or *b*, immediately (figs. 4 and 7, 1st dose) or some time after (fig. 6); and *c*, while the arterial pressure is still well maintained (fig. 6, 1st dose) or *d*, not until the arterial pressure falls to quite a low level (fig. 5, 2nd dose). In all of these cases there is a certain, although by no means complete, parallelism between the portal pressure and the arterial pressure. In one case (fig. 5, 1st dose) the portal pressure remained high even after the arterial pressure had returned to the initial level.

The very marked effects that these doses of adrenalin have upon the portal circulation suggest the advisability of carefully examining them in order to determine whether they are of significance in relation to the failure of the circulation. Physiologically, a continuous elevation of the portal pressure can be effected *a*, through a rise in arterial pressure; *b*, through a diminution in arterial resistance in the portal area; *c*, through a rise in caval pressure; *d*, through contraction of the musculature of the portal veins themselves; and *e*, through an increase in the resistance to flow through the liver.

a and b. The injection of adrenalin, of course, raises the arterial pressure but at the same time markedly increases the resistance in the arterioles. Whether or not the blood flow into the portal area would increase, therefore, would depend upon the balance that is struck between these two effects. Our inflow determinations show conclusively that the constriction, in the intestinal area, at least, is so extreme that despite the rise in arterial pressure the adrenalin must restrict the blood flow. It is, of course, conceivable that the other pathways into the portal system are not constricted by the adrenalin to the same extent as is the intestinal pathway and that it is through the former that the portal pressure is raised. We have no direct observations bearing on this possibility.

The measurements of the blood flow in the portal veins as effected by adrenalin that are recorded in the literature help to settle this question, though the effects of brief injections only have been studied. With large doses Schmid (13) invariably obtained a marked reduction in blood flow in the portal vein; in one of his instances the blood flow stopped completely for a few seconds. On the other hand, Burton-Opitz (14) finds an increase in portal flow during the phase of high arterial pressure. This increase, though, was never very striking, and evidently very inconstant, as may be indicated by the following values taken from his published data:

EXPERIMENT NO.	BLOOD FLOW, CUBIC CENTIMETERS PER SECOND	
	Before	At crest of arterial pressure
3	4.50	4.54
4	3.05	3.20
5	3.43	3.37
	3.25	2.56

Upon the whole, it seems fair to conclude that the blood flow through the portal area is not increased during the prolonged administration of adrenalin. Granting this, it follows that adrenalin constricts the whole of the splanchnic bed. The rise in portal pressure, therefore, is referable neither to a premised splanchnic dilatation nor to the rise in arterial pressure; though, of course, during the period of constriction, variations in arterial pressure must effect variations of the same sign in the portal pressure.

c. It has already been pointed out that the systemic venous pressure ordinarily is not increased by the prolonged injection of adrenalin; therefore we must seek elsewhere for the cause of the high portal pressure.

d. Edmunds (15) has observed that strips of the portal vein contract when immersed in a solution of adrenalin. Occasionally we have observed during the height of the adrenalin action, a sausage-like appearance of the branches of the portal vein, rings of constriction so complete as to quite obliterate the lumen and to offer an appreciable resistance to the passage of the blood, alternating with segments filled with blood. If this were constant in occurrence it might help to explain the high pressure recorded in the splenic vein; but in many instances these evidences of contraction of the veins are absent.

e. If constriction of the large portal radicles is not the cause of the high portal pressure there remains as the only possible explanation of the condition an increase in the resistance to the passage of blood through the liver. This question has recently been discussed by Bainbridge and Trevan (12). The possibilities in the case are: *a*, constriction of the minute portal radicles in the liver; *b*, constriction of the hepatic or sublobular veins; and *c*, narrowing of the hepatic capillaries. Bainbridge and Trevan are disposed "to regard narrowing of the capillary channels by swelling of the liver cells as the most probable explanation of the obstruction produced by adrenalin to the blood flow through the liver." The evidence they offer in favor of this view is that adrenalin causes the liver volume to increase, while histological examination of clamped-off pieces of liver shows the interlobular and intralobular veins to be engorged with blood.

Our own experiments throw relatively little light on the condition of the blood vessels of the liver. We have not examined the liver during the height of the adrenalin action. The gross appearance presented by the liver at the close of experiments has been rather variable. Occasionally all of the marks of engorgement are evident; more often its edges have been sharp, though in such instances the parenchyma on section often seemed to be bloody. In one case we weighed the liver and found it to be at the lower limit of the normal range. Microscopical examination has been made in only one case and that was one in which the liver at autopsy was found to be engorged. In this instance the hepatic capillaries, upon the whole, were dilated and contained an excess of blood. The corpuscles did not seem to be so closely packed as they are in the intestinal vessels of shocked animals. The liver cells were not swollen.

It is inconceivable, indeed, that the liver cells could swell so rapidly under the influence of adrenalin as to cause the practically instantaneous increase in liver volume and portal pressure which are pictured by Bainbridge and Trevan, the more so when it is recalled that even a comparatively small dose of adrenalin may cause a marked increase in portal pressure (see fig. 7, *a* and *b*). As a matter of fact, we are not convinced that the method employed by Bainbridge and Trevan for the purpose of following the volume of the liver is entirely satisfactory. For an increase in arterial pressure, by increasing the turgidity of the liver lobes, might, depending upon conditions, increase the pressure upon the balloon between the lobes. However this may be, the complete parallelism of their "volume" and pressure curves to each other

make it incumbent upon them to prove that they are actually recording volume changes. Better methods (15) seem to indicate that in the dog, at least, the liver volume is reduced by small, brief intravenous injections of adrenalin. If it could be shown that the engorgement of the liver that sometimes occurs after the administration of adrenalin is slow in development, another possible cause of the swelling of the liver cells and of the engorgement of the liver capillaries observed by Bainbridge and Trevan would be the effect upon the tissues of the marked slowing of the blood stream that accompanies the prolonged injection of adrenalin. The process would then be similar to the one which in our opinion accounts for the engorgement of the intestinal capillaries.

Assuming, for purposes of discussion, that the portal action of the adrenalin is the cause of the shock-like failure of the circulation, let us try to discover the mechanisms that might bring it about.

1. When the portal pressure is high, such large amounts of blood might be trapped in the portal area as to lead to serious depletion of the systemic vessels. It is here that the exact location of the increased resistance in the liver might be of some significance. For if the obstruction were beyond the capillaries a very much larger volume of blood would be trapped in the portal area than if the obstruction were in the portal venules. Bainbridge and Trevan (12) have suggested that the distention of the liver and portal area might be a contributory factor. But in certain cases, at least, the trapping of blood cannot be sufficient to be the sole cause of circulatory failure, for the rise in portal pressure may be transitory even in cases that die (fig. 4). Furthermore, the liver, at least at the time of death, as has been said, often does not contain more than its normal quota of blood. Nor are the tributary veins of the portal area always distended during the injection; often they seem relatively empty, though the pressure in them may be high. And finally, experiments to be described later show that a mere rise in portal pressure even when maintained for hours does not embarrass the circulation nearly to the same extent as does the administration of adrenalin.

2. A high portal pressure might reduce the blood volume by increasing filtration of plasma. As a matter of fact, Bainbridge and Trevan find that during the prolonged injection of adrenalin the lymph flow through the thoracic duct is increased. There can be no doubt but that the administration of adrenalin increases filtration pressure in the splanchnic capillaries; therefore, a part of the increase in lymph forma-

tion may be accounted for here. But Bainbridge and Trevan, finding that the lymph "becomes slightly more concentrated," conclude that a part of it comes from the liver. This observation they take as further evidence in support of their contention that the portal obstruction is in or beyond the liver capillaries. There is, however, another possible explanation of the increase in concentration of the lymph, and that is that the extreme and prolonged slowing of the blood stream so damages the hepatic capillary walls (as well as those of other organs) that they become more permeable to the plasma. Again, if we admit for the moment that the portal obstruction is in or beyond the portal capillaries, the filtration would be most rapid at the time the portal pressure is highest, which, as a rule, is during the early stages of the injection. The figures of Bainbridge and Trevan show, however, that the lymph flow steadily increases as long as the injection of adrenalin continues. This observation tends to confirm our view that the part of the lymph that increased concentration indicates is formed in the liver, passes out of the blood stream, not as a result of increased filtration pressure, but rather as a result of increased permeability of the capillary walls.

But after all, those portions of the blood fluids that reach the thoracic duct play no part in the processes that lead to failure of the circulation, for they soon are returned to the blood stream. Only those portions that are retained in the tissues or in the body cavities are of significance in this connection. We have seen no indications of edema of the organs, though during the injection of adrenalin beads of fluid have been seen to form on the surface of the liver. At autopsy the peritoneum occasionally seems to contain a slight excess of fluid.

3. The other striking effect the injection of adrenalin has upon the portal circulation is to slow the blood stream. Here attention should be called to the magnitude of this slowing. The inflow determinations show that even salt solution under a pressure considerably higher than the normal arterial pressure may scarcely flow through either the splanchnic or somatic capillaries during the height of adrenalin action. It is obvious, therefore, that when blood takes the place of salt solution and when two areas of increased resistance (splanchnic and portal) replace the one the inflow has to contend with there can be but little blood flowing through the splanchnic (or the somatic) area.

There are, therefore, good reasons, for believing that failure of the circulation after the administration of adrenalin is the consequence, mainly, of changes wrought in the tissues by asphyxia. In favor of this view is the fact that death has occurred whenever the constriction

has been extreme and long-lasting, and that recovery has occurred whenever the dose of adrenalin failed to maintain a high grade of constriction. Furthermore, the slowing of the circulation in our cases has lasted sufficiently long to lead to failure of the circulation through the same mechanism which, we believe, operates to cause the circulation to fail when the vena cava or the aorta is partially occluded (16). There is, therefore, no need of going outside of this mechanism to find an explanation of the failure of the circulation that follows the long-continued administration of adrenalin, though, of course, such trapping of blood and such filtration of plasma as may occur will contribute to that end.

Pathology. This conclusion is supported by the autopsy findings, for the most striking lesion found is the same marked dilatation of the capillaries and venules of the villi of the intestines that was found by us in shock as produced by exposure of the abdominal viscera, and by partial occlusion of the vena cava or of the aorta. To be sure, the circulatory disturbance resulting from the injection of adrenalin is just the one that would tend to produce this picture, for the high pressure developed in the portal area, at least early in the period of injection, must have the effect of increasing the pressure in the splanchnic capillaries, notwithstanding the sharp fall in pressure that must occur in the constricted arterioles. But that this probably is not the mechanism by which the dilatation is effected, but rather the slowing of the blood flow, is the conclusion that is justified by the results described in the preceding paper of this series (16). In the gross, as well as microscopically, the intestines, stomach and spleen present very much the same appearance as they do in shock as induced by the other methods we have employed.

Does adrenalin produce shock?

Having described and discussed certain of the manifestations of large and long-continued doses of adrenalin the question now arises—are there any reasons for regarding as shock the condition thus induced? If we exclude those cases that die suddenly as a result of stoppage of the respiration or of the heart, we think it can be asserted that at least the type of circulatory failure that is illustrated by experiment 44 (fig. 4), of which we have several instances, in which the pressure gradually falls until the animal dies, bears a close resemblance to the failure of the circulation resulting, for instance, from exposure of the intestines. The fact, furthermore, that animals in this condition may not require an anesthetic, although the eye reflex is still present, if this sign is of any sig-

nificance, lends additional support to the position first taken by Bainbridge and Trevan that shock can be induced by adrenalin. We do not agree with Henderson and collaborators in their conclusion (17) that adrenalin causes death *only* through acute cardiac dilatation or pulmonary edema, though it must be admitted that such accidents (at least the former) often bring the experiment to a close before shock-like failure of the circulation has had a chance to develop.

THE EFFECTS OF OBSTRUCTING THE PORTAL RADICLES IN THE LIVER

Accumulation of blood in the portal area forms the basis of a number of the hypotheses of shock. It is in this way that shock is supposed to result from the splanchnic dilatation of vasomotor paralysis or inhibition; from temporary partial occlusion of the inferior vena cava (18); from constriction or obstruction of the portal hepatic capillaries (12); and from constriction of the latter region and also of the splanchnic capillaries (19). In many of the experiments we have described, a rise in portal pressure, slight and transitory (cava occlusion) or marked and sometimes long-lasting (adrenalin injection), has been a possible factor in the failure of the circulation.

In an effort to reproduce as closely as possible the conditions premised by the hypotheses that attribute shock to an increase in portal resistance, we have had recourse to a method of obstructing the flow of blood through the smaller vessels that has long been practised in physiology, namely, by injecting into the stream of blood flowing toward an organ a suspension of lycopodium spores. These spores have a rough surface and about three to four times the diameter of the red blood corpuscles, and plug the vessels proximal to the point where they break up into capillaries. The increased resistance is thus placed in the situation in which constriction normally occurs, though, if Bainbridge and Trevan are right in their view (and we do not, as has been seen, regard their evidence as conclusive) that the obstruction occurs in the capillaries of the liver, the amount of blood that the injected spores trap in the portal area will not be quite so great as would be held by complete closure of the capillaries.

Method

The suspension of lycopodium spores has been injected into the gastrolienic vein in both the dog and the rabbit. The carotid pressure has been followed in all experiments, and in some of the experiments on the dog the femoral or intestinal inflow rate, the portal pressure and the jugular pressure, also.

Experimental

We will describe and discuss our experiments not in the order in which they were done, nor as entities, but in the way that facilitates the development of the subject.

In *experiment 53* (*fig. 8*), the first, and a relatively small, dose of lycopodium, *a*, raised the portal pressure from the normal of 8 mm. Hg. to a plateau which, in the course of 70 minutes, declined from 12 to 11 mm. Hg. The vasomotor tone was immediately increased and the increase continued for about 25 minutes when,

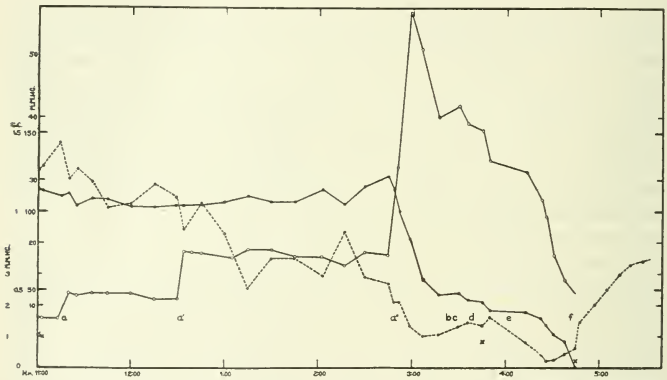

Fig. 8. Experiment 53. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○—; jugular pressure, x x x. *a*,*a'*, *a''*, lycopodium injected; *b*, accidental injection of carbonate; *c*, artificial respiration; *d*, artificial respiration stopped; *e*, inflow readings transferred to other femoral; *f*, heart stopped.

upon the whole, it decreased slightly but did not return to the normal level. The arterial pressure was affected but slightly, falling from 110 to 102 mm. Hg. Evidently the vasoconstriction sufficed to almost compensate such altered distribution of the blood as was determined by this dose of lycopodium.

Then another dose was administered, *a'*. This raised the portal pressure from 11 to 19 mm. Hg. and, barring certain fluctuations, evidently attributable to passing fluctuations in vasomotor tone, this level was maintained for 2 hours. The inflow rate again was decreased. The general trend of the inflow rate during this period was downward, and of the arterial pressure upward. Evidently at the end of the period vasoconstriction perfectly compensated even this degree of portal obstruction.

We may for the present pass by the last part of this experiment and turn to another experiment (no. 60, fig. 9) in which to all intents and purposes a single, moderately large dose of lycopodium was given. As a matter of fact the lycopodium was given repeatedly in small doses until the desired grade of obstruction was obtained. The immediate effect upon the portal pressure was a tremendous rise from the normal of 6 to 8 mm. Hg., to 41 mm. Hg. But in the course of the next 40 minutes the pressure steadily fell almost to 15 mm. Hg. and, excepting temporary fluctuations, again undoubtedly due to temporary fluctuations in the vasomotor tone, the portal pressure did not vary more than 1 mm. above or below 16 mm. Hg. during the course of the next 3 hours 40 minutes, when the experiment was terminated by stabbing the ventricles *b*. In this experiment the vasomotor tone was followed in the intestines as well as in the leg. We call attention to the fact that the general trend of the tone in these two regions was toward parallelism. The parallelism to be sure was not perfect; but when divergences occurred they were not any larger than one would expect considering that the comparable readings were not made simultaneously. Bearing this in mind, it is seen that

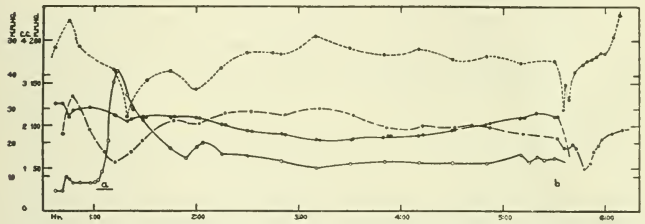

Fig. 9. Experiment 60. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, · · ● · · ● · ·; intestinal inflow, - - ●- - ●- - ; portal pressure, —○— ○—. *a*, injecting lycopodium; *b*, heart punctured.

simultaneously with the rise in portal pressure the peripheral resistance increased and the arterial pressure fell, though not more than 15 mm. Hg. During the rapid decline in portal pressure the vasomotor tone diminished but the arterial pressure, after recovering somewhat, remained quite stationary.

We pause here to discuss the mechanism of these occurrences. It is important to ascertain first why the portal pressure steadily falls after the first rise. This fall cannot be attributed to splanchnic constriction because, during this period, the intestinal arterioles are dilating; nor can it be attributed to a change in arterial pressure, for this is rising slightly or is stationary. Neither can it be explained upon the basis of a fall in systemic venous pressure, for in other experiments (no. 53, fig. 8) it was found that this pressure is altered but little by lycopodium injections. An increase in the capacity of the portal area cannot be

invoked as an explanation of the fall in portal pressure because the change in tone of the arterioles and in the arterial pressure indicates that the circulation is improving, which could not be the case if blood were passing out of effective circulation. The only acceptable explanation must provide a gradually widening pathway out of the portal area. Three ways in which this might occur suggest themselves: *a*, The resistance to the passage of blood through the portal venules might diminish as a result of the washing out of the obstructing spores; *b*, the high portal pressure might open up by distention both plugged and unplugged venules. Both of these possibilities are rendered highly improbable by the fact that the same rise and fall in the portal pressure is seen (fig. 11) even when the dose of lycopodium without doubt is large enough to completely occlude the liver capillaries; and by the fact that after any dose large enough to raise the portal pressure above 15 to 20 mm. Hg., the portal pressure subsequently falls and comes to rest somewhere between 15 and 20 mm. Hg. irrespective of the size of the dose. *c*, The foregoing observation, on the other hand, strongly supports another possible explanation, namely, that as a result of the high portal pressure a collateral circulation is established, by way of which the portal blood can reach the systemic veins with ever increasing facility. The pathways that might be developed under these circumstances are familiar to the anatomist; they are the ones opened in man by any chronic portal obstruction. Upon the basis of our experience it can be concluded that with normal arterial pressure and splanchnic resistance, the resistance of the fully opened collaterals is such as to maintain a portal pressure of from 15 to 20 mm. Hg.

Returning now to the analysis of experiment 60 (fig. 9) we believe, on the basis of the foregoing discussion, that during the rapid fall in portal pressure blood is being restored to the general circulation. It is this that accounts for the maintenance of a constant arterial pressure despite a diminishing peripheral resistance. The peripheral resistance continued to diminish until, by about 3.10, it had become quite normal. At this time, though, the arterial pressure was definitely below normal (84 mm. Hg.). It could hardly have been otherwise; for the portal area at this time must have contained more than its normal volume of blood, and the peripheral resistance was normal. During the rest of the experiment, and until the animal was killed, the peripheral resistance and the arterial pressure both slowly increased, the former finally becoming somewhat above normal, the latter, quite normal.

This then represents the extent of the circulatory disturbance that results from holding the portal pressure, for the most part, above 14 mm. Hg. for a period of 4 hours 20 minutes by partially obstructing the portal capillaries. It must be concluded, therefore, that the amount of blood that is withheld from effective

circulation by such distention of the portal area as is caused by a rise of the portal pressure to 14 mm. Hg. is not so marked but that it can be compensated almost perfectly and indefinitely (4½ hours). We further infer that this degree of portal obstruction does not result in nutritional or mechanical disturbances of such a grade as to lead to any considerable reduction in total blood volume.

Even the rabbit, with its very much longer intestinal tract, may withstand perfectly similar grades of portal obstruction. This is clearly shown in experiment 56 (fig. 10). The injection of lycopodium here raised the portal pressure from 8 to 16 mm. Hg. The portal pressure then fell gradually to 12.5 mm. Hg. and then rose again to 13.5 mm. Hg. After a transitory rise, the arterial pressure fell from 74 to 66 mm. Hg., but rose again to 72 as the portal pressure fell, and did not vary very much from that level during the rest of the period.

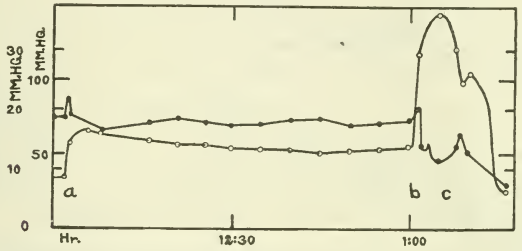

Fig. 10. Experiment 56. Circulatory effects of injecting lycopodium spores into the portal vein. Rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, small dose of lycopodium given; *b*, large dose given; *c*, artificial respiration necessary.

The *effect of very large doses of lycopodium spores* may now be considered. This is illustrated by figure 11 (exp. 51). A small dose was given at *a*. As it evidently was not going to be sufficient for our purposes, a very large dose was given at *b*. Considering the effects of the larger dose only, it is seen that the rise in portal pressure again is immediate but that the fall to the plateau is very much more protracted than after smaller doses. There is a profound fall in arterial pressure (to almost 40 mm. Hg.) and a very marked peripheral constriction. The portal circulation is at its worst shortly after 1.00, for the difference between the arterial and portal pressures here amounts to only 10 mm. Hg.; presumably the pressure gradient in the splanchnic area is practically eliminated. But now the circulation improves for a time, presumably as a result of the opening up of byways between the portal area and the systemic veins. Only by invoking some such process is it possible to explain the combination of a fall in portal pressure, a rise in arterial pressure, and an increase in inflow rate. By about 2.20, disregarding a passing fluctuation, conditions have become fairly stable. The arterial pressure is at about 50 mm. Hg., the portal pressure at 15 to 16 mm. Hg., and the inflow rate somewhat below the normal range. The jugular pressure rose throughout the course of the experiment, but the rise amounted to less than 1 mm. Hg. The

picture as a whole can be accounted for perfectly if it is assumed that there has occurred a reduction in the effective blood volume.

Around 3.00 p.m. a change for the worse begins. The arterial pressure falls slowly; the portal pressure rises slowly, and the inflow rate steadily increases. A slow giving way of the vasomotor center, presumably under the influence of the low arterial pressure, satisfactorily explains these changes. At about 4.20 the heart gives evidence of failing, the arterial pressure and the portal pressure both

Fig. 11. Experiment 51. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○— (lower curve on same scale as arterial pressure, upper curve on larger scale); jugular pressure, x x x. *a*, injecting lycopodium, very large dose; *b*, injecting lycopodium, very large dose; *c*, inflow readings transferred to other femoral; *d*, heart becoming irregular.

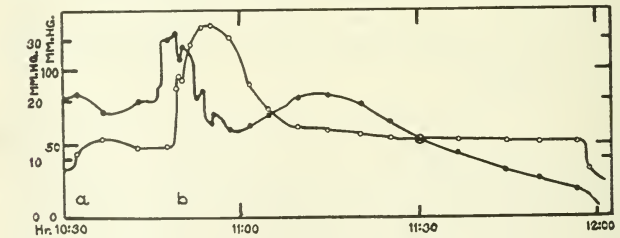

Fig. 12. Experiment 57. Circulatory effects of injecting lycopodium spores into the portal vein. Rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, injecting a small dose of lycopodium; *b*, injecting a large dose.

fall rapidly, the vasomotor tone rapidly gives way, and the animal soon dies. It is worth while emphasizing that at no time during the course of this experiment could the ether be dispensed with. Whenever the mask was removed, even after the heart had become irregular (4.20), the animal soon began to struggle.

Figure 8 (exp. 53) shows that a large dose of lycopodium given a dog (at a'') which already has for some hours been under the influence of moderate doses, brings about circulatory failure much more quickly than when it is given at the

beginning of an experiment. The character of the response, though, is alike under both conditions. Thus, in this case we see the tremendous rise in portal pressure followed by a steady and comparatively rapid decline; an immediate sharp fall in arterial pressure followed by a later sharp decline when the level of 50 mm. Hg. is reached; and a further sharp vasoconstriction.

A similar response is obtained from the rabbit when a large dose is given after a small dose has been acting for some time (see fig. 10, exp. 56). But to a larger dose given early in an experiment, the rabbit (fig. 12, exp. 57) seems to succumb much more rapidly than does the dog, though, as nearly as can be judged in the absence of inflow determinations, the response in the rabbit is similar in kind to that given by the dog. As in the case of the dog (fig. 11), we see the rise in portal pressure followed by a fall to a fairly well-maintained level of about 12 to 15 mm. Hg., and a fall in arterial pressure to 50 mm. Hg. followed by a rise which then gives way to a steady fall that leads to death.

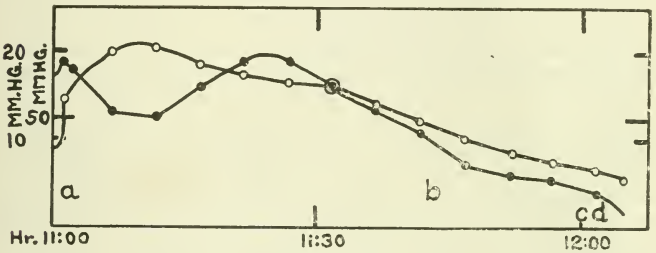

Fig. 13. Experiment 54. Circulatory effects of injecting lycopodium spores into the portal vein. Sick rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, moderate dose of lycopodium injected; *b*, respiration shallow, heart irregular; *c*, respiration stopped; *d*, heart stopped.

As might have been anticipated, it was found that the response given by sick rabbits is much more profound than that given by well animals. This is illustrated by figure 13 (exp. 54). The dose of lycopodium suspension given was regarded as moderate. The curve, though, is quite like the one (fig. 12) just described; but it took only an hour for the circulation to fail.

DISCUSSION

These experiments show that an obstruction of the portal radicles in the liver that raises the portal pressure to a much higher level than it is raised by the Janeway and Jackson method of obstructing the vena cava (18), and quite as high as it is raised by the injection of adrenalin, in the dog at least, does not, even after more than 4 hours have elapsed, result in any very considerable alteration in the effectiveness of the cir-

culation as evidenced by the arterial pressure and by the tone of the vasomotor center. A similar statement applies to the results obtained in the rabbit, also. Complete portal embolism in the dog immediately impairs the general circulation, as is evidenced by a low arterial pressure, and eventually (in $3\frac{1}{2}$ hours) causes the vasomotor center to fail. But even in this event the animal does not become apathetic, and therefore does not present the complete picture of shock. In the rabbit, the fatal issue develops more rapidly than in the dog, and the rabbit, if not strong, may rapidly succumb to even a moderate portal obstruction.

Inasmuch as the portal obstructions we have produced by the injection of lycopodium without doubt have been quite as marked as any that could be effected by any physiological or acute pathological process (embolism and thrombosis excepted) we are forced by our results to conclude that acute portal obstruction due to the action of some physiological mechanism, and the consequent accumulation of blood in the splanchnic area, can scarcely be regarded as a cause of shock. Even if it should turn out that the seat of the action of adrenalin is in or beyond the hepatic capillaries, and even if it should be shown that the adrenalin obstructs the collateral, as well as the direct portal circulation, we would still regard our experimental results as sufficient to justify the statement that a portal obstruction *per se* cannot reasonably be regarded as the cause of the failure of the circulation following the injection of adrenalin.

As a matter of fact, we have in the action of adrenalin upon the systemic arterioles all that is necessary to account for the failure of the circulation, namely, the consequences of an extreme and general slowing of the blood stream; though, of course, such portal obstruction as may occur after adrenalin injection will be contributory, both through mechanical distention of vessels and through filtration of plasma, to the general slowing of blood flow due to constriction.

GENERAL SUMMARY AND CONCLUSIONS

1. The injection of adrenalin for a period of 20 to 30 minutes at such a rate as to maintain a high arterial pressure invariably constricts the arteries of both the somatic and the splanchnic areas. With large doses this constriction may be maximal and outlasts the injection period, it may be, for as long as 2 hours. This continued constriction is partly due to central action.

2. After sufficiently large doses, the arterial pressure, barring occasional intercurrent phenomena, falls steadily and slowly until the animal dies.

3. The jugular pressure, during or after large injections, shows no constant alteration, at least not of a kind that indicates inefficiency of the heart.

4. The heart may become irregular for a while and occasionally stops suddenly while the arterial pressure still is high.

5. The respiration after large doses often is slowed and may suddenly or gradually fail.

6. The portal pressure is increased, often markedly, during the injection, and may remain high subsequently; but not uncommonly it soon returns to the normal level.

7. The rise in portal pressure undoubtedly is caused by an obstruction in the liver which is so marked as to back up even the small amount of blood that is entering the portal system through the strongly constricted splanchnic arterioles.

8. The circulation may fail suddenly through stoppage of the heart due to direct action of the adrenalin, or to the indirect action of respiratory failure. But more often the failure of the circulation is gradual, the pressure steadily falling until the animal dies. The arterial constriction induced by the adrenalin then lasts until the end. In such instances a reduced blood volume, either real or effective, seems to be the main factor at fault. Apathy, as well as the other signs of shock, are present.

9. Evidence is presented indicating that accumulation of blood in the portal area as a result of the increased portal-hepatic resistance is not in itself the cause of the failure of the circulation. For marked obstruction of the hepatic radicles in the liver by the injection of a suspension of lycopodium spores may not lead to the shock-like failure of the circulation that is seen after adrenalin.

10. The failure of the circulation is rather to be attributed to the extreme slowing of the blood flow throughout the body caused by the constricting action of the adrenalin on the arteries. It is concluded that the cause of the failure is the same as is operative after temporary partial obstruction of the vena cava or of the aorta. This conclusion is justified by the fact that the most striking lesion found in animals dying as a result of any of these three procedures is alike, and consists of tremendous engorgement of the capillaries and venules of the villi of the intestines.

BIBLIOGRAPHY

(1) Medical Research Committee: Memorandum upon surgical shock, Brit. Med Journ., 1917, i, 381.
(2) Bartlett: Journ. Exper. Med., 1912, xv, 415.
(3) Erlanger, Gesell and Gasser: This Journal, 1919, xlix, 90.
(4) Hartman: Endocrinology, 1918, ii, 122.
(5) Hoskins and Gunning: This Journal, 1917, xliii, 299.
(6) Hartman and McPhedran: This Journal, 1917, xliii, 311.
(7) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(8) Gunning: This Journal, 1917, xliii, 395.
(9) Gruber: This Journal, 1918, xlv, 302.
(10) MacWilliam: Proc. Roy. Soc., 1902, lxx, 109.
(11) Elliott: Journ. Physiol., 1905, xxxii, 401.
(12) Bainbridge and Trevan: Journ. Physiol., 1917, li, 460.
(13) Schmid: Arch. f. d. gesammt. Physiol., 1909, cxxvi, 165.
(14) Burton-Opitz: Quart. Journ. Exper. Physiol., 1912, v, 329.
(15) Edmunds: Journ. Pharm. Exper. Therap., 1914, vi, 569.
(16) Erlanger and Gasser: This Journal, 1919, xlix, 151.
(17) Henderson, Prince and Haggard: Journ. Amer. Med. Assoc., 1917, lxix, 965.
(18) Janeway and Jackson: Proc. Soc. Exper. Biol. and Med., 1915, xii, 193.
(19) Cannon: Boston Med. Surg. Journ., 1917, clxxvi, 859.

PHYSIOLOGICAL STUDIES ON PLANARIA

I. Oxygen Consumption in Relation to Feeding and Starvation

L. H. HYMAN

From the Hull Zoölogical Laboratory, University of Chicago

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For a number of years the physiology of Planaria and other lower invertebrates has been extensively investigated in this laboratory. As a result of these investigations certain fundamental conceptions have been reached. These conceptions are in part concerned with the metabolic gradients of organisms and their relation to the phenomena of polarity, symmetry, individuation, regeneration, reproduction and development; and in part with the relation between metabolic rate and regeneration, age, starvation and other physiological conditions. These conclusions and the evidence from which they are derived have been presented by Professor Child in numerous papers and in his two books *Senescence and Rejuvenescence* and *Individuality in Organisms*.

In reaching these conceptions, various methods have been employed. Two of these are referred to in this paper and require explanation. One of them is called by us in the direct susceptibility method and consists in observing the time of death of organisms or parts of organisms in lethal concentrations of certain substances or under lethal conditions such as lack of oxygen, extreme temperatures, etc. A great variety of substances has been employed for this purpose, including cyanides, acids, bases, salts, anesthetics and dyes; all of them yield practically identical results, but we have found that cyanides constitute the most delicate and precise indicators of susceptibility differences. We believe that the time of death in such solutions or under such conditions is a direct measure of the metabolic rate of the protoplasm so tested. The chief grounds for this belief are: all conditions which are known to increase the general metabolic rate also increase the susceptibility to these solutions; in the case of the cyanides at least there is a large body of evidence, which I have reviewed in a recent

paper (1), which demonstrates that cyanides depress metabolic rate, and further we have recently shown (1), (2), that potassium cyanide decreases to a very marked degree both the carbon dioxide production and the oxygen consumption in Planaria; and finally the susceptibility data check completely with the results obtained by the second method referred to. This method consists in the determination of the carbon dioxide production of organisms or parts of organisms either by means of the Tashiro biometer or by means of an indicator, chiefly phenolsulphonephthalein. The carbon dioxide production furnishes the same conclusions regarding metabolic rates as does the susceptibility method, wherever comparable conditions are present.

In consideration of a misunderstanding which has arisen among other workers regarding the susceptibility method, it becomes necessary to emphasize the fact that the method in general measures only the susceptibility of the superficial structures of the body, since as a rule it is these only whose time of death can be accurately observed. Hence the time of death in these toxic solutions is, strictly speaking, a measure of the metabolic rate of the body wall and nervous system only and not of the total metabolic rate. It becomes a measure of the total metabolic rate only when comparable internal conditions exist. Hence when these internal conditions are altered as by introducing food into the intestine, the susceptibility of the surface will obviously not measure the metabolic rate of the intestine nor the total metabolic rate, since this is the sum of the activity of all parts of the body. Therefore the objections of Lund (3) to the susceptibility method on the grounds that in Paramecium the susceptibility is increased by short periods of starvation but the total oxygen consumption and carbon dioxide production are decreased are without basis and without point. In Protozoa obviously only the condition of the ectoplasm can be determined by the susceptibility method since as soon as the ectoplasm dies and disintegrates, the entoplasm scatters and can no longer be observed. In the cases of other organisms, however, it is sometimes possible to observe the time of death of both the intestine and the body wall separately and therefore to draw a correct conclusion regarding the total metabolism.

The expression "metabolic rate" seems to require definition. The word metabolism is generally taken to signify the sum of all the energy-producing and substance-producing processes occurring in the body. Each such process may, of course, have a rate of its own, and in order to find out the total metabolic rate at any one time it would be

necessary for strict accuracy to determine the rate of each process separately. This procedure is practically impossible and probably unnecessary, since it happens that in the animal body the majority of such processes are energy-consuming, that is, they use up oxygen and give off carbon-dioxide. It is therefore generally adequate to measure the rate of respiratory exchange in organisms in order to obtain some idea of the total metabolism. In the higher organisms, other methods are also at hand, as urine analysis, blood analysis, heat production, etc., which, however, cannot be applied to the lower forms. I wish to point out further that the rate of oxygen consumption and carbon-dioxide production are adequate measures of the rates of processes not in themselves essentially oxygen-consuming, such as the contraction of muscles; and that they furnish information about apparently unrelated conditions such as the irritability and conductivity of nerves. For these reasons we use the term metabolic rate instead of the more specific expression respiratory exchange, since we believe such respiratory rate to be an index of many other metabolic and physiological conditions besides oxidation. We also believe that the susceptibility method measures other metabolic conditions besides the rate of oxidation. We use the expression metabolic rate to indicate the rate of the large general chemical processes occurring in the body and not with reference to conditions specific to one organ or part nor with reference to special conditions. Thus the rate of the heart beat is an index to general physiological states such as age, sex, nutrition, etc., but obviously it would be foolish to attempt to draw conclusions regarding these states from the rate of the heart beat under special conditions where it had been altered, as after exercise. It is in precisely the same sense that we speak of metabolic rate, in reference to long-lasting and general processes, common to all of the cells of the body.

As previously stated, in our work on the physiology of the lower forms we have employed chiefly the method of differential susceptibility and the measurement of carbon-dioxide production. The present series of papers is concerned with the rate of oxygen consumption under various physiological conditions and therefore constitutes a check upon the results obtained by the other methods. The work has unfortunately been greatly hampered and delayed by the accidental loss in the spring of 1918 of our entire stock of Planaria, numbering many thousands. This has necessitated the use of somewhat smaller lots of worms than would ordinarily have been the case.

The present paper reports the results of my experiments on the rate of oxygen consumption of Planaria in feeding and starvation; experiments on oxygen consumption with respect to age and regeneration are also completed. It is gratifying to state that these results confirm the conclusions reached by the other methods.

METHOD

These experiments were performed upon *Planaria dorotocephala* and *Planaria velata*, two common species of flatworm, both of which have been used as the chief experimental material in the investigations mentioned in the opening paragraph. The method was the same as that described in a recent paper (1) except that, owing to the smaller stocks available, smaller receptacles and samples were employed. Briefly each lot of worms to be tested was placed in a 500 cc. Erlenmeyer flask filled air-tight with water, and allowed to respire in this flask at constant temperature for the desired length of time. A sample of the water was then withdrawn and analyzed for oxygen content by Winkler's method, and the difference between the oxygen content of this sample and a control sample similarly treated gave the oxygen consumption of the worms. In most cases where time and circumstances permitted, each test was repeated, so that two separate determinations of the oxygen consumption of a given lot of worms were obtained.

In order to compare the rates of oxygen consumption of the same lot of worms at different intervals it is necessary to know their weight. The oxygen consumption of these organisms is directly proportional to their weight, other factors being equal. This appears clearly in the experimental tables, and is in fact so axiomatic as to require no further discussion. The following method of weighing the worms was found most suitable. The animals were collected in a funnel of filter paper, being washed down to the point of the funnel by a pipette. After the water had drained through, the filter paper was opened out and spread flat on top of two or three layers of moist filter paper until all excess water had been absorbed, small pieces of dry filter paper being also placed under the mass of worms to aid the process. The worms were then scraped up with a dry scalpel blade into a small glass receptacle, previously balanced on the scales, and rapidly weighed. Weighings were made to the third place only. This method has been found more rapid and accurate than procedures in which the worms are

weighed in water, since it is almost impossible to avoid adding or withdrawing water. Usually some worms are injured through drying but as the weighings were nearly always made at the conclusion of the experiment, this was not of any consequence. If, however, it was necessary to weigh the worms before the experiment, the injured worms were collected and weighed separately and this weight then subtracted from the first weight. The injured worms were always discarded. Tests have shown that this method of weighing is sufficiently accurate for the purpose at hand. In these tests the same lot of worms was returned to water, re-collected and weighed again. Such duplicate weighings are not, however, strictly comparable, since the worms lose weight after weighing owing to the secretion of mucus due to the stimulation of drying. If this mucus is removed, the weight of the worms will be less than it was at the first trial; if retained, it may be more, as the mucus absorbs water. A fair idea of the loss due to the weight of the mucus can be obtained by drying it on filter paper and weighing it separately. The following are duplicate weighings of the same lot of worms: 0.192, 0.186 gram (mucus retained); 0.527, 0.532 (mucus retained); 0.458, 0.451 (mucus removed); 0.763, 0.746 (mucus removed, separate weight of the dried mucus, 0.014); 0.817, 0.802 (mucus removed, separate weight of the dried mucus, 0.014).

Eight lots of *Planaria dorotocephala* and three lots of *Planaria velata* were used in the experiments. Each lot, consisting of individuals of approximately the same size and condition, was selected from one of the general laboratory stocks. The oxygen consumption of each lot was then determined at various intervals after feeding and through a period of starvation, the lot being weighed after each such determination. The water was changed at intervals and the lots examined from time to time and all individuals noticeably larger or smaller than the average, all injured individuals and all posterior products of fission removed and discarded. When Planaria is placed in a clean receptacle, it is likely to undergo fission. This can be obviated to some extent by avoiding the use of clean dishes and placing the worms only in dishes previously occupied by worms. However, it is necessary to examine the dishes at intervals and pick out the products of fission. In the present experiments, all of the posterior products of fission have been removed and the anterior ones have been retained only when they constituted nearly the whole of the original worm. Thus these experiments deal only with whole worms of small size or anterior zoöids. As shown by Child's work (4), the posterior zoöids behave

somewhat differently during starvation than whole worms or anterior zoöids. As starving planarians will eat each other if possible, it is advisable to employ large receptacles for the starving stocks and to remove all individuals which vary much in size from the average of the stock. As starvation proceeds, the heads of the worms, especially if they were rather small individuals at the start, are likely to disintegrate, and new heads are then regenerated. Such individuals were removed during the early stages of the experiments but not at the end, as many of the individuals in some of the stocks were in this condition.

Since movement increases both the oxygen consumption and carbon dioxide production, movement is a disturbing factor in any experiments on the rate of respiratory exchange. In most of our experiments on Planaria, we remove the heads of the individuals to be tested several hours before the test, since decapitated worms remain perfectly quiet. This procedure was not thought advisable in the present experiments since the subsequent regeneration of the heads would introduce an additional factor. Hence it was necessary to observe carefully the degree of movement. If the worms are placed in the experimental flask several hours before the test, and if the flask is darkened during the test, movement can be largely eliminated. Movement is most likely to occur during the middle of the starvation period, recently fed worms or greatly starved worms being generaliy inclined to inactivity. Hence the figures obtained during the middle of the starvation period are probably slightly too high on account of movement. At the end of the starvation period at the last test made, in order to eliminate this factor completely, the heads were cut off, unless they had previously disintegrated as a consequence of starvation. Hence the final figures on the rate of oxygen consumption after prolonged starvation are entirely free from this difficulty.

Further details regarding the worms are given in the following records of the experiments.

EXPERIMENTAL RESULTS WITH PLANARIA DOROTOCEPHALA

Planaria dorotocephala is the species which has been employed in most of the investigations carried out by Professor Child and his students. Large stocks of this worm are kept constantly in the laboratory and fed three times a week on liver. From these general stocks eight lots, designated as A, B, F, G, H, K, M and N were selected

for the present experiments. The members of each lot were of approximately equal size and had been kept under the same conditions for some time preceding the experiments. Details regarding the make-up of each lot and the data obtained upon them are given in the following descriptions and accompanying tables. Six of the eight lots were fed and the effect of feeding as well as that of starvation on the oxygen consumption determined.

In the tables, the first column gives the length of time since feeding. The second column gives the actual figures of the oxygen consumption in a given period of time of the lot of worms on the day designated in the first column. In most cases two figures, the results of two separate determinations, are presented. The third column gives the weight of the lot of worms just after testing. The last column in each table is a calculation of the average oxygen consumed in cubic centimeters in two hours by 0.5 gram of Planaria. This calculation to a given weight and time is, of course, necessary in order to compare the rate of oxygen consumption at different periods of starvation. The time period and weight chosen for this calculation are, of course, purely arbitrary, selected as most nearly corresponding with the weights and time periods actually used in the experiments.

A word may be needed in explanation of the recorded weights. In the case of lots A, B, F, G and H, not all of the individuals in each lot were used each time in the early part of the experiments, a circumstance which accounts for the variability in weight. In lots K, M and N, the entire lot was used at each test, and the recorded weights therefore illustrate the loss of weight of these lots during starvation, with one increase due to feeding. I was somewhat at a loss to know how to regard the increase in weight resulting from ingestion of food. I finally decided that such increase should not be calculated in the results since this additional weight from the ingested food can hardly be regarded as respiring protoplasm, at least not within the first twenty-four hours after feeding. Hence in the feeding experiments the worms were weighed just before feeding, and this weight was used for calculating the rate of oxygen consumption after feeding.

The temperature of all experiments was 22 ± 0.5°C.

Record of lot A (*table 1*). Lot A consisted of worms about 10 mm. long selected from a stock which had been in the laboratory for four or five months (the exact date of collection was not known). They were last fed on March 3, 1919. On March 8, five days after feeding,

the average oxygen consumption of 0.5 gram in two hours was 0.30 cc.; on March 15, twelve days after feeding, it was 0.26; on March 29, after twenty-six days of starvation it was 0.36; and on April 29, after about two months' starvation, 1.50 cc. of oxygen was consumed. At this time when the experiment was concluded, the size and number of the worms was greatly reduced and most of them had lost their heads so that movement could not have played any rôle in the final result.

TABLE 1

Record of lot A

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
days	*cc.*	*grams*	*cc.*
5	0.30 in 2 hours	0.464	**0.30**
	0.26 in 2 hours		
12	0.28 in 2 hours	0.499	**0.26**
	0.24 in 2 hours		
26	0.22 in 2 hours	0.302	**0.36**
57	0.15 in 3 hours		
	0.16 in 3 hours	0.034	**1.50**

TABLE 2

Record of lot B

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
days	*cc.*	*grams*	*cc.*
5	0.46 in 2 hours	0.712	**0.31**
	0.42 in 2 hours		
12	0.26 in 2 hours	0.566	**0.23**
	0.28 in 2 hours		
26	0.23 in 2 hours	0.464	**0.24**
56	0.13 in 2 hours	0.147	**0.47**
	0.15 in 2 hours		

Record of lot B (*table 2*). Lot B was selected from a stock collected in February, 1919. They were slightly larger than the worms in the preceding stock, about 12 to 13 mm. long. The worms were last fed on March 5. On March 10, five days after feeding, the oxygen consumed in two hours by 0.5 gram was 0.31 cc.; on March 17, twelve days without food, it was 0.26 cc.; on March 31, after twenty-six days without food, it was 0.24; and at the end of the experiment, May

1, after nearly two months of starvation, it was 0.47. The worms were much reduced in size but had retained their heads. These were cut off several hours before the final test.

Record of lot F (*table 3*). This lot of worms was taken from a mixed stock which had been used for various other purposes. Worms in this stock had been collected during the fall and winter. The members of this lot were last fed on March 10. They were 12 to 15 mm. in length. On March 18, eight days after feeding, they consumed 0.25 cc. of oxygen in two hours as calculated for 0.5 gram weight. They

TABLE 3

Record of lot F

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
	cc.	*grams*	*cc.*
8 days	0.17 in 1½ hour		
	0.17 in 1½ hour	0.444	0.25
Fed immediately after above determination			
1 to 3 hours	0.34 in 1½ hour	0.444*	0.50
3 to 5 hours	0.28 in 1½ hour	0.444*	0.42
2 days	0.18 in 1½ hour	0.486	0.25
	0.19 in 1½ hour		
7 days	0.21 in 2 hours	0.413	0.22
	0.17 in 2 hours		
21 days	0.12 in 2 hours	0.214	0.28
48 days	0.17 in 3 hours†	0.075	0.80
	0.19 in 3 hours		

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

were then fed on liver and their oxygen consumption tested one to three hours after feeding. It was now 0.50 cc. Three to five hours after feeding it had fallen to 0.42 cc. It must be noted that the weight of these worms was taken before feeding and they were not weighed again after feeding as it was considered that the increased weight resulting from the intake of food could not properly be calculated with respiring protoplasm. It was therefore considered fairer to omit the increase in weight following feeding. On March 20, two days after feeding, the oxygen consumption had fallen to 0.25 cc.; on March 25, a week since feeding, it was 0.22; on April 8, three weeks of star-

vation, it was 0.28; and on May 5, when the experiment was concluded, after a fast of seven weeks, it was 0.80. The condition of the worms was similar to that in the preceding stocks. The number remaining was so few that it was necessary to combine those from lots G and H with those of lot F for the final determination on May 5. These three lots came from the same stock and had been treated in an identical manner throughout the course of the experiment.

Record of lot G (table 4). The worms in this lot came from the same stock as those of lot F, were of the same size and were handled

TABLE 4

Record of lot G

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
8 days	0.17 in 1½ hour 0.18 in 1½ hour	0.494	**0.23**
	Fed immediately after above determination		
1 to 3 hours	0.29 in 1½ hour	0.466*	**0.40**
3 to 5 hours	0.26 in 1½ hour	0.466*	**0.37**
2 days	0.13 in 1½ hour 0.20 in 1½ hour	0.423	**0.26**
7 days	0.17 in 2 hours 0.16 in 2 hours	0.344	**0.24**
21 days	0.11 in 2 hours	0.176	**0.31**
48 days	0.17 in 3 hours† 0.19 in 3 hours	0.075	**0.80**

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

throughout in the same way. On March 18, eight days since the last feeding, they consumed 0.23 cc. of oxygen as calculated for 0.5 gram weight for two hours. They were then fed on liver, and one to three hours after feeding they consumed 0.40 cc.; three to five hours after feeding, 0.37 cc. On March 20, two days later, their oxygen consumption was 0.26; on March 25, one week after feeding, it was 0.24; on April 8, three weeks without food, it was 0.31; and on May 5, seven weeks without food, it was 0.80. As noted for lot F, this final figure was obtained on the combined individuals from lots F, G and H.

Record of lot H (*table 5*). This lot was the same as to origin, size and handling as the preceding lots F and G. On March 18, eight days since the last feeding, the oxygen consumption was 0.24 cc. in two hours per 0.5 gram weight. The worms were then fed, and during the first to third hour after feeding they consumed 0.44 cc. of oxygen; during the third to the fifth hour after feeding, 0.42 cc. Two days later, their oxygen consumption had fallen to 0.33 cc.; a week after feeding, it was 0.24; after three weeks without food, it was 0.31; and after seven weeks' starvation, it was 0.80. The three lots F, G and H

TABLE 5

Record of lot H

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
8 days	0.16 in 1½ hour 0.16 in 1½ hour	0.440	**0.24**
	Fed immediately after above determination		
1 to 3 hours	0.28 in 1½ hour	0.421*	**0.44**
3 to 5 hours	0.27 in 1½ hour	0.421*	**0.42**
2 days	0.18 in 1½ hour 0.21 in 1½ hour	0.387	**0.33**
7 days	0.18 in 2 hours 0.16 in 2 hours	0.346	**0.24**
21 days	0.12 in 2 hours	0.192	**0.31**
48 days	0.17 in 3 hours† 0.19 in 3 hours	0.075	**0.80**

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

were, as can be readily seen, run simultaneously and were always under the same conditions. At the end of the starvation experiment they were greatly reduced in size and most of them had lost their heads, thus eliminating movement as a factor in the final determination.

Record of lot K (*table 6*). Lots K, M and N were three lots selected from the same stock and run in all respects under the same conditions. These three lots were tested with especial care, and the data presented for them are hence possibly more exact than the data for the five preceding lots. They were selected from a new stock, collected on March 11, 1919. They were last fed on March 24. The worms of

these lots were larger than in the preceding cases, 15 to 18 mm. long. In consequence of this larger size, they were not reduced so much at the end of the experiment, and all had retained their heads throughout the period of starvation. The possible disturbing factor of regeneration of the head is therefore eliminated in the case of these three lots. These worms were also so active at the conclusion of the experiment that it was necessary to decapitate them before testing their rate of oxygen consumption. In these three lots then movement has been completely eliminated as a factor in the final results. In short, the

TABLE 6

Record of lot K

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.23 in 1½ hour	0.589	**0.25**
	Fed immediately after above determination		
2 to 4 hours	0.46 in 1½ hour	0.579*	**0.52**
7 to 8½ hours	0.48 in 1½ hour	0.579*	**0.54**
24 hours	0.30 in 1½ hour	0.579*	**0.32**
3 days	0.32 in 2 hours	0.623	**0.25**
7 days	0.27 in 2 hours	0.543	**0.24**
21 days	0.21 in 1½ hour†	0.325	**0.38**
	0.16 in 1½ hour		
48 days	0.11 in 2 hours	0.112	**0.53**
	0.13 in 2 hours		

* Weight taken before feeding, see text.

† Considerable movement during this determination.

data for these three lots seem to me to be absolutely dependable and to be free from all possible sources of error. The entire stock was used in each case for each determination, and hence the weights given are a fair picture of the loss of weight of Planaria during starvation. Of course a few individuals were discarded from time to time on account of injury. Fission, however, was practically absent in these lots.

The oxygen consumption of lot K on March 28, four days after feeding was 0.25, calculated as before for two hour periods and for 0.5 gram of weight. The worms were then fed and the oxygen consumption two to three and a half hours after feeding was 0.52; seven

to eight and a half hours after feeding it was 0.54. On the next day, twenty-three to twenty-four hours after feeding, it was 0.32. On March 31, about three days after feeding, it was 0.25; on April 4, a week without food, it was 0.24; April 18, three weeks without food, it was 0.38; and at the conclusion of the experiment, on May 15, after seven weeks' starvation, it was 0.53. It should be noted that the worms were rather active during the determination of April 18, and hence the figures obtained upon this date are slightly too great, particularly the first of the two successive determinations. During the

TABLE 7

Record of lot M

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.29 in 1½ hour	0.761	**0.25**
	Fed immediately after above determination		
2 to 4 hours	0.58 in 1½ hour	0.747*	**0.51**
7 to 8½ hours	0.54 in 1½ hour	0.747*	**0.48**
24 hours	0.37 in 1½ hour	0.747*	**0.33**
3 days	0.43 in 2 hours	0.779	**0.28**
7 days	0.32 in 2 hours	0.671	**0.23**
21 days	0.23 in 1½ hour†	0.424	**0.31**
	0.17 in 1½ hour		
48 days	0.13 in 2 hours	0.159	**0.45**
	0.16 in 2 hours		

* Weight taken before feeding, see text.

† Considerable movement during this determination.

second analysis they were for the most part inactive and hence this figure is more nearly correct. As already stated the heads of the worms were cut off for the final determination so that movement was completely eliminated.

Record of lot M (*table 7*). All the remarks made with reference to lot K apply to this lot also. Four days after the last feeding, lot M consumed 0.25 cc. of oxygen; on the same day, two to three and one-half hours after feeding, this figure rose to 0.51 cc.; and seven to eight and one-half hours after feeding, it had fallen slightly to 0.48. The next day it was 0.33 cc.; on the third day after feeding, 0.28; after a

week without food, 0.23; after three weeks, 0.31; and after seven weeks' starvation, 0.45.

Record of lot N (*table 8*). This lot is similar in all respects to the two preceding lots. These worms consumed on the fourth day after feeding 0.23 cc. of oxygen per 0.5 gram weight in two hours; on the same day shortly after feeding, the oxygen consumption rose to 0.51, and fell a few hours later to 0.49. The next day it was 0.31; the third day after feeding, 0.27; a week after feeding, 0.22; after three weeks without food, 0.28; and after seven weeks without food, 0.56.

TABLE 8

Record of lot N

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.27 in 1½ hour	0.769	**0.23**
	Fed immediately after above determination		
2 to 4 hours	0.58 in 1½ hour	0.758*	**0.51**
7 to 8½ hours	0.56 in 1½ hour	0.758*	**0.49**
24 hours	0.36 in 1½ hour	0.758*	**0.31**
3 days	0.45 in 2 hours	0.832	**0.27**
7 days	0.32 in 2 hours	0.706	**0.22**
21 days	0.19 in 1½ hour 0.19 in 1½ hour	0.446	**0.28**
48 days	0.14 in 1½ hour 0.14 in 1½ hour	0.125	**0.56**

* Weight taken before feeding, see text.

EXPERIMENTS WITH PLANARIA VELATA

Planaria velata is a turbellarian flatworm found in temporary pools in the spring in the Chicago region. Associated with the peculiar conditions of life which exist in temporary pools, the life cycle of this animal is exceptionally interesting. It has been investigated by Child, to whose paper on the subject the reader is referred (5). The animal emerges from cysts in the spring when the temporary ponds fill with water, grows rapidly and soon attains adult size. It then ceases to feed and posterior portions of its body drop off, secrete a mucous cyst about themselves, and pass into a quiescent state within this cyst.

This process continues until the entire worm has formed a series of cysts. After about four weeks, in case the pond still contains water, the worms emerge from the cysts, but they are now completely regenerated whole worms of small size. This cycle is repeated until the pond dries up, whereupon the cysts remain quiescent until the following spring.

I performed three experiments with this species. In each case the experiment was interrupted by the formation of cysts. The young worms which emerge from the cysts are of course in a state of extreme starvation. As, however, not all of them emerge at once, it was necessary to feed those which emerged first in order to keep them alive until a number of individuals sufficient for a determination of their oxygen consumption had appeared. The young starved worms were therefore fed two or three times at long intervals. Since this feeding was entirely inadequate to bring about growth, it is certain that at the time of testing the young worms were still in a state of extreme starvation, and that their oxygen consumption therefore truly represents starvation metabolism. In addition it must be noted that these worms have also undergone regeneration. Now as I shall show in the second paper of this series, regeneration also greatly increases the rate of oxygen consumption. Hence, in these experiments with *Planaria velata*, the end result is a combination of the effects of both starvation and regeneration on the metabolic rate. It is not in this particular case possible to separate these two factors, although one can prevent this species from undergoing encystment by beginning to starve the worms before they have attained adult size. This I attempted to do but evidently growth had already progressed too far when I began the experiments and all of the worms encysted.

Record of lot C (*table 9*). This lot of *Planaria velata* was taken from a collection of March 7, 1919. They were fed once after being received in the laboratory, on March 10. The individuals selected for the experiment were 10 to 12 mm. long. On March 13, three days after feeding, they consumed 0.31 cc. of oxygen in two hours per 0.5 gram weight. On the same day, two to three and one-half hours after feeding, the oxygen consumption was 0.43. It should be noted that only a small proportion of the lot fed, as they were evidently already on the verge of encystment. On March 15, two days after feeding, the oxygen consumption was 0.32; on March 20, a week without food, it was 0.29. The worms now rapidly encysted and in a week or two the dishes were a mass of small round cysts. Late in

TABLE 9

Record of lot C

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.32 in 1½ hour 0.42 in 1½ hour	0.745	**0.31**
Fed immediately after above determination			
2 to 3½ hours	0.46 in 1½ hour	0.707*	**0.43**
2 days	0.28 in 1½ hour 0.30 in 1½ hour	0.602	**0.32**
7 days	0.19 in 1½ hour	0.428	**0.29**
Worms encysted and emerged as young worms, see text			
75 days	0.10 in 3 hours 0.14 in 3 hours	0.058	**0.68**

TABLE 10

Record of lot D

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.44 in 1½ hour 0.39 in 1½ hour	0.774	0.35
Fed immediately after above determination			
2 to 3½ hours	0.50 in 1½ hour	0.754*	0.49
2 days	0.37 in 1½ hour 0.37 in 1½ hour	0.648	0.38
7 days	0.22 in 1½ hour	0.525	0.27
Worms encysted and emerged as young worms, see text			
70 days	0.19 in 3 hours 0.21 in 3 hours	0.140	0.70

*Weight taken before feeding, see text.

April and throughout May, the young worms were emerging. They were picked out on May 3, and fed at that time; then again on May 10, and fed; and again on May 21. On May 26, those which had emerged since were added to the lot of young worms and at this time the heads of all were cut off to prevent movement. On May 27, the final test of the rate of oxygen consumption was made. It should be noted therefore that lot C at that time was made up of young worms, some of which had been fed three times, some twice, some once, and some not at all. As already noted the feedings were at such long intervals that the worms were in a state of starvation. The oxygen consumption on May 27 was 0.68.

Record of lot D (*table 10*). This lot of worms was identical in make-up and kept under the same conditions as the preceding lot. On March 13, three days after feeding, the oxygen consumption was 0.35 cc.; on the same day a few hours after feeding (most of the worms refused to feed), it was 0.49. Two days later, it was 0.38; and after a week without food, it was 0.27. Encystment now ensued as related in the preceding case. The emerged worms were fed on May 3 and May 10 and tested on May 22, so that the lot finally tested contained some worms which had been fed once, some twice and some which had not received any food. The oxygen consumption on May 22 was 0.70 cc. The heads were not removed but the worms certainly were no more active than in the earlier tests on this lot, so that the results are dependable.

Record of lot E (*table 11*). This lot is identical with lots C and D. On March 13, three days after feeding, the oxygen consumption was 0.34; on the same day, a few hours after feeding, it rose to 0.53. More of the individuals in this case took food than in the case of the two preceding lots. Two days later, the oxygen consumption had fallen to 0.35 cc.; and a week after feeding it was down to 0.21. After emergence from the cysts, the young worms were fed on May 3 and May 10, and tested on May 22, some of the lot at the time of the test having therefore fed twice, others once, and others not at all. On this day, the oxygen consumption was 1.05.

On May 26, all worms which had emerged in lots C, D and E since May 22 were collected into one lot and their heads removed. No members of this lot had been fed, and they had therefore been without food for seventy-five days. Their rate of oxygen consumption was tested on May 27. They consumed 0.16 and 0.17 cc. of oxygen in two tests, each lasting three hours. They weighed 0.067. The

oxygen consumption as calculated for two hours and for 0.5 gram weight was 0.80. In this experiment, then, movement had been completely eliminated by decapitation, and none of the worms had received food. The rate of oxygen consumption of such emerged starved worms was more than twice as high as that of the same worms a few days after feeding. Both regeneration and starvation are involved in this increase, as already noted.

TABLE 11

Record of lot E

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.31 in 1½ hour	0.593	0.34
Fed immediately after above determination			
2 to 3½ hours	0.47 in 1½ hour	0.581*	0.53
2 days	0.29 in 1½ hour	0.494	0.35
	0.24 in 1½ hour		
7 days	0.12 in 1½ hour	0.374	0.21
Worms encysted and emerged as young worms, see text			
70 days	0.18 in 3 hours	0.60	1.05
	0.20 in 3 hours		

* Weight taken before feeding, see text.

CONCLUSIONS AND DISCUSSION

The data obtained from eleven different lots of Planaria, belonging to two species, are in complete agreement with each other and serve to demonstrate the following facts regarding the oxygen consumption of Planaria during feeding and starvation.

1. The oxygen consumption is increased after feeding. The amount of this increase depends directly upon the number of individuals which take in food. Thus in the case of lots K, M and N, presented in tables 6, 7 and 8, where practically all of the individuals fed, the increase is more than 100 per cent, while in lots F, G and H, in which some individuals did not feed, the increase ranges from 80 to 100 per cent, and in lots C and D, where the majority of the individuals

refused to feed, the increase is only 40 per cent. In lot E, worms comparable in every way to those of lots C and D, more of the worms were observed to apply themselves to the meat and a correspondingly greater increase, about 60 per cent, in rate of oxygen consumption was found.

2. This increase in the rate of oxygen consumption due to feeding begins to fall within a relatively short period of time after feeding. A slight decrease is noticeable seven to eight hours after feeding, and twenty-four hours after feeding the rate has fallen off very markedly. The rate continues to fall more slowly from this time on and reaches a minimum value one to two weeks after the last feeding.

3. After the rate of oxygen consumption has reached a certain minimum value, it begins to rise. This rise is distinctly noticeable three weeks after feeding.

4. The oxygen consumption continued to rise as long as the experiments were continued. After seven to eight weeks without food, the oxygen consumption is two to five times as great as it is in the same worms several days after the last feeding.

5. Starvation is therefore a means of increasing the rate of oxygen consumption of Planaria. After a long period of starvation the organism has a metabolic rate like that of young worms and resembles them in shape and proportions, color, rapidity of movement and in general reactions and behavior. Child has shown (4) that the carbon-dioxide production of young worms is invariably greater than that of old worms per unit weight. I have recently found (results to be published shortly) that similarly the oxygen consumption of young worms is invariably higher than that of old worms, per unit weight, both being in a condition of adequate nutrition. The difference between fed young and old worms is not so great as that between fed and starved worms. Starved worms are therefore metabolically like young worms, when both are compared with old fed worms.

These conclusions merit further discussion, with reference to experiments on Planaria by other methods and experiments on other forms.

The effect of feeding and starvation on *Planaria dorotocephala* and *Planaria velata* has already been studied by Child by means of the susceptibility method and through carbon-dioxide production (4), (5), (6), (7). A significant difference exists between the results obtained by these two methods. The susceptibility continually increases during the period of starvation and is not increased by feeding, while both the carbon-dioxide production and the oxygen consumption

are increased by feeding, decrease during the earlier stages of starvation, and increase only in the later stages of starvation. As already emphasized in the introductory remarks, susceptibility concerns chiefly the metabolic condition of the superficial structures of the body. The metabolic rate of these structures is then not increased by feeding but increases throughout the period of starvation. On the other hand, the susceptibility of the intestine, which can be observed separately in Planaria, is increased by feeding, falls after feeding, and rises again in the later stages of starvation. The susceptibility of the intestine therefore runs parallel to the total metabolism as measured by both carbon dioxide production and oxygen consumption. It is therefore certain that the increase in the rate of respiratory exchange seen after feeding in Planaria is due entirely to the increased activity of the digestive apparatus and the subsequent decrease in total metabolism during the early stages of starvation is due to the decreased activity of the digestive tract.

The data at hand permit us to draw certain conclusions as to the factors involved in the increase of metabolic rate after feeding. I have already mentioned that the effect of feeding on total metabolism decreases rapidly so that within twenty-four hours after a single feeding, the rate had fallen to a marked extent. Now it is certainly impossible for Planaria to digest a meal completely in this short length of time. The intestine of Planaria remains filled with food undergoing digestion certainly for as long as a day after feeding and probably much longer. If Planaria is fed on blood clot, the corpuscles can still be recognized within the intestine on the following day. If these worms are fed at intervals of two days, only a portion of them will take food on each occasion. Hence it is fairly certain that in Planaria the rise in rate of oxygen consumption after feeding cannot be attributed to assimilation or similar anabolic processes, since such processes would be going on even more rapidly twenty-four hours after feeding than a few hours after feeding; yet by the next day the oxygen consumption has fallen markedly. Further any increase in assimilative processes ought to be detectable in other parts of the body also but the susceptibility method demonstrates that the metabolic rise following feeding involves the intestine only. One may therefore conclude that in Planaria the increase in metabolic rate following feeding is attributable to the activity of the intestine, including probably such processes as the secretion of digestive enzymes by the entoderm, amoeboid movements of the entoderm cells, and absorption of food through the entoderm, all processes which

are known to involve an increased rate of respiratory exchange. It is probable, however, that all of these activities combined are not adequate to account for the increase observed. One must therefore postulate a *direct stimulation* of the entoderm cells by the food or its products, causing an increase in the intrinsic metabolic rate of these cells, not due either to formation of new protoplasm or to oxidation of the food products, although the latter process may of course play some rôle. A similar conclusion has been reached in regard to mammals, as will be considered below.

All three methods, susceptibility, carbon-dioxide production and oxygen consumption, demonstrate that in Planaria the total metabolism is markedly increased in long-continued starvation. The relative susceptibilities of fed and starved animals have been tested by Child not only with cyanide, alcohol and other anesthetics, but also recently with lack of oxygen, a method to which none of the objections possible with the toxic solutions can be raised. It may therefore be concluded that starved worms are in a metabolic condition identical with that of young worms, and that starvation is a means of rejuvenescence. This matter has been so fully treated in Child's book that further discussion of it is superfluous.

The oxygen consumption in starvation rises much more rapidly than does the carbon-dioxide production, and runs more in accord with the results obtained by the susceptibility method than does the latter. It may therefore be suggested that the chemistry of the oxidation processes is different during starvation than in a condition of adequate nutrition. The oxygen consumption rises but the carbon-dioxide production increases less rapidly so that the organism is apparently carrying on oxidations which do not involve evolution of carbon-dioxide. Perhaps this is due to the fact that it is using its own tissues as a source of energy while ordinarily it is using ingested food. It seems reasonable to suppose that the former can be burned more economically than the latter.

Experiments on the metabolism of other animals in relation to feeding and starvation are scanty in number, with the exception of mammals. Among the Protozoa, tests have been made on Paramecium only. Barratt (8) first showed that the carbon-dioxide production of Paramecium is decreased during the early stages of starvation. Recently Lund (3), (9) has confirmed this observation and has performed more extended experiments on the rate of carbon-dioxide production and oxygen consumption in feeding and early starvation in this organism.

The rates of both processes were increased by feeding and decreased in early starvation. Susceptibility to cyanide (of the ectoplasm) was not increased by feeding but increased in starvation. The rise in metabolic rate following feeding is therefore as in Planaria due solely to the activity of the entoplasm, and the fall in rate in early starvation is attributable to decreased activity of the entoplasm. The results with Paramecium as far as they go are therefore identical with our results on Planaria. Lund's experiments were not carried on for a sufficient length of time to determine whether or not there is an increased metabolism in prolonged starvation; this is probably impractical as one would have to know the weight of the organisms. Lund states that the increase in metabolic rate after feeding is the result of "ingestion, digestion and assimilation of the food by the cell leading to growth." It is probable that the activity involved in Paramecium in sweeping food into the gullet and in the formation of food vacuoles would account for a small part of this increase. Digestion may be ruled out, as digestion is chemically a process of hydrolytic cleavage involving neither oxygen consumption nor carbon-dioxide production. It is probable that what Lund really means is the secretion of digestive enzymes, an activity undoubtedly involving an increase in respiratory exchange. Whether assimilation is concerned in the result cannot be decided from the data presented, since the animals were supplied with food throughout the experiment and were continually ingesting food throughout the experiment. If assimilation and growth are really involved in the increased metabolism noted, then they must affect the ectoplasm also. However, the susceptibility method showed that the metabolic rate of the ectoplasm was not increased by feeding, but only by starvation. It may further be pointed out that assimilation as commonly understood and certainly that resulting in the formation of new protoplasm is chemically a process of dehydration, involving no increase in rate of respiratory exchange. It seems very likely therefore that in Paramecium the factors mentioned by Lund are not adequate to account for the increased metabolism observed in feeding but that as in Planaria and as has been concluded in regard to mammals, a direct stimulation of the digestive apparatus by the food is involved. Oxidation of the products of digestion may be a factor also.

The effects of feeding and starvation on Hydra have been tested in this laboratory but by the susceptibility method only. We have found (10) that in this animal the ingestion of food causes a local

increase in susceptibility. This increase is confined solely to the small region of the column which contains the food and the susceptibility of the rest of the animal is unaffected. Soon after ingestion, the susceptibility falls in this local region. The susceptibility of the entire animal is greatly increased by starvation (one or two weeks), and such starved animals resemble in susceptibility and behavior newly released buds. Conditions in Hydra are therefore like those in Planaria.

Some data on other coelenterates are available also through the work of Vernon (11). In some of these forms the rate of respiratory exchange was tested on successive days without food but the experiments were unfortunately carried on for a few days only. In two species of ctenophores and two species of Salpa (a pelagic tunicate), the rate of respiratory exchange was found to increase daily when the organisms were kept without food. Such an immediate increase in the absence of food has not been noted in other organisms but may be accounted for in these forms by their extreme delicacy and lack of solid structures which do not permit the storage of excess food; they are hence probably in a state of starvation very soon after cessation of feeding. In other similar coelenterates, two kinds of medusae, the rate of respiratory exchange remained about the same during the period tested, three to four days without food. In these watery coelenterates, then, the metabolic rate ceases to fall or may even rise in very short periods of starvation.

In other marine organisms, nudibranchs, octopus, Amphioxus and teleosts, Vernon noted a fall in metabolic rate in the early days of starvation. These forms, whose anatomy permits of food storage, are therefore similar to Planaria, as far as the data permit a comparison.

For some time I have been studying the effect of starvation on the metabolic rate of the smaller aquatic oligochaetes, chiefly Tubifex. In this worm, the susceptibility to cyanide increases continuously throughout a period of starvation extending over two months. The carbon-dioxide production, however, falls in the early days of starvation, and increases later. This increase occurs much earlier than in the case of Planaria, a fact which we attribute to the simpler structure of the digestive tract in Tubifex as compared with Planaria. In general, however, except for this detail, the metabolic conditions in these worms, in relation to starvation, are like those found in Planaria.

In fish, Wells (12) has tested the susceptibility to cyanide during starvation. The susceptibility was found to decrease during the early

part of the starvation period and to increase subsequently. Here, contrary to the findings on the lower organisms, the decrease in metabolism in early starvation shows up with the susceptibility method. In the lower forms, it is the susceptibility of the intestine alone which exhibits such a decrease. In these lower forms, susceptibility is measured by the time of death and disintegration of the body surface; in fish by the time of death of the organism as a whole. In this latter case, as Child has suggested, the time of death is probably a more definite measure of the total metabolic rate, since in vertebrates, the cyanide circulating in the blood causes death chiefly by its effect upon internal parts. In fish, then, as in lower invertebrates, the metabolic rate is increased in prolonged starvation.

The data upon mammals are so extensive that it would be out of place to attempt to review them in this brief paper. They are adequately presented in Lusk's *The Science of Nutrition*, in his various papers on animal calorimetry published in the *Journal of Biological Chemistry*, and in the publications of the nutrition laboratories at Middletown and Boston. This work on mammals agrees with the work on the lower forms. In mammals, the metabolic rate is increased after the ingestion of food. This increase is greatest in the case of ingestion of protein, less in the case of carbohydrates or fats. The cause of the increased metabolism after protein ingestion has been carefully investigated by Lusk and his associates (13) who came to the conclusion that a direct stimulation of the cells by the metabolic products of amino-acids was responsible for the results. Only certain of the amino-acids have this "specific dynamic action." Neither processes of deamination nor direct oxidation of ingested protein by the cells could be regarded as responsible since the increase was greatest shortly after ingestion of the protein or amino-acids before a sufficient time had elapsed, as shown by urinea nalysis, for the metabolizing of the ingested materials to have occurred. Some carbohydrates, such as glucose, also increased the metabolic rate, and the same was true of fat. In both of these cases, the increase was probably due to an actual oxidation of the foods after they had reached the cells (14). In mammals, then, ingestion of food almost invariably increases the metabolic rate, either through a direct stimulating effect of the constituents of the food upon the cells, or through the oxidation of the newly available nutritive molecules. The activity of the digestive glands and digestive apparatus apparently plays a negligible rôle in the observed metabolic increase.

In mammals as in other forms the metabolic rate soon begins to fall after ingestion. In the experiments quoted above the maximum effect in the case of protein food was observable two to three hours after ingestion, remained high up to about fourteen hours after ingestion, and then gradually fell. The carbon-dioxide production and oxygen consumption began to fall within eight to ten hours after ingestion. In the case of carbohydrate food, the increase is at the maximum during the second to fifth hours after ingestion, and in the case of fat attains a maximum by the sixth hour.

Studies on prolonged fasts in mammals have shown that the metabolism continues to fall during the early days of the fast. Soon however the metabolism attains a nearly constant level and after that begins to rise. The carbon-dioxide production, the oxygen consumption and the total heat production, as calculated per kilogram of body weight, rise in the later periods of prolonged fasts. These data are given in Benedict's report on a fast of thirty-one days by the subject L (15) and in this publication similar results are reported from prolonged fasts in dogs. In L, it is also important to note that the rate of the heart beat was distinctly accelerated toward the end of the fast.

In mammals, therefore, including man himself, the effect of starvation is the same as in Planaria and other lower organisms. As a result of starvation, all organisms are restored to a metabolic condition resembling that of younger animals. It has been the general impression of men who have undergone long fasts, and observations on dogs confirm this, that they are in a better physiological state after than before fasting.

One point still remains to be discussed. It might be maintained that the increased metabolism per unit of weight observed in fasting is only apparent, since it might be supposed that the decrease in weight was due solely to a loss of non-respiring materials. If, however, this were the case, the metabolism as calculated per unit body weight ought to increase in the early days of the fast when this non-respiring material is disappearing most rapidly. This, however, is not the case. Further, the heart loses less material than almost any other part of the body, and hence the increase in the rate of the heart beat in starvation cannot be attributed to loss of non-respiring materials but must represent a real increase in the basic metabolism of the heart protoplasm. That the intensity of cellular activity is really increased in fasting is the conclusion reached by Benedict from his study of the metabolism of L in fasting.

The increased cellular metabolism in fasting is probably, as Child has long maintained, due to the removal from the cells of reserves, structures and deposits of one kind or another which interfere with metabolic processes. The removal of these hindrances to metabolism restores the cell to an undifferentiated condition like that of young cells and permits a metabolic activity like that of such cells.

SUMMARY

1. The oxygen consumption of Planaria is increased markedly after the ingestion of food.

2. This increase is maintained only for several hours after ingestion of food, and the oxygen consumption then begins to fall. By the following day the metabolism has decreased again to a marked degree.

3. The oxygen consumption continues to fall in the early days of starvation, reaching a minimum value within the first two weeks.

4. The oxygen consumption then begins to rise and at the end of a period of prolonged starvation it is much higher than in animals starved only a few days.

5. Data quoted from the work of other investigators show that in other organisms the effects of feeding and starvation on metabolic rate are essentially the same as in Planaria.

6. It may therefore be concluded that starvation increases the metabolic rate of organisms, and that starved organisms are metabolically in a condition similar to that of young organisms.

BIBLIOGRAPHY

(1) Hyman: This Journal, 1919, xlviii, 340.
(2) Child: This Journal, 1919, xlviii, 372.
(3) Lund: This Journal, 1918, xlvii, 167.
(4) Child: This Journal, 1919, xlviii, 231.
(5) Child: Biol. Bull., 1913, xxv, 181.
(6) Child: Arch. f. Entw.-Mech., 1911, xxi, 537.
(7) Child: Arch. f. Entw.-Mech., 1914, xxxviii, 418.
(8) Barratt: Zeitschr. f. allg. Physiol., 1905, v, 66.
(9) Lund: This Journal, 1918, xlvii, 318.
(10) Child and Hyman: Biol. Bull., 1919, xxxvi, 183.
(11) Vernon: Journ. Physiol., 1895, xix, 18.
(12) Wells: Biol. Bull., 1916, xxxi, 441.
(13) Williams, Riche and Lusk: Journ. Biol. Chem., 1912, xii, 349; Lusk: Ibid., 1912, xiii, 155.
(14) Lusk: Journ. Biol. Chem. 1912, xiii, 27; Murlin and Lusk: Ibid., 1915, xxii, 15.
(15) Benedict: Carnegie Inst. of Washington Publ., 1915, no. 203.

SUSCEPTIBILITY TO LACK OF OXYGEN DURING STARVATION IN PLANARIA

C. M. CHILD

From the Hull Zoölogical Laboratory, the University of Chicago

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In the course of investigation of the physiological condition of *Planaria dorotocephala* during starvation, the following facts have already been ascertained and recorded in earlier papers: first, that the susceptibility of ectoderm and body wall to concentrations of cyanide and various other agents which are lethal within a few hours increases during starvation (1), (3); second, that susceptibility of the alimentary tract to the same agents decreases rapidly in relation to that of the body wall during the earlier stages of starvation, but later shows some increase (5); third, that CO_2 production also decreases rapidly at first, but later shows some increase (5); fourth, that starved animals appear to be extremely sensitive and show distinctly less capacity for acclimation to certain low concentrations of cyanides and other agents than fed animals, this capacity decreasing during the course of starvation (1), (3). Numerous facts indicate that susceptibility to cyanides and many other toxic agents in sufficient concentration to kill without permitting any marked degree of acclimation or development of tolerance is in a general way and under at least a wide range of conditions, a rough criterion of metabolic condition, the susceptibility varying directly with, though not necessarily proportionally to the rate of metabolism or probably oxidation (2), (3), (4). Consequently the increase in susceptibility of ectoderm and body wall during starvation suggests that the rate of oxidation in these organs increases as starvation progresses, while the decrease in susceptibility of the alimentary tract during the early stages of starvation suggests a decrease in rate in this organ in the absence of food. It has been pointed out in another paper (5) that the data on susceptibility and those on CO_2 production during starvation are not in conflict when the alimentary tract is taken into account. It has been shown that the marked decrease in CO_2 production during the first few days of starvation is evidently due, at least in large part, to decrease in the

metabolic activity of the alimentary tract in the absence of food, and that in advanced stages of starvation, when the alimentary tract, as well as other organs, is undergoing reduction, there is a distinct increase in total CO_2 production. The conclusion suggested by the experimental data on susceptibility and CO_2 production is that ectoderm and body wall, which maintain or even increase their functional activity during starvation, show an increase in oxidation rate, while the alimentary tract at first undergoes a marked decrease in rate in consequence of decreased function, but later, when undergoing reduction, may also show some increase in rate. According to this interpretation, the increase in susceptibility of ectoderm and body wall during starvation and the decrease in CO_2 production during earlier stages, followed by increase in later stages, are not in any way contradictory. Susceptibility of ectoderm and body wall is a criterion of conditions in these parts only, while CO_2 production is a measure of conditions in the body as a whole. Since the CO_2 production of the alimentary tract is evidently a large part of the total, the changes in the alimentary tract are the chief factor in determining the changes in total CO_2, and only in later stages of starvation, when the alimentary tract is undergoing reduction, is the increase in CO_2 production sufficient to balance the earlier decrease, resulting from decrease in functional activity in the alimentary tract. At present this seems to be the only logical and satisfactory interpretation of the facts.

Whatever the course of the changes in physiological condition during starvation in *Planaria*, it is certain that animals which are again fed after a period of starvation are, as regards susceptibility, capacity for acclimation, CO_2 production, rate of growth and all other distinguishable characteristics, physiologically younger than at the beginning of the starvation period (1), (3) (4, chap. VII), (5), being in approximately the same physiological condition as well-fed growing animals of their own size or somewhat smaller. In other words, the decrease in size during starvation serves roughly as a measure of the degree of regression or rejuvenescence which the animals have undergone.

The present paper is concerned with another aspect of the problem of starvation and its effects in *Planaria*, viz., the changes in susceptibility to lack of oxygen. Since oxygen is essential to the life of *Planaria*, susceptibility to lack of oxygen may be expected to show some relation to the fundamental metabolic condition. Moreover, various authors have observed that the action of cyanides seems in certain respects to be similar to that of lack of oxygen and I have found that cyanide in-

creases susceptibility to lack of oxygen, i.e., cyanide and lack of oxygen are additive in their action (7). And finally, susceptibility to lack of oxygen is the reaction of the animal to the absence of a factor essential for its fundamental metabolism, rather than to the presence of a toxic agent, and the possible complicating conditions involved in the use of toxic agents for determination of susceptibility are absent.

METHOD

Since the chlorination of the Chicago city water supply renders this water injurious to *Planaria*, water pumped from a well is used for all stocks and all experiments with the animals. This water, which before exposure to the air has a very low oxygen content, usually $\frac{1}{10}$ cc. per liter or less, has served as the basis for the experiments on susceptibility to lack of oxygen, but the procedure has been modified in various cases by introducing more or less oxygen into the water and thus prolonging the survival time.

The usual method of procedure is as follows: the animals to be compared, e.g., well-fed animals and animals starved a certain length of time, or starved animals and animals fed after starvation, etc., are placed together in a pyrex tube about 10 mm. in diameter, closed by fusion at one end, holding 5 to 6 cc. of water and calibrated in cubic centimeters, and this tube is sealed without air bubbles at any desired level, so that the animals are confined in 1, 2 or more cubic centimeters of water. Since the data are purely comparative, the placing of the two or more lots to be compared in the same tube provides identical conditions as regards oxygen for all the animals and avoids many complications. Since the animals to be compared in each case are in the same water it maks no essential difference whether the different lots are of equal weight, and in consequence of the oxygen consumption of all the animals in the tube the oxygen concentration is undergoing continuous decrease and the most susceptible animals must die first. Of course the actual survival time of the lots and the amount of difference between them will vary with different conditions in the tubes but the essential point, i.e., whether one lot is more or less susceptible than another, is readily determined by this method. It is not even necessary to know the oxygen content of the water in a particular experiment, if the precautions mentioned below concerning CO_2 are observed.

Several methods of sealing the tubes have been used. One of these which avoids exposure of the water to air, consists in the insertion of

a small tight plug of absorbent cotton to any desired level in the tube while both tube and cotton are held well below the surface of a large volume of the water coming directly from the pump and then sealing above the cotton with melted paraffine. Since leakage occasionally occurs around the paraffine, particularly if changes in temperature occur, the entrance of air is prevented by placing a tightly fitting soft rubber tube 2 to 3 cm. long over the open end of the glass tube, filling both glass and rubber tube with the water used and closing with a screw clamp. Air may of course be excluded by the rubber tube and clamp alone, but the animals are likely to creep into the rubber tube out of the light and die there, and since they tend to stick to surfaces when they begin to die they cannot readily be removed to the glass tube, where they can be seen. The cotton plug serves merely to avoid exposure of the water in the tube to the air and to remove the possibility of injuring the animals by temperature changes when the melted paraffine is run in. If care is taken to keep the animals at the bottom of the tube the paraffine may be run in directly on the surface of the water, though this necessitates exposure of this small surface to the air for a short time.

In most cases lots of 3 to 5 worms each are used, and when starved and well-fed animals are compared there is no difficulty in distinguishing the animals of the two lots, even when they are of the same size. In cases where it may be difficult to distinguish animals of different lots, the tips of the posterior ends of one lot may be cut off. This or some other slight injury does not alter the susceptibility to any appreciable degree, except in the immediate region of the injury.

Since the animals not only consume oxygen, but produce CO_2, it is necessary to make certain that death results from lack of oxygen and not from accumulation of CO_2. The well water when first pumped contains a slight excess of CO_2, and pH = 7.4 $\pm$. It was determined experimentally by running washed CO_2 into flasks of well water containing a little of the proper indicator and some planarians and sealing at different values of pH, that death of the animals from CO_2 does not begin to occur until pH = 5.5 to 5.0 approximately. Susceptibility to CO_2, as to other agents, decreases with advancing age and differs with nutritive and other conditions, so that the minimum lethal concentration of CO_2 differs somewhat for different animals. By adding to the water a little of the proper indicator it is possible to determine the pH at which the animals die in water containing various amounts of oxygen. In this way it has been found that in the low oxygen water

directly from the well they usually die while pH > 7.0, while with the various degrees of aeration used, death almost always occurs before the pH has decreased below 6.0. In other words, even in the aerated water the animals die before they have produced enough CO_2 to kill any of them and in the low oxygen water they die when or before the accumulation of CO_2 has decreased the pH to neutrality. As regards the low oxygen water then there can be no doubt that it is lack of oxygen, not CO_2, that kills, and even in the aerated water lack of oxygen is at least the chief if not the only factor in producing death, although the increasing H ion concentration may alter the amount of difference in susceptibility of the different lots.

In many series, however, a little NaOH has been added to the water in order to avoid all possibility of injurious H ion concentration before the animals are killed by lack of oxygen. With the low oxygen water NaOH is unnecessary but, as noted above, in aerated water, the H ion concentration sometimes approaches the danger point before the animals die from lack of oxygen. The lower limit of lethal concentration of NaOH for the animals is between m/450 and m/500 so that the well water may be made up to any concentration below m/500 with the certainty that the NaOH is not itself lethal. The well water made up to NaOH m/500 has a pH = 9 to 9.5 according to the amount of CO_2 in the water and with lower concentrations of NaOH the pH of course lies between 9.5 and 7.4. The addition of NaOH to the water increases the susceptibility to lack of oxygen even when the concentration is far below the lethal limit, e.g., m/1000 or m/2000, and in general the survival times in water with a given oxygen content decrease as the NaOH concentration increases. In the higher concentrations of NaOH, e.g., m/750 made up in low oxygen water the animals may die from lack of oxygen at pH 8.6 or above and in aerated water at pH 7.0 or above. The use of NaOH in this way makes it possible to bring about death from lack of oxygen at any point within a considerable range of pH on the alkaline side of neutrality.

As might be expected, it is found that the amount of difference in susceptibility of two lots of animals differs with the volume of water used, the oxygen content, the number and size of the animals in the water, the pH at the beginning and the amount of change in pH before death occurs, and some of these factors are mutually related. In no case, however, within the limits indicated, do these factors alter the general susceptibility relations of the lots compared, i.e., the susceptibility of a lot in a particular physiological condition is always greater than

that of another lot in a different condition, in all the modifications of the experimental method described, though the amount of difference may vary to a considerable degree with the different conditions of experiment. Some of these differences will appear in the data presented below, but since the paper is primarily concerned with the effect of starvation on susceptibility to lack of oxygen, the point of chief importance is the increase or decrease in susceptibility with change in nutritive condition, and the degrees of difference with different ranges of H ion concentration and other experimental conditions have to do with other problems. In all cases recorded below where susceptibilities are directly compared, the experimental conditions, except as regards nutrition or physiological age, are either identical or the differences are noted. With very few exceptions only lots in the same tube are directly compared.

Two criteria may be used in determining susceptibility to lack of oxygen; first, the loss of muscular coördination with consequent loss of ability to attach the body to the glass, and the cessation of motor activity which soon follows; second, by the disintegration of the body, both methods giving similar comparative results. As regards both these changes, the body of each individual, except in the more advanced stages of starvation, shows a gradient in susceptibility similar to the gradient in susceptibility to cyanides and other agents, the head region being most susceptible (6). In the later stages of starvation the body is usually almost as susceptible as the head, sometimes more so.

The data on susceptibility are most simply presented in graphic form, and the method adopted for graphing is briefly as follows: since the effects of lack of oxygen, both as regards cessation of movement and disintegration usually begin at the head and progress in an orderly manner along the body, it is possible to distinguish more or less arbitrarily three or four stages in the course of each of these processes. Assigning to each of these stages a numerical value, e.g., stage I, 10; stage II, 20, etc., it is then possible to obtain a numerical value for the average condition of each lot of worms at any given time during the experiment by multiplying the number of worms in each stage by the numerical value for that stage, adding the products and dividing their sum by the number of worms in the lot. These values may then be plotted as ordinates against times as abscissas. By measuring the ordinates downward from a distance above the axis of abscissas equal to the longest possible ordinate, the curves of susceptibility appear as descending curves which reach the axis of abscissas when all worms of the lot have attained the final stage, or by reversing the order of the numerical values

of the stages and measuring upward from the axis of abscissas the same result is obtained. In the following figures the curves are all drawn as descending curves. This method of graphing susceptibility data is described in detail elsewhere (4, p. 81, footnote). With this method the difference in time measured on the axis of abscissas between the upper end and the base of the curve represents the entire time from the beginning to the end of the process of change concerned, either loss of motility or disintegration, and the course of the curve represents in a general way the progress of the change from the head along the body. The less the distance of the curve from the axis of ordinates, the greater the susceptibility, and vice versa. The following graphs are plotted in this way, the time intervals indicated on the axis of abscissas being two hours each and the divisions on the axis of ordinates representing the stages.

It should be noted that these data on susceptibility to lack of oxygen concern ectoderm and body wall and probably the nervous system, but not the alimentary tract. For the loss of motility, changes in cilia, muscles or the nervous system or all three are responsible, but as regards disintegration, oxygen must pass through the body wall to reach the alimentary tract, and when the oxygen content of the water becomes so low that it is not sufficient to maintain the life of ectoderm and body wall, it is probable that very little oxygen is reaching the alimentary tract. As a matter of fact the alimentary tract appears to disintegrate at about the same time as the body wall, but under the conditions of the experiment this fact can have little or no significance.

The standardization of starvation stocks has been discussed in an earlier paper (5), and it need only be said here that the methods of standardization described have been followed with all material used in these experiments, in order that all animals of a given lot should be as nearly as possible alike, not only as regards size and nutritive condition, but as regards the occurrence of fission and regulation.

THE INCREASE IN SUSCEPTIBILITY DURING STARVATION

It may be stated at once that all experiments performed, twenty-two in number, each consisting of two or three lots of from three to five worms each in the same tube or small flask, show that susceptibility of ectoderm and body wall to lack of oxygen increases during starvation, at least up to four months, longer periods not having been used. Since the results are essentially the same in all cases, the differences being

merely differences in the degree of susceptibility, dependent on the conditions of different experiments, it is quite unnecessary to give data or graphs for all series. The following series show the character of the results and some of the differences under different experimental conditions.

Series 708. Figure 1.

Lot I. 3 animals 15 mm. Well fed.

Lot II. 3 animals, originally 15 mm., starved 37 days. At time of experiment 12 to 13 mm.

Lot III. 3 animals, originally 15 mm., starved 99 days, now 7 mm.

These three lots were sealed in a tube in 3 cc. of m/1000 NaOH in well water of low oxygen content.

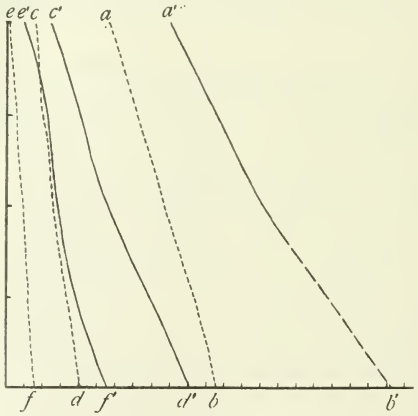

Fig. 1

The curves *ab*, *cd*, *ef* (fig. 1) show the susceptibility to lack of oxygen of lots I, II and III respectively in terms of loss of motor activity and the curves *a'b'*, *c'd'* and *e'f'* show the susceptibility in terms of disintegration. The broken line in the lower part of *ab* indicates that observations were concluded before all animals of this lot were entirely disintegrated.

The fed animals of lot I are least susceptible, those of lot II, starved 37 days, much more susceptible and those of lot III, starved 99 days, most susceptible to lack of oxygen, both as regards loss of motor activity and disintegration. Moreover, the relative susceptibilities of the different lots are very nearly the same in terms of loss of motor activity as in terms of disintegration. This is a perfectly characteristic series. With higher oxygen content, greater volume of water or lower alkalinity the susceptibility curves lie farther to the right and farther apart than in figure 1 but their relative positions are not greatly changed.

Series 614. Figure 2.

Lots I a, II a. Each 3 animals, 25 mm., well fed.

Lots I b, II b. Each 3 animals originally 25 mm.; starved 39 days and underwent fission once during starvation; length at time of experiment 12 to 14 mm.

Lots I a and I b were sealed in one tube in 5 cc. NaOH m/3000 and lots II a and II b in another tube in 5 cc. NaOH m/2000, both slightly aerated.

The curves in figure 2 show the susceptibilities in terms of disintegration as follows: *ab* lot I a; *cd* lot I b; *ef* lot II a; *gh* lot II b. In both pairs of lots the animals starved 39 days are far more susceptible than the well-fed animals.

Since the two pairs of lots were in two different tubes, they are perhaps not strictly comparable, although, except as regards concentration of NaOH, conditions in the two tubes must have been very nearly identical, both containing the

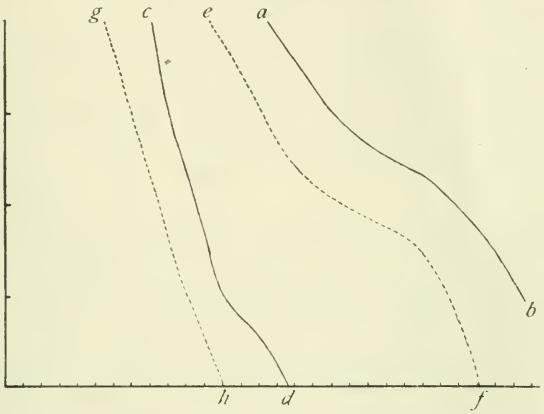

Fig. 2

same number of worms of the same two sizes and the same nutritive conditions in the same volume of water. Figure 2 shows that the susceptibility in NaOH m/2000 (curves *ef*, *gh*) is somewhat greater than in NaOH m/3000. While slight differences in weight, physiological condition, oxygen consumption or oxygen content may exist in the two tubes, the differences in susceptibility indicated by the differences in position of the two pairs of curves, is too great to be the result of such incidental differences and is undoubtedly due, at least in large part, to the difference in concentration of NaOH in the two tubes, the susceptibility being greater in the higher concentration. Other series have given similar results.

Series 715. Figure 3.

Lot I. Posterior halves of animals originally 25 mm. Starved 59 days; underwent regulation to whole animals during early stages of starvation; at time of experiments 8 to 9 mm.

Lot II. Posterior halves of animals originally 25 mm. Starved 121 days; underwent regulation to whole animals during early stages of starvation; at time of experiment 4 to 5 mm.

The two lots of three animals each were placed together in a tube in 2 cc. of low oxygen water made up approximately to NaOH m/500 with as little exposure to air as possible and containing 1/150,000 phenolsulphonephthalein.

Curve *ab* of figure 3 shows the disintegration of lot I, curve *cd* that of lot II, the more advanced stage of starvation being the more susceptible. The high susceptibility of both lots is due to the high concentration of NaOH. This concentration, m/500, was not lethal for animals from the same stocks as the experimental animals in a tube open to air. In this experiment the animals died from lack of oxygen before the pH had fallen below 8.6.

Fig. 3

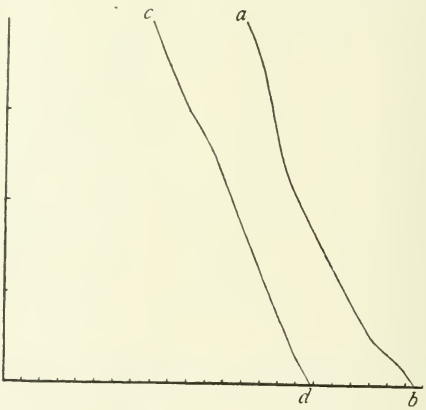

Fig. 4

Series 737. Figure 4.

Lot I. Four animals 16 mm., well fed.

Lot II. Four animals originally 16 mm., starved 18 days; not appreciably reduced in length.

Both lots together in a tube in 7 cc. water containing 1/150,000 phenolsulphonephthalein, but no alkali; water aerated; pH at beginning 7.9.

The curve *ab* in figure 4 shows the disintegration of lot I, the fed animals, curve *cd* that of lot II, the animals starved 18 days. As in other experiments the susceptibility of the starved is greater than that of the fed animals. Since no alkali was added in this series and the water contained considerable oxygen, it became distinctly acid, pH 6.0, before all the animals died. Under these conditions the susceptibility of both lots is less and the difference between the two lots greater than in water with very low oxygen content or with higher alkalinity.

The point of chief interest, however, is that, even with the short starvation period of eighteen days and with no appreciable decrease in size, the susceptibility of the starved animals is distinctly higher than that of the fed animals which represent as nearly as possible the condition at the beginning of starvation.

DIFFERENCES IN SUSCEPTIBILITY TO LACK OF OXYGEN BETWEEN YOUNG AND OLD ANIMALS

It has been shown elsewhere that susceptibility to KNC and rate of CO_2 production decrease in *Planaria* with advancing physiological age and that starving animals show changes in the opposite direction (3), (4), (5), i.e., according to these criteria of physiological age the starving animals become younger. The data of the preceding section show that susceptibility to lack of oxygen increases during starvation and in order to determine whether in this respect also the starving animals undergo changes in the opposite direction from those of progressive development and advancing age, it is necessary to determine what changes in susceptibility to lack of oxygen occur in relation to age in well-fed growing animals.

Comparative determinations of susceptibility to lack of oxygen in animals of different age have been made many times and by different persons in this laboratory, the experiment having been used in laboratory classwork. In these experiments where all animals are well fed, size of animals is used as the most satisfactory criterion of physiological age, for the larger animal has undergone more growth and if a progressive senescence occurs in the species, the larger animal must be physiologically older than the smaller. In all experiments with well-fed animals, the smaller younger individuals are more susceptible to lack of oxygen than the larger and older. Graphs of a few characteristic experiments are given in figure 5.

Series 665.

Lot I. 5 animals, 25 mm. Lot II. 5 animals, 6 to 7 mm.

Both lots from well-fed laboratory stock together in 125 cc. of slightly aerated water. Curve *ab* shows susceptibility of lot I, the old animals, curve *cd* that of lot II, the young animals. The long survival times, 65 hours for lot I and 31 hours for lot II, are due to the large volume of water.

Series 664.

Lot I. 5 animals, 20 to 25 mm. Lot II. 5 animals, 7 mm.

Both lots from well-fed laboratory stock, together in 125 cc. of low oxygen water made up to NaOH m/2000 with as little exposure to air as possible. Curve *ef* shows susceptibility of lot I, curve *gh* that of lot II.

Series 751.

Lot I. 5 animals, 18 mm. Lot II. 5 animals, 7 mm.

Both lots from a stock freshly collected in midwinter and fed only three times in the laboratory. Such worms contain less nutritive reserves than animals which have been a long time in the laboratory. Both lots together in 5 cc. of water made up to NaOH m/750 and slightly aerated. Curve *ij* shows susceptibility of lot I, curve *kl* that of lot II.

Series 754.

Lot I. 5 animals, 20 mm. Lot II. 5 animals, 5 mm.

From same stock as preceding series. Both together in 3 cc. of low oxygen water. Curve *mn* shows susceptibility of lot I, curve *op* that of lot II

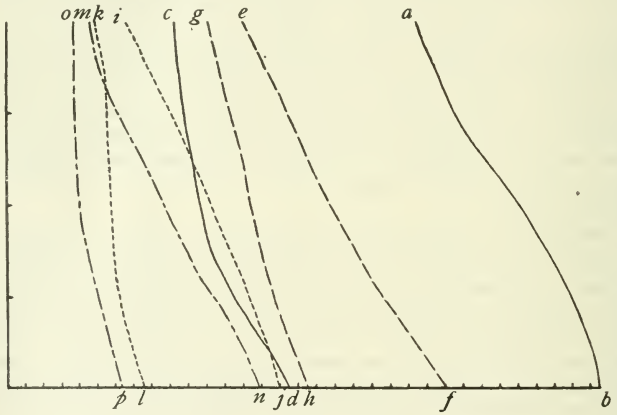

Fig. 5

In these four series the ages compared, the volume, oxygen content and alkalinity of the water differ, consequently only the two curves of each pair are comparable, but in all series the younger animals are distinctly more susceptible to lack of oxygen than the older, though the actual susceptibility and the degree of difference differ with the conditions. There can be no doubt that this difference in susceptibility to lack of oxygen between animals of different size and therefore of different physiological age, indicates a real difference in physiological condition. Moreover, since these data make it clear that susceptibility to lack of oxygen decreases with advancing age and since it was shown in the preceding section that susceptibility to lack of oxygen increases during starvation, it is evident that the changes during starvation are

opposite in direction to those during growth and progressive development, i.e., as regards susceptibility to lack of oxygen, the starving animals become progressively younger.

COMPARISON OF STARVED AND FED ANIMALS OF THE SAME SIZE

The starving animals decrease in size and it is of interest to determine how the rate of increase in susceptibility to lack of oxygen compares with the rate of decrease in size. To obtain evidence on this point the starved, reduced animals are compared with well-fed, growing ani-

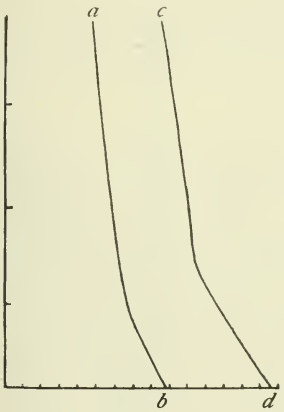

Fig. 6

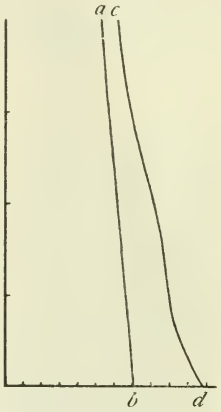

Fig. 7

mals of approximately the same size. Only five experiments of this sort have been performed, but the results are the same in all cases, the starved animals being somewhat more susceptible than the young fed animals. The data of three series are given.

Series 755. Figure 6.

Lot I. 3 animals, starved 42 days. Reduced from 8 mm. to 5 mm.

Lot II. 3 animals from well-fed laboratory stock; 6 mm. (smallest of stock).

Both lots together in 4 cc. of low oxygen water. Curve *ab*, figure 6, shows susceptibility of lot I, curve *cd* that of lot II. Here the fed worms of lot II were slightly larger than the starved animals of lot I.

Series 757. Figure 7.

Lot I. 3 animals, starved 42 days. Reduced from 8 mm. to 5 mm.
Lot II. 3 animals from well-fed laboratory stock; 5 mm.
Both together in 3 cc. low oxygen water. Curve *ab*, lot I; curve *cd*, lot II.
Series 761. Figure 8.
Lot I. 3 animals, starved 79 days. Reduced from 10 to 12 mm. to 4 mm.
Lot II. 3 animals from freshly collected stock fed three times in the laboratory; 4 mm.
Together in 3 cc. low oxygen water; Curve *ab*, lot I; curve *cd*, lot II.

In all three series the higher susceptibility of the starved worms is evident: the differences in the three are dependent upon the differences in size and nutritive condition of the fed, as compared with the starved

Fig. 8

Fig. 9

animals and upon differences in volume and oxygen content of the water, possibly also to some extent upon the stage of starvation (see p. 403).

Although in the earlier work on susceptibility to KNC attention was not particularly directed to this point, it was noted that the susceptibility of starved animals was frequently somewhat higher than that of fed animals of the same size (3, p. 433), and one series is given here.

Series 557 III. Figure 9.
Lot I. 10 worms, starved 81 days. Reduced from 10–12 mm. to 5–6 mm.
Lot II. 10 worms, well-fed; 5 to 6 mm. From freshly collected stock.
Curve *ab*, figure 9, shows the susceptibility of lot I, curve *cd* that of lot II to KNC m/1000.

With the method and scale of graphing, which is the same as that of the preceding figures, the differences in susceptibility appear to be slight, but actually the susceptibility of the starved worms is only about 75 per cent that of the fed animals of the same size. Similar differences have been observed in many other KNC series, but they seem to occur more frequently when the animals were of medium size at the beginning of starvation than when they were very large. It is possible that in the extreme stages of starvation which occur when very large animals are reduced by starvation to small size, the increase in susceptibility does not keep pace with the decrease in size, but further work is necessary to settle this point. It is certainly true that animals after one to three months of starvation are very commonly more susceptible to KNC and in all cases thus far observed are more susceptible to lack of oxygen than fed, growing animals of the same size.

CONCLUSION

The experimental data demonstrate that during starvation susceptibility to lack of oxygen increases, at least in ectoderm and body wall, the method not being suitable for determining conditions in the alimentary tract. This increase in susceptibility parallels very closely the increase in susceptibility to KNC which occurs during starvation. In both KNC and lack of oxygen it is primarily susceptibility of ectoderm and body wall that is determined, though in KNC the susceptibility of the alimentary tract may be roughly determined as differing either more or less from that of ectoderm and body wall (5, p. 253). The data on CO_2 production, however, represent CO_2 production of the alimentary tract as well as that of ectoderm and body wall, and it has been pointed out (5) that the decrease in CO_2 production during the earlier stages of starvation is very largely, if not wholly due to the decrease in metabolic activity of the alimentary tract in the absence of food. In the later stages of starvation even the total CO_2 production increases.

The data on susceptibility to lack of oxygen constitute a further contribution to the problem of starvation. The experiments with animals of different age show that susceptibility to KNC (3), (4) and lack of oxygen and rate of CO_2 production (5) decrease with advancing age, and Doctor Hyman has found that oxygen consumption also decreases during senescence. In short, all the evidence indicates that susceptibility to lack of oxygen in fed, growing animals varies directly with rate of oxidation. In starving animals susceptibility to KNC and to lack of

oxygen increases, CO_2 production also increases, except for the initial decrease noted above, and Doctor Hyman's work shows that oxygen consumption also increases after an initial decrease due to the same factor as the decrease in CO_2 production.

The only logical conclusion on the basis of all the evidence is that the rate of oxidation increases during starvation, in ectoderm and body wall from the beginning and in the alimentary tract in later stages after the initial decrease due to decreased functional activity. If this conclusion is correct, susceptibility to lack of oxygen is, as might be expected, in some degree a measure of rate of oxidation in ectoderm and body wall.

The only other possibility as regards the starving animals is that certain oxidative reactions which require a higher oxygen concentration than those characteristic of fed, growing animals, occur in increasing proportion as starvation advances, but there is at present no evidence in favor of this hypothesis. Apparently the starving planarian oxidizes its own substance with increasing rapidity as starvation advances and the products of metabolism accumulated during growth and progressive development are broken down and removed. The inactive alimentary tract undergoes atrophy and resorption more rapidly than ectoderm, body wall and nervous system and undoubtedly constitutes the chief source of nutrition for the other functionally active organs after the reserves are exhausted. As regards the rate of its fundamental metabolic reactions, the starving animal unquestionably becomes progressively younger, and when it is again fed after a period of starvation, it is in essentially the same physiological condition, as regards susceptibility to KNC (3), (4) and lack of oxygen, rate of CO_2 production (5) and oxygen consumption (Hyman), as well as rate of growth, as a fed, growing animal of the same size. If feeding is continued, such an animal passes again through the progressive stages of the life history of the species from the point at which feeding began. The experimental data on the effect of feeding after starvation have not yet been presented in detail and must be reserved for another paper.

SUMMARY

1. Susceptibility of ectoderm and body wall of *Planaria dorotocephala* to lack of oxygen, as measured either by loss of motility or by disintegration, increases progressively during starvation, up to at least four months.

2. The susceptibility to lack of oxygen of the animal reduced by starvation is about the same as, or slightly higher than that of a fed, growing animal of the same size.

3. The change in susceptibility to lack of oxygen during starvation is in the opposite direction from that which occurs during growth and progressive development in fed animals, and in the light of the facts already at hand concerning CO_2 production, oxygen consumption and susceptibility to KNC, must be considered as evidence of an increase in rate of oxidation during starvation.

BIBLIOGRAPHY

(1) Child: Arch. f. Entwickelungsmech., 1911, xxxi, 537.
(2) Child: Journ. Exper. Zoöl., 1913, xiv, 153.
(3) Child: Arch. f. Entwickelungsmech., 1914, xxxviii, 418.
(4) Child: Senescence and rejuvenescence, Chicago, 1915.
(5) Child: This Journal, 1919, xlviii, 231.
(6) Child: Arch. f. Entwickelungsmech., 1913, xxxvii, 158.
(7) Child: This Journal, 1919, xlviii, 372.

QUANTITATIVE STUDIES ON THE RATE OF RESPIRATORY METABOLISM IN PLANARIA

II. The Rate of Oxygen Consumption during Starvation, Feeding, Growth and Regeneration in Relation to the Method of Susceptibility to Potassium Cyanide as a Measure of Rate of Metabolism

GEORGE DELWIN ALLEN

From the Department of Animal Biology, University of Minnesota

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INTRODUCTION

A review of the literature concerning the rôle of what has been designated "rate of metabolism" in senescence, rejuvenescence and gradients along the body axes in lower animals ((1) to (9) and other references there cited), will show that little or nothing is known which is based on direct quantitative measurements of metabolism in the animals concerned. It has been assumed that relative susceptibility of an organism to the toxic action of potassium cyanide and other substances is a measure of rate of metabolism or rate of oxidation. This assumption has been used extensively as the basis for inferences regarding the metabolism in Planaria and other animals. The only attempts to measure the metabolism in these forms by methods which do not involve assumptions are certain tests reported by Child, (3, pp. 422, 434; 4, pp. 73, 161, 202) of carbon dioxide production by Planaria in Tashiro's "biometer." These estimations were few in number, comparative not strictly quantitative, and we cannot judge from the account how carefully they were controlled. The purpose of the present paper is to present quantitative information regarding the oxidations in Planaria in relation to starvation and feeding, increase in size of the body, and regeneration; and secondly, to compare these results with the reported differences in the susceptibility of Planaria to potassium cyanide.

The term metabolism is commonly used in animal calorimetry to refer to the decomposition of organic compounds (total katabolism)

yielding energy. It may be considered either from the standpoint of the quantities of the different foodstuffs decomposed and the completeness of this decomposition or from the standpoint of the quantities of energy yielded. The latter basis is used for expressing metabolism in a single quantitative term. Lusk, for example, says with regard to the "exact measurement of the metabolism":

> Thus heat may become a measure of the total activity of the body. It is derived from the total metabolism and must be dependent on it and be a measure of it (10, p. 32).

From this standpoint the oxidations are considered important as a source of energy and as a measure of the energy production. The term "rate of metabolism" seems to be used in this sense in some of the references on senescence and axial gradient, especially where studies of the metabolism of man and mammals are quoted (e.g., (4, pp. 63, 65, 271–273, 297–298)). The following statements seem to imply this meaning of the term.

> There can be no doubt that the rate of metabolism or, more specifically, of oxidations in these animals increases during the course of starvation (3, p. 435).
>
> It has been shown that a relation exists between susceptibility and metabolic activity, more specifically the oxidations or energy-freeing reactions (6, p. 50).

It is not certain, however, that the term is always used in the literature concerning "rate of metabolism" as equivalent to the rate of oxidations or of energy production. A distinction seems to be drawn between them in accordance with the following statement:

> While oxidations are fundamental metabolic reactions, and serve in a general way as a measure of metabolic activity, a considerable range of variation in the different reactions which go to make up the metabolic complex may undoubtedly exist (4, p. 72).

A fact which must be kept in mind in using the term metabolism is that while energy transformation in the cell may be large, the corresponding quantity of material transformed may be small. This is true of respiratory metabolism, while on the other hand in a large number of metabolic processes the quantity of material being transformed is relatively very large while the energy change is hardly measurable. As pointed out by Lund (11, p. 168):

It is very doubtful if the rate of oxidations as measured by carbon dioxide production or oxygen consumption can *even approximately*[1] be used as a measure of the rate of total metabolism (either of matter or energy[1]) in a cell, for it has never been shown that the speed of such processes as hydrolyses and changes in colloidal constitution are correlated to the speed of oxygen consumption or carbon dioxide production.

Furthermore, these processes are of a reversible character and matter and energy may pass through them repeatedly before appearing in the external exchanges of the animal. It is evident from these considerations that the terms metabolism and rate of metabolism may be and are used in different senses. On the one hand they may refer strictly to the rate of oxidations or more generally to the rate of energy production as measured by oxygen consumption, carbon dioxide production or heat liberation. On the other hand they may refer in a loose way to the rate of all metabolic processes, involving the complications just mentioned. These two usages are by no means equivalent. It is of vital importance to any profitable discussion of the rate of metabolism in organisms that it shall be clearly understood precisely what is under discussion. This matter cannot be over-emphasized. It is not a question of quibbling over the propriety of one definition as contrasted with another, which is a very unessential matter. But it is extremely important that there shall not be ambiguity as to meaning at any given point, and that an unconscious and unwarranted translation of fact into inference shall not be made. Statements regarding the rate of metabolism may be true when the term means one thing while the same statements may be untrue when the term means something else. If an established fact regarding the rate of metabolism in one sense is used as the basis for an inference regarding the rate of metabolism in a different sense, then great care must be exercised that the inference does not become confused with the fact. The present paper is concerned with the rate of metabolism in the sense of rate of energy production, with the usual qualifications concerning errors in the different methods of direct and indirect calorimetry, and of the possibility of other sources of energy than that represented by the respiratory exchange (see Krogh, (12), chap. i). This statement is made for the purpose of avoiding any ambiguity, and of guarding against the reading into the discussions of more than is intended.

[1] Italics and parenthesis mine.

METHODS

Two species of Planaria, P. maculata and P. agilis,[2] were used in the experiments reported in this paper. The general method for determining the amount of oxygen consumed was to place worms in a bottle of tap water of known content of dissolved oxygen for a stated period of time, and then to remove the worms and analyze the water by the Winkler method for the amount of oxygen remaining. A large carboy was filled with tap water and stirred to make sure that the amount of dissolved oxygen was uniform throughout. The bottles used in an experiment were filled in rapid succession, and the average of the analyses of three or four blanks containing no worms during the experiment was taken to represent the amount of oxygen in each of the experimental bottles at the beginning of the experiment. All bottles were of the same capacity, 132 cc. During analysis the amount of water displaced by the 2 cc. of reagents added is lost. As explained in a previous paper (13, p. 98) removing worms at the end of a test may remove enough water to make it difficult to close bottles after adding the reagents without leaving air bubbles. This can be prevented by adding more reagents or by inserting a glass rod of known volume. In the experiments summarized in tables 3, 4, 5, 6, 8, 12 and 13 the reagents were diluted with an equal volume of distilled water and 2 cc. instead of 1 cc. of each were used. In the other experiments, glass rods of 2 cc. volume were used. The latter method appears preferable although it is unnecessary, if the worms in a single bottle are not too numerous and do not cling too firmly to the glass. The experimental methods are described in more detail in the previous paper. It was shown in that paper (13, table 1) that when 28 bottles were filled with aerated tap water and analyzed at once, the maximum difference between individual analyses did not exceed 0.08 cc. thiosulphate. In the experiments reported previously 15 sets of three or four blanks were analyzed. In no case was the maximum difference between duplicate analyses greater than 0.06 cc. thiosulphate. For the experiments reported in the present paper 37 sets of blanks were analyzed. In one case a difference of 0.12 cc. thiosulphate was noted between duplicate analyses, but this was apparently an error in the reading. In only one other instance did the maximum difference amount to so much as 0.09 cc. thiosulphate. For all the experiments of both papers the average

[2] I am indebted to Dr. Ruth Higley for verifying the identification of these species.

difference beteen the maximum and the minimum analyses in a series of blanks was 0.046 cc. thiosulphate. One drop of thiosulphate from the burette used averages 0.06 cc. in volume. (Titrations were made in a porcelain dish.) These figures therefore indicate the limits of experimental error in the method used and enable one to form a judgment of the accuracy of the data presented in the tables and curves.

The methods of weighing worms have not been described. In the earlier experiments reported in this paper, worms were removed individually to a glass plate in a drop of water, the excess water was absorbed with filter paper and the worm was transferred by means of a probe to a moistened filter paper in a weighing dish. All of the worms which were together in the same bottle in a respiration test were weighed at once, not individually. The error from evaporation was found to average 0.0005 gram. In later experiments a better method was followed. All the individuals from one bottle were transferred in a drop of water to a glass cover slip, the excess water was absorbed with filter paper, and the cover slip was dropped quickly into a weighing bottle half filled with water. When a weighing bottle with a well-ground stopper was used, the error from evaporation was only 0.0002 gram and was much less variable than in the earlier method. These average errors were added to the observed weight in each case.

In order to test the limits of error in the method just described, a single set of worms was weighed repeatedly, as shown in table 1. The worms were weighed three times in rapid succession, and then were allowed to rest undisturbed for a few hours before the next series of weighings. It appeared at once that the first determination in a series was always highest and that the later ones were successively lower. The difference between the first and the third weight was sometimes over 6 per cent. This apparent loss was not permanent, however. After each period of rest the first weight was considerably higher than the last, and almost as high as the first weight of the preceding series. Figure 1 shows these relations graphically. These peculiar results are due, probably, to differences in the amount and character of the mucus. At the moment when excess water is removed from the group of worms on the cover slip, thicker mucus will retain more water; and since care must be exercised not to injure worms by excessive drying, they cannot safely be freed entirely from mucus. Figure 1 shows that the first weights of the different sets make a very regular series decreasing with time and starvation, in which individual variation did not reach 2 per cent. In order to weigh worms under uniform conditions in experi-

ments, all sets were left undisturbed under dark cloth for some time, each set in a separate dish of water, and each set was taken for weighing with as little disturbance to the others as possible.

In experiments extending over a considerable period of time, the worms were weighed at intervals and the weights were plotted on a graph as in figure 2. The weight of each lot of worms at the middle of

TABLE 1

Showing variation among successive weights of a set of Planaria agilis. Five large worms were weighed three times in rapid succession, then were left undisturbed for several hours under dark cloth and were weighed again as before, etc.

DATE	TIME	WEIGHT
July 2	10.50 a.m.	0.0294
	11.06 a.m.	0.0283
	11.24 a.m.	0.0281
July 2	1.30 p.m.	0.0292
	1.46 p.m.	0.0284
	2.02 p.m.	0.0275
July 2	4.44 p.m.	0.0285
	4.58 p.m.	0.0278
	5.12 p.m.	0.0271
July 3	8.41 a.m.	0.0276
	9.04 a.m.	0.0271
	9.17 a.m.	0.0265
July 3	4.28 p.m.	0.0272
	4.40 p.m.	0.0267
	4.52 p.m.	0.0259
July 4	9.19 a.m.	0.0258
	9.34 a.m.	0.0256
	9.48 a.m.	0.0251

the test period was read off from the graph and taken as the average weight during the test. Figure 2 shows the weight curves for the worms of table 7. These worms were undergoing starvation and progressive decrease in size during the experiment. Inspection of the curves shows that the maximum error in individual weights was not over 3 per cent. Inspection of all the weight curves of the experiments in this paper shows that individual variations rarely exceeded 6 per cent.

In the case of figure 2 and table 7, none of the worms had to be discarded because of fission or accidental injury. In some other cases, worms underwent fission during the experiment. Such a worm was removed at once and a new weight was obtained. In later experiments, substitutes were kept ready for replacing such individuals. The substitutes were treated like the worms in the experiment except that their oxygen consumption was not measured unless they took the place of worms in experimental bottles.

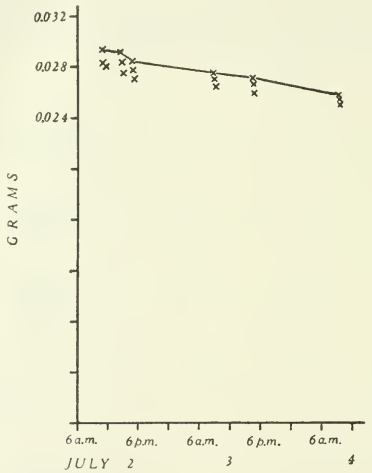

Fig. 1. Showing variation in successive weights of the same set of Planaria agilis. Abscissas, time in 6-hour periods. Ordinates, weight in grams. Solid line connects the first weights of successive series. See table 1.

During the experiments summarized in tables 7, 9 and 14, the respiration bottles were immersed in a water bath within a constant temperature oven at 20°C. Other experiments were carried out at the same temperature in a constant temperature bath.

The test periods were relatively long and the number of worms in each bottle was small. The worms of tables 7, 9 and 14, spent most of the time for six weeks in the respiration bottles. The results represent, therefore, almost continuous measurement of their metabolism through that period, and may be taken as the "normal metabolism" as the term is

used by Krogh (12, p. 116). They do not represent rigidly "standard metabolism" (Krogh, (12), chap. iv) or "basal metabolism" of other authors, because locomotion was not absolutely eliminated, but when Planaria are left undisturbed for several hours they remain at rest for most of the time so that experimental periods of long duration approach nearly to the conditions for "standard metabolism" (see (13), table 9).

In all experiments at least three parallel determinations upon different worms of the same history have been made. Along with the average result, the individual determinations are also given to show the range of variation among them.

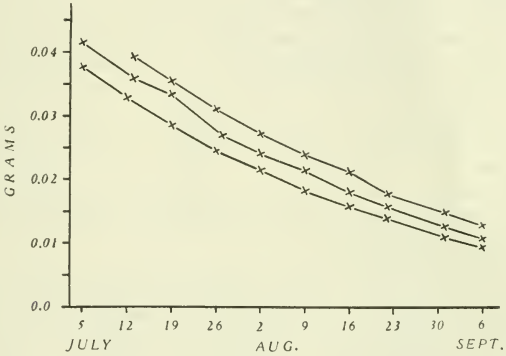

Fig. 2. Curves show decrease in weight of three sets of Planaria agilis of 5 worms each, during 9 weeks of starvation. Abscissas, time in weeks indicated by dates. Ordinates, total weight of 5 worms in grams. See table 7.

OXIDATIONS DURING STARVATION

The rate of metabolism of man and other higher vertebrates falls during the early stages of starvation. Johansson, Landergren, Sonden and Tigerstedt (14) found a decline from 33.15 to 31.20 calories per kilo during the first three days of starvation in man. The heat production during the third to fifth days inclusive remained constant. Among the numerous experiments of Benedict upon *The influence of inanition on metabolism* (15), are three of four days' duration, a fourth of five days' and a fifth of seven days' duration. In general they show a fall in the heat production during these periods, whether it is calculated per unit

of weight or of surface, although in most cases the determinations for the first day were lower than those for the two or even three days following. This fall in heat production during the first few days of starvation is probably due to disappearance of food reserves.

The best observations on man show that metabolism remains fairly constant during prolonged starvation, when calculated per unit of weight or of surface. Lehman, Mueller, Munk, Senator and Zuntz (16) found the respiratory metabolism of Cetti, the professional faster, to remain practically uniform during a fast of 11 days, when calculated per unit of body weight. In Benedict's very complete study of the metabolism in a man starving for 31 days (17), both the respiratory metabolism and the heat liberation, when calculated either per unit of weight or of surface, fell to a practically constant level. Benedict concluded that there was a tendency for the metabolism to divide into three periods: the first period, extending nearly to the middle of the fast, being characterized by a rapidly falling metabolism; the second period, of approximately ten days, showing a comparatively constant metabolism, and the third period, the last week of the fast, showing a general tendency toward an increase (17, pp. 372, 384, 391). The tendency for metabolism to increase during later starvation was slight and of doubtful significance.

Zuntz (18) reports for a dog living for a year in a state of undernutrition, that the heat production, calculated either per unit of weight or surface, fell almost continuously till the eleventh month and rose sharply in the twelfth month toward the end of life. On the other hand Benedict (17, p. 355) quotes experiments by Awrorow on the metabolism of dogs starving for 44 days and 61 days respectively which show a sharp fall in the rate of heat production per unit weight during the first few days, i.e., from about 60 to 48 calories, followed by a gradual rise to a level somewhat above the original, i.e., to 60 to 72 calories, just before a premortal fall.

Warm-blooded animals, however, with their regulatory mechanisms may present special conditions as regards metabolism during starvation. Turning to the cold-blooded animals, the heat liberated by starving frogs per cubic centimeter of frog was found by Hill (19) to decrease during the first two weeks to a practically constant level during the next two weeks. Brunow (20) found a fall in respiratory metabolism in the crayfish during prolonged starvation. A knowledge of the rate of oxidations in Planaria during inanition would add materially to our limited information upon this subject in the cold-blooded animals.

During the first few days of starvation in Planaria, as in other animals, there is a decrease in the rate of oxidations probably due to the diminishing effects of the residual food reserves from the last of the feedings. Table 2 gives a comparison of the rates of oxygen consumption by two lots of worms measured simultaneously, one lot fed daily

TABLE 2

Showing that Planaria agilis after three weeks starvation have a lower rate of oxygen consumption than unstarved worms of the same size. Worms of medium size with short tail because of recent fission; 9 individuals in each bottle. "Well-fed worms" were fed beef liver daily, missing an occasional day, up to the day before the test. "Starved worms" were well fed up to 22 days before the experiment. Temperature 20.4° C. One cubic centimeter thiosulphate equals 0.1711 cc. oxygen at N. T. P. Test period 12 hours. Weighed once within 8 hours after the test

PART	BOTTLE	BLANKS (NO WORMS PRESENT)						
		cc. thio.			*average*			
A	1	4.25						
	2	4.30			*4.26*			
	3	4.24						
	4	4.26						
		I. WELL-FED (STARVED 1 DAY)			II. STARVED 3 WEEKS			
		(1)	(2)	(3)		(4)	(5)	(6)
		Oxygen consumed by the 9 worms in each bottle	Weight of the 9 worms in each bottle	Oxygen consumption per gram per 24 hours	Bottle	Oxygen consumed by the 9 worms in each bottle	Weight of the 9 worms in each bottle	Oxygen consumption per gram per 24 hours
		cc. thio.	*grams*	*cc. oxygen*		*cc. thio.*	*grams*	*cc. oxygen*
B	1	1.17	0.0709	5.6	1	0.73	0.0821	3.0
	2	1.11	0.0723	5.3	2	0.69	0.0784	3.0
	3	0.99	0.0626	5.4	3	0.56	0.0692	2.8
	4	1.03	0.0694	5.1	4	0.68	0.0740	3.1
	5	1.06	0.0636	5.7	5	0.72	0.0755	3.3
	6	1.16	0.0740	5.4	6	0.74	0.0830	3.1
Average		*1.09*	0.0688	*5.4*		*0.69*	0.0770	*3.1*

up to the day before the experiment and the other lot starving for three weeks previous to the test. The worms were of approximately the same weight. The well-fed worms absorbed 1.09 cc. thiosulphate equivalent of oxygen in 12 hours while the worms starved for three weeks absorbed only 0.69 cc. thiosulphate equivalent of oxygen. Cal-

culated per gram of body weight, the well-fed worms consumed 5.4 cc. of oxygen per 24 hours and the worms starved for three weeks only 3.1 cc.

After the first few days of starvation of Planaria, during which the food reserves from the last feedings are effective in increasing the oxidations, the oxygen consumption per unit of weight remains practically constant for a long period of time. Table 3 shows a comparison between Planaria starved for 63 days and others starved for 7 days. The former worms, during the first four days in the laboratory, underwent natural fission, because of their large size and the sudden deprival of food. Only the anterior pieces were used in the experiment. They measured 10 to 13 mm. in length. At the time of the respiration determinations, these were reduced in length to 7 to 8 mm. The second lot of worms starved for only a little over 7 days, were 14 to 19 mm. long at the time of the test. Three successive tests were made. The worms were weighed before the first test and after the last test and the average weight during each period was determined by interpolation, as explained in the section on methods. In each of the three tests the worms after long starvation and those after short starvation absorbed the same amounts of oxygen per gram body weight per 24 hours. While the worms of shorter starvation period were in this laboratory for only six days before the comparisons with those worms which were in the laboratory for 62 days, there was no reason for supposing that they were different from the first lot at the beginning of the starvation period, except that they did not undergo fission. The results of this experiment are confirmed by those of later experiments, tables 4, 5, 6 and 7, in which worms were under continuous observation during the starvation period.

For determining the effects of size, feeding and regeneration upon the oxidations, the rate of oxidation during starvation is the proper standard of reference. All such experiments reported in this paper have been controlled by simultaneous determinations of the oxygen consumption by normal, starving worms, and the continuous observations on these controls give a picture of the course of the oxidations during prolonged starvation. These observations are given in tables 4, 5, 6 and 7. Since the effects of previous feeding were to be avoided in the experiments for which these worms were controls, the worms were starved for several days before beginning observations.

In the experiment recorded in table 4, Planaria maculata that had been starving for several weeks were observed for 34 days during which

TABLE 3

Showing that Planaria agilis after 63 days of starvation have the same rate of oxygen consumption as worms starved for only 7 days. The former worms underwent natural fission 62 to 59 days previous to the first test, because of their large size and sudden removal of food. The anterior pieces from this fission, measuring 10 to 13 mm. in length 59 days before the first test were reduced in length to 7 or 8 mm. at the time of the first test; 10 worms in each bottle. The worms of short starvation period measured 14 to 19 mm. in length; 5 worms in each bottle. The lengths of the three successive tests were, in order, 49, 49 and 46.25 hours. Temperature 20° C. plus or minus 0.15° C., except a temporary fall to 16° C., during the third test, which accounts for the low values in column 10. One cubic centimeter thiosulphate equals 0.1710 cc. oxygen at N. T. P. Worms weighed before the first test and after the third, and the average weight during each period determined by interpolation.

PART	SET	BOTTLE	NUMBER OF WORMS IN EACH BOTTLE	FIRST TEST: OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	FIRST TEST: WEIGHT OF THE WORMS IN EACH BOTTLE	FIRST TEST: OXYGEN CONSUMPTION PER GRAM PER 24 HOURS	SECOND TEST: OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	SECOND TEST: WEIGHT OF THE WORMS IN EACH BOTTLE	SECOND TEST: OXYGEN CONSUMPTION PER GRAM PER 24 HOURS	THIRD TEST: OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	THIRD TEST: WEIGHT OF THE WORMS IN EACH BOTTLE	THIRD TEST: OXYGEN CONSUMPTION PER GRAM PER 24 HOURS
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
A	Blanks (no worms present)	1		Blanks: 4.56 cc. thio.			Blanks: 4.42 cc. thio.			Blanks: 4.29 cc. thio.		
		2		4.53			4.44			4.25		
		3		4.54			4.45			4.25		
		4		4.54			4.44			4.24		
				Blanks: average 4.54			Blanks: average 4.44			Blanks: average 4.26		
B	I	1	5	At end of 7 days starvation: 1.60 cc. thio.	0.0318 grams	4.3 cc. oxygen	At end of 7 days starvation: 1.53 cc. thio.	0.0304 grams	4.3 cc. thio.	At end of 11 days starvation: 1.13 cc. oxygen	0.0290 grams	3.6 cc. oxygen
		2	5	1.68	0.0332	4.4	1.52	0.0311	4.2	1.02*	0.0244*	3.8
		3	5	1.76	0.0399	3.8	1.66	0.0384	3.7	1.41	0.0369	3.5
		Average..		*1.68*	*0.0350*	*4.1*	*1.57*	*0.0333*	*4.1*	*1.19*	*0.0301*	*3.6*
	II	1	10	At end of 63 days starvation: 0.57	0.0116	4.2	At end of 65 days starvation: 0.52	0.0110	4.1	At end of 67 days starvation: 0.42	0.0104	3.7
		2	10	0.57	0.0132	3.7	0.62	0.0123	4.4	0.49	0.0115	3.9
		3	10	0.62	0.0126	4.3	0.58	0.0120	4.2	0.46	0.0113	3.7
		Average ..		*0.59*	*0.0125*	*4.1*	*0.57*	*0.0118*	*4.2*	*0.46*	*0.0111*	*3.8*

* 4 worms.

time 12 tests of the oxygen consumption were made, each of about 46 hours' duration. Column 6 shows a steady decline in the average amount of oxygen consumed by the 8 worms in each bottle from 0.105 to 0.037 cc. per 24 hours. In figure 4 the oxygen consumption by the worms during the 34 days of observation, is shown as the control for a

TABLE 4

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms starving for several weeks preceding first test and during the experiment; 8 worms in each bottle. One cubic centimeter thiosulphate equals 0.1766 cc. oxygen at N. T. P. Worms weighed November 20, 27, December 1, 4, 11, 18, 25. Average weight for each test period determined by interpolation. Control for table 8. See figures 4 and 5

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT), AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE, AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE PER 24 HOURS, AVERAGE	WEIGHT OF THE 8 WORMS IN EACH BOTTLE, AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
							Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*grams*	*cc oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
November 20–22....	46.17	19.8–20.3	4.58	1.11	*0.105*	0.0152	5.5	6.1	9.0(?)	*6.9*
November 24–26....	48.00	20.0–20.6	4.47	0.93	*0.085*	0.0134	7.0	5.6	6.2	*6.3*
December 1– 3....	45.92	20.0–20.3	4.55	0.79	*0.075*	0.0108	7.1	8.3	5.9	*7.0*
December 4– 6....	46.15	20.0±00.1	4.29	0.72	*0.068*	0.0099	6.7	8.6	6.0	*6.9*
December 6– 8....	46.13	20.0–20.2	4.02	0.63	*0.060*	0.0093	5.6	7.1	6.5	*6.4*
December 8–10....	46.00	19.8–20.1	4.10	0.64	*0.061*	0.0089	6.5	7.2	6.9	*6.8*
December 11–13....	46.17	20.1–20.3	4.28	0.57	*0.054*	0.0081	6.3	7.2	6.5	*6.7*
December 13–15....	46.25	20.0–20.2	4.21	0.46	*0.044*	0.0075	6.1	5.9	5.4	*5.8*
December 15–17....	46.05	20.0–20.3	4.25	0.58	*0.055*	0.0069	7.5	9.2(?)	7.5	*8.0*
December 18–20....	46.17	20.0–20.3	4.25	0.45	*0.043*	0.0061	6.6	7.5	7.0	*7.0*
December 20–22....	46.05	20.3–18.7	4.31	0.50	*0.048*	0.0058	8.0	9.5(?)	7.6	*8.2*
December 22–24....	51.23	19.9–20.1	4.34	0.43	*0.037*	0.0054	6.3	6.5	7.6	*6.8*

feeding experiment. Column 7, table 4, shows a similar loss of average weight from 0.0152 to 0.0054 gram per 8 worms. Column 11 shows a fairly *uniform rate* of oxygen consumption of 6.5 to 7.0 cc. *per gram weight* during the 34 days of starvation. These last figures are represented graphically in figure 5. Columns 8, 9 and 10 show the varia-

tion in determinations of the individual bottles which indicates the limits of error in the experiment.

Table 5 gives similar observations upon starving Planaria maculata during a period of 27 days. The worms were starved 15 days previous

TABLE 5

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms 10 to 13 mm. in length on January 8; starving for 15 days previous to the first test and during the experiment; 8 worms in each bottle. Weighed January 12, 14, 15, February 5 and 13. One cubic centimeter thiosulphate equals 0.1698 cc. oxygen at N. T. P. See figure 9. For further data on control, see table 12

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	WEIGHT OF THE 8 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
						Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
January 12–14	41.37	19.9–20.1	4.20	1.20	0.0249	4.5	5.0	4.9	*4.8*
January 15–17	42.66	20.0	4.19	1.20	0.0229	5.2	4.9	5.2	*5.2*
January 18–20	43.23	19.9–20.1 (20.6)*	4.32	1.05	0.0214	4.6	4.6	5.1	*4.7*
January 20–22	46.48	19.9–20.1 (20.3)*	4.66	0.98	0.0205	4.4	4.1	4.5	*4.3*
January 22–24	42.45		4.46	0.81	0.0194	4.1	3.8	4.6	*4.1*
January 24–26	46.90	19.9–20.0 (19.0)*	4.18	0.83	0.0185	4.1	3.8	4.3	*4.0*
January 26–28	45.44		4.30	0.80	0.0175	4.2	3.9	4.5	*4.2*
February 5–9	94.77	16.7–22.6	4.18	1.18	0.0114	4.5	4.4	4.8	*4.6*

* For a short time.

to the experiment. The average rate of oxygen consumption per gram body weight, column 10, was less uniform in this case. The data of column 10 are represented in figure 9, as the control for an experiment in which worms were cut in two and allowed to regenerate. It may be seen that the curves representing the rates of oxygen consumption by

anterior pieces and posterior pieces parallel the irregularities in the curve of the normal worms, indicating some common factor or factors, probably other than the stage of starvation, affecting all worms simultaneously. Table 6 and figure 10 record results of respiration tests on Planaria maculata during 24 days of starvation. The worms were starving for 4 days preceding the experiment. These again were control

TABLE 6

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms 7 to 10 mm. long February 25; starving for 5 days preceding the first test and during the experiment; 10 worms in each bottle. Weighed March 2, 5, 11, 12, 18, 30. One cubic centimeter thiosulphate = 0.1698 cc. oxygen at N. T. P. See figure 10. For further data on controls, see table 13

(1)	(2)	(3)	(4)	(5)	6	(7)	(8)	(9)	(10)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 10 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	WEIGHT OF THE 10 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
						Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
March 2– 4.....	41.40	20.0–20.5	4.59	0.79	0.0160	5.3	5.0	4.8	*5.0*
March 5– 7.....	45.00	20.0–20.2	4.48	0.77	0.0149	4.8	4.8	4.8	*4.8*
March 7– 9.....	47.30	20.0–20.2 (20.5)*	4.54	0.73	0.0136	5.1	4.5	4.9	*4.8*
March 9–11.....	45.65	20.0–20.4	4.56	0.74	0.0123	5.8	5.1	5.5	*5.5*
March 12–14.....	46.38	20.1–20.5	4.65	0.61	0.0106	5.2	5.1	5.4	*5.2*
March 14–16.....	46.16	20.0–20.5	4.70	0.59	0.0096	5.8	5.5	5.4	*5.6*
March 16–18.....	45.00	20.1–20.6	4.68	0.55	0.0087	6.5	5.9	5.4	*5.9*
March 19–21.....	42.90	20.0–	4.62	0.37	0.0076	5.2	5.0	4.1	*4.8*
March 21–29.....	191.09	20.0–20.5	4.47	1.53	0.0062	6.0	5.3	5.1	*5.4*

* For a short time.

data for an experiment in regeneration and again the experimental worms showed individual fluctuations paralleling those of the control.

The experiment recorded in table 7 was the last and most complete series of continuous observations upon starvation. These worms, Planaria agilis, were starved for 7 days before the beginning of the experiment. Three determinations a week were made for six weeks

TABLE 7

Showing uniform rate of oxygen consumption by Planaria agilis during starvation. Worms 16 to 17 mm. long July 5; 5 worms in each bottle; starving for 7 days preceding the first test and during the experiment. July 6 to 19, 1 cc. thiosulphate = 0.1768 cc. oxygen at N. T. P. July 20 to August 16, 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P. Worms weighed weekly, namely July 5, 13, 19, 26, August 2, 9, 16, 22, 31 and September 6. See figures 2, 3, 6, 7 and 11. For further data on controls, see table 9

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 5 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED BY THE 5 WORMS IN EACH BOTTLE PER 24 HOURS. AVERAGED	WEIGHT OF THE 5 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS			
							Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6– 8.....	45.15	22.0–20.0	3.68	1.55	*0.150*	0.0401	3.7	3.8	3.8	*3.7*
July 8–10.....	43.08	20.0–19.6	4.09	1.38	*0.141*	0.0387	3.5	3.6	3.8	*3.6*
July 10–12.....	45.25	20.4–19.9	4.28	1.38	*0.134*	0.0374	3.5	3.5	3.7	*3.6*
July 13–15.....	45.75	20.2–19.4	3.96	1.26	*0.121*	0.0355	3.4	3.4	3.5	*3.4*
July 15–17.....	45.00	20.5–18.8	3.85	1.20	*0.116*	0.0344	3.3	3.3	3.5	*3.4*
July 17–19.....	45.00	20.4–19.2	3.81	1.14	*0.111*	0.0332	3.1	3.3	3.4	*3.3*
July 20–22.....	42.25	19.6–20.0	3.79	1.01	*0.104*	0.0313	3.3	3.2	3.5	*3.3*
July 22–24.....	43.50	20.0–19.7	3.80	1.02	*0.102*	0.0300	3.3	3.4	3.5	*3.4*
July 24–26.....	42.00	19.7–20.0	3.63	0.92	*0.095*	0.0287	3.2	3.2	3.4	*3.3*
July 27–29.....	45.10	19.6–20.2	3.79	0.93	*0.089*	0.0269	3.3	3.3	3.3	*3.3*
July 29–31.....	45.00	19.6–19.8	3.55	0.87	*0.084*	0.0259	3.2	3.3	3.3	*3.2*
July 31–August 2	43.00	19.8–	3.96	0.84	*0.085*	0.0249	3.3	3.4	3.6	*3.4*
August 3– 5....	45.25	19.6–19.9	3.92	0.82	*0.079*	0.0235	3.3	3.3	3.5	*3.3*
August 5– 7....	45.07	19.3–20.0	3.79	0.79	*0.076*	0.0226	3.2	3.3	3.6	*3.4*
August 7– 9....	45.07	20.0–20.7	3.93	0.83	*0.080*	0.0216	3.4	3.7	3.9	*3.7*
August 10–12....	45.42	19.8–19.6	3.73	0.71	*0.067*	0.0204	2.9	3.8	3.4	*3.3*
August 12–14....	45.33		3.84	0.68	*0.065*	0.0196	3.2	3.2	3.5	*3.3*
August 14–16....	45.00	19.6–19.7	4.02	0.66	*0.063*	0.0188	3.3	3.3	3.5	*3.4*
September 8–10	45.27	20.0–	4.44	0.77*	*0.073**	0.0158*	4.3	4.5	4.3	5.1	4.9	*4.6*
September 10–12	45.00	20.4–20.0	4.39	0.71*	*0.068**	0.0151*	4.2	4.5	4.6	5.0	4.8	*4.5*
September 12–14	45.00	19.7–	4.39	0.63*	*0.060**	0.0144*	3.8	3.3	3.7	5.0	4.4	*4.2*

*Ten worms in each bottle.

without interruption. During this time the average consumption per 5 worms per 24 hours decreased from 0.150 to 0.063 cc. oxygen, i.e., to about 40 per cent of the original. The average weight fell, also, during this time from 0.0401 to 0.0188 gram per 5 worms. The fall in oxygen consumption followed very closely, therefore, the loss in weight. This is shown graphically in figure 3 where the average amounts of oxygen consumed are plotted along with the average weights. In general the two curves parallel each other but the curves of oxygen consumption show a greater initial decline, probably representing the remnant of the great fall in oxidations of the earliest stages of starvation due to residual food. When the oxygen consumption per gram weight is calculated, as in table 7, column 11, it is found to be practically constant at about 3.25 to 3.5 cc. after the first week. This is shown graphically in figure 7, where these worms served as the control for an experiment in feeding. The unusual rise on August 7 to 9, figure 7, was thought to be due to disturbing changes in the composition of the tap water. Similar irregularities have been noted in several experiments, under conditions which seem to point to the composition of the tap water as the cause. After an interruption of three weeks, the determinations of oxygen consumption were resumed. Since the worms had become very small, they were redistributed in the bottles and more individuals were added from reserve stock so as to make 10 in each bottle. These reserve worms had the same history as the others. The oxygen consumption per gram weight of body showed a marked rise over previous determinations. It averaged 4.6, 4.5 and 4.2 cc. per 24 hours in three tests. At the same time it was found that worms were beginning to die. They were very susceptible to injury in the manipulations of weighing and analysis. This interfered with continued observation of the rate of oxygen consumption and it was considered that the abnormal conditions of the worms at this time made the observed rise in oxidations of doubtful significance.

Regarding tables 4, 5, 6 and 7, it should be said that they are given in the order in which the experiments were performed. In the last experiment the procedure and manipulations were naturally better so that the results are more uniform. The last experiment was of longer duration, also, and the Planaria agilis, which were used in this case, were found generally more satisfactory for such experiments than Planaria maculata.

From all the above observations on starvation in Planaria, we conclude that the *actual* consumption of oxygen at constant temperature

of 20°C. falls continuously throughout the period of starvation, at first more rapidly, and later more slowly. The body weight also decreases continuously during starvation, figure 3. On the other hand, *the oxygen consumption per gram body weight* (*rate of oxidations*) falls during a short period of the first few days of starvation, due probably to residual food, and then becomes uniform for a long period thereafter. During this long period of uniform rate of oxidations the worms may undergo reduction to one-half or less of their original weight. This

Fig. 3. Curves show decrease in oxygen consumption by Planaria agilis compared with decrease in weight during 6 weeks of starvation. Abscissas, time in weeks indicated by dates. Ordinates at the left, weight of 5 worms in grams. Ordinates at the right, oxygen consumption by 5 worms per 24 hours in cc. oxygen. Broken line represents average weight of three sets of worms weighed once a week. Solid line represents average oxygen consumption by the same three sets of worms, three tests made each week. See table 7 and figure 2.

distinction between the early period of a few days of starvation, during which food reserves from the last feedings are effective in increasing oxygen consumption, and the later period of several weeks, during which the rate of oxidations is uniform, is very important. In the first place, the fact that the rate of oxidations is uniform during a long period of starvation (6 weeks) will serve as an important basis for the study of respiratory metabolism in these forms. In the second place, the fact that food reserves alter the respiratory metabolism for several days at the beginning of starvation makes this period unsuited for such studies.

According to the experiments above and those in the following section on oxidations after feeding, this preliminary period during which food reserves alter the respiratory metabolism may last for a number of days in P. maculata and P. agilis. *In any case before the "basal" or "standard metabolism" has been reached, the rate of oxidations is not a proper basis for comparisons.*

The respiratory metabolism during extreme starvation, when the body has been reduced to a minute fraction of the original weight by starvation alone unaccompanied by fission, has not been studied, and the statements above are not intended to include such later stages.

OXIDATIONS AFTER FEEDING

It is well known that the ingestion of food results in increased oxidations and heat production in higher animals. Planaria can take in at one meal a very large amount of suitable food material, such as boiled egg yolk, clotted blood or beef liver. Several experiments show that such a meal results in a great increase in the oxygen consumption. Table 8 shows an experiment in which P. maculata were fed clotted blood. Columns 4 and 5 give the oxygen consumption of the control worms which were starving throughout the experiment. The control is described in more detail in table 4. The experimental worms were fed blood clots on December 3. Column 7, table 8, shows that the actual amount of oxygen absorbed, which was decreasing progressively before feeding, doubled in the first test following the meal, then declined rapidly, and later more slowly. Figure 4 shows these relations. Column 8, table 8, shows that the worms ingested about 60 per cent of their own weight at this meal. When the rate of oxygen consumption per gram body weight was calculated as in column 12, it was found to rise suddenly from 6.7 to 9.6 cc. oxygen per gram weight and then to fall rapidly to its former level and to remain fairly constant as shown in figure 5. In this calculation the food in the digestive tract is included in the total body weight, thus making the rate of oxygen consumption appear less than its true value. If the weight of food is subtracted from the total body weight for the first test period following the meal, the rate of oxygen absorption per gram of empty body weight becomes 16.5 cc., as shown in table 8, column 12. Upon this basis of calculation, then, the ingestion of food resulted in an immediate increase in the rate of oxygen consumption of over 140 per cent.

TABLE 8

Showing great increase in oxygen consumption by Planaria maculata after feeding clotted blood. Worms not fed for several weeks preceding the first test; 8 worms in each bottle. Experimental worms fed blood clots on December 3. Control worms not fed. Weighed November 20, 27, December 1, 3, 4, 11, 18 and 25. One cubic centimeter thiosulphate = 0.1766 cc. oxygen at N.T.P. See figures 4 and 5. For further data on controls, see table 4.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
			CONTROL WORMS STARVING THROUGH WHOLE EXPERIMENT		EXPERIMENT. WORMS FED BLOOD CLOTS DECEMBER 3						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Oxygen consumed by the 8 worms in each bottle per 24 hours. Average	Oxygen consumption per gram weight per 24 hours. Average	Oxygen consumed by the 8 worms in each bottle. Average	Oxygen consumed by the 8 worms in each bottle per 24 hours. Average	Weight of the 8 worms in each bottle. Average	Oxygen consumption per gram body weight per 24 hours			
								Individual determinations			Average
	hours	*°C.*	*cc. oxygen*	*cc. oxygen*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
November 20–22	46.17	19.8–20.3	0.105	6.9	1.00	*0.095*	*0.0155*	6.1	6.3	5.9	*6.1*
November 24–26	48.00	20.0–20.6	0.085	6.3	0.99	*0.090*	*0.0135*	6.5	7.8	5.9	*6.7*
December 1–3	45.92	20.0–20.3	0.075	7.0	0.80	*0.076*	*0.0113*	6.8	7.0	6.4	*6.7*
						Experimental worms fed blood clots December 3					
							0.0101)*	(15.7)*	(15.7)*	(17.7)*	(*16.5*)*
December 4–6	46.15	20.0±0.1	0.068	6.9	1.67	*0.159*	*0.0165*	9.3	9.6	9.8	*9.6*
December 6–8	46.13	20.0–20.5	0.060	6.4	1.41	*0.134*	*0.0153*	9.0	9.3	8.1	*8.7*
December 8–10	46.00	19.8–20.1	0.061	6.8	1.10	*0.105*	*0.0141*	7.6	7.9	7.0	*7.4*
December 11–13	46.17	20.1–20.3	0.054	6.7	0.91	*0.086*	*0.0126*	6.9	7.4	6.4	*6.8*
December 13–15	46.25	20.0–20.2	0.044	5.8	0.78	*0.074*	*0.0118*	6.1	6.4	6.3	*6.2*
December 15–17	46.05	20.0–20.3	0.055	8.0	0.75	*0.071*	*0.0110*	6.9	6.9	5.9	*6.5*
December 18–20	46.17	20.0–20.3	0.043	7.0	0.66	*0.063*	*0.0099*	6.4	7.4	5.6	*6.3*
December 20–22	46.05	20.3–18.7	0.048	8.2	0.67	*0.064*	*0.0093*	6.8	7.8	6.2	*6.8*
December 22–24	51.23	19.9–20.1	0.037	6.8	0.57	*0.048*	*0.0085*	5.8	6.1	5.4	*5.7*

* Weight of ingested food subtracted from total body weight.

Table 9 gives similar data for an experiment in which P. agilis were fed beef liver. Data for the control worms, which were starving throughout the experiment, are given in columns 4 and 5, and more fully in table 7. After feeding upon liver, July 20, the amount of oxygen absorbed by the experimental worms increased from 0.096 to 0.167 cc. per 5 worms per 24 hours, column 7, table 9. The body weight in-

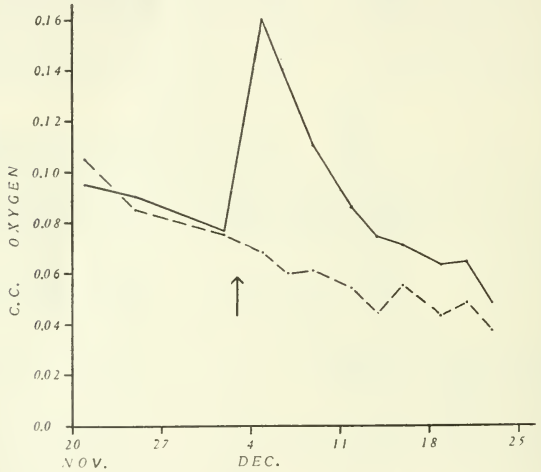

Fig. 4. Curves show decrease in oxygen consumption by starving Planaria maculata, and increase in oxygen consumption after feeding. Abscissas, time in weeks indicated by dates. Ordinates, cc. oxygen consumed by 8 worms in 24 hours. Broken line represents average of three sets of worms, starving throughout the experiment. Solid line represents average of three sets of worms, fed once on December 3. The arrow indicates when the experimental worms were fed. See table 8.

creased from 0.0276 to 0.0353 gram per 5 worms and the rate of oxygen consumption per gram of total body weight, including the ingested food, increased from 3.5 to 4.7 cc., column 12. Deducting the weight of food, the rate of oxygen consumption per gram of empty body weight becomes 6.4 cc. for the first test after feeding, an increase of over 80 per cent. These relations are shown graphically in figures 6 and 7. Inspection of these curves shows that the oxidations rise suddenly with

the taking in of food and decline rapidly during the next week or ten days to a constant level. In figure 6, the curve of the absolute amount of oxygen consumed by the experimental worms ran parallel to the control for two weeks preceding the meal. After feeding, it rose sharply, then declined rapidly during the next 10 to 12 days, and then continued parallel with the control, but at a relatively higher position. This might indicate one of two things, either that the meal had resulted in a certain amount of growth so that the animals which were formerly smaller than the controls became slightly larger, or that the meal re-

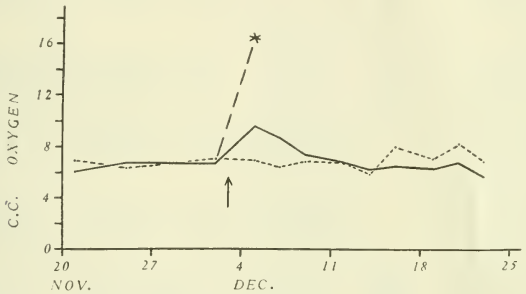

Fig. 5. Curves show practically constant rate of oxygen consumption by Planaria maculata during starvation and increase in rate of oxygen consumption after feeding. Abscissas represent time in weeks indicated by dates. Ordinates represent rate of oxygen consumption in cc. of oxygen per gram total body weight per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. Solid line represents the average of three sets of worms fed once on December 3 (time of feeding indicated by the arrow). * represents the rate of oxygen consumption by fed worms in cc. oxygen per gram empty body weight (weight of body if no food had been ingested) per 24 hours. See table 8 and figure 4.

sulted in a permanent increase in the speed of oxidations. Figure 7 and tables 9 and 7 indicate that the former interpretation is correct. A comparison of the average weights given in table 9, column 8, and table 7, column 7, shows that the experimental worms did weigh less than the control worms during the two weeks before the feeding, but that after feeding they did not become reduced again to the weight of the controls. Moreover, figure 7 shows that after August 1 the rate of oxygen consumption by the two lots of worms per gram total body weight ran parallel for 15 days, or till the end of the experiment.

TABLE 9

Showing marked increase in the oxygen consumption by Planaria agilis after feeding beef liver. Worms 15 to 16 mm. long July 5; without food for 7 days preceding the first test; 5 worms in each bottle. Experimental worms fed beef liver July 20. Control worms not fed. Weighed weekly, namely July 5, 8, 12, 19, 20, 26, August 2, 9 and 16. One cubic centimeter thiosulphate = 0.1768 cc. oxygen at N.T.P. from July 6 to 19. One cubic centimeter thiosulphate = 0.1751 cc. oxygen at N. T. P. from July 20 to August 16. See figures 6 and 7. For further data on controls, see table 7.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
			CONTROL WORMS STARVING THROUGH WHOLE EXPERIMENT		EXPERIMENT. WORMS FED BEEF LIVER JULY 20						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average	Oxygen consumption per gram weight per 24 hours. Average	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average.	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average	Weight of the 5 worms in each bottle. Average	Oxygen consumption per gram body weight per 24 hours			
								Individual determinations			Average
	hours	°C.	*cc. oxygen*	*cc. oxygen*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6–8	45.15	22.0–20.0	0.150	3.7	1.37	*0.133*	*0.0346*	3.9	3.9	3.7	*3.8*
July 8–10	43.08	20.0–19.6	0.141	3.6	1.22	*0.124*	*0.0332*	3.7	3.9	3 7	*3.7*
July 10–12	45.25	20.4–19.9	0.134	3.6	1.24	*0.120*	*0.0317*	3.9	3.8	3.7	*3.8*
July 13–15	45.75	20.2–19.4	0.121	3.4	1.09	*0.105*	*0.0298*	3.6	3.4	3.4	*3.5*
July 15–17	45.00	20.5–18.8	0.116	3.4	1.03	*0.099*	*0.0287*	3.4	3.5	3.5	*3.5*
July 17–19	45.00	20.4–19.2	0.111	3.3	0.99	*0.096*	*0.0276*	3.5	3.4	3.5	*3.5*
							Experimental worms fed beef liver July 20				
							*(0.0259)**	*(6.4)**	*(6.1)**	*(6.7)**	*(6.4)**
July 20–22	42.25	19.6–20.0	0.104	3.3	1.62	*0.167*	*0.0353*	4.8	4.5	4.9	*4.7*
July 22–24	43.50	20.0–19.7	0.102	3.4	1.37	*0.137*	*0.0298*	4.7	4.2	4.9	*4.6*
July 24–26	42.00	19.7–20.0	0.095	3.3	1.15	*0.118*	*0.0289*	4.2	4.0	4.1	*4.1*
July 27–29	45.10	19.6–20.2	0.089	3.3	1.06	*0.102*	*0.0273*	3.8	3.7	3.8	*3.7*

July 29–31	45.00	19.6–19.8	0.084	3.2	0.95	*0.091*	*0.0264*	3.5	3.4	3.4	*3.5*
July 31–August 2	43.00	19.8–	0.085	3.4	0.88	*0.089*	*0.0255*	3.6	3.5	3.4	*3.5*
August 3– 5	45.25	19.6–19.9	0.079	3.3	0.89	*0.085*	*0.0241*	3.4	(lost)	3.4	*3.4*
August 5– 7	45.07	19.3–20.0	0.076	3.4	0.83	*0.080*	*0.0230*	3.6	3.5	3.4	*3.5*
August 7– 9	45.07	20.0–20.7	0.080	3.7	0.86	*0.083*	*0.0220*	3.9	3.6	3.7	*3.8*
August 10–12	45.42	19.8–19.6	0.067	3.3	0.72	*0.068*	*0.0207*	3.4	3.2		*3.3*
August 12–14	45.33		0.065	3.3	0.73	*0.070*	*0.0198*	3.5	3.6		*3.5*
August 14–16	45.00	19.6–19.7	0.063	3.4	0.68	*0.065*	*0.0190*	3.5	3.4		*3.4*

* Weight of ingested food substracted from body weight.

It is evident, therefore, that a single feeding increases the oxidations in Planaria by 80 to 140 per cent and that this increase disappears during the first 10 to 12 days of starvation, the rate of oxidations falling sharply at first and then more slowly to reach a constant level. This result explains why the oxidations in well-fed worms decrease during the first stages of starvation (see expers. 2 and 7 and figs. 3 and 7).

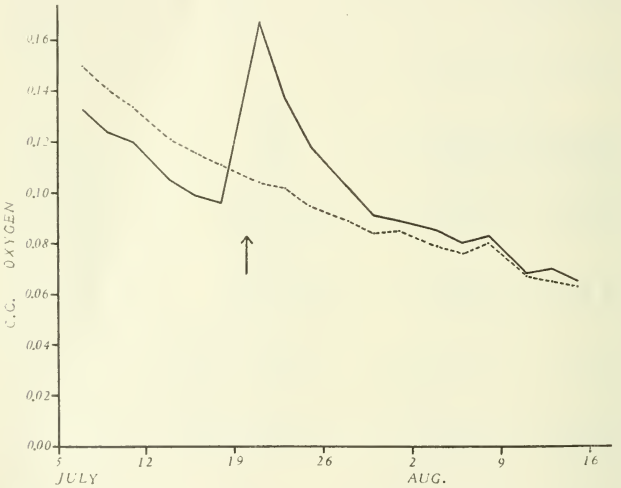

Fig. 6. Curves show decrease in oxygen consumption by Planaria agilis during starvation and increase after feeding. Abscissas represent time in weeks, indicated by dates. Ordinates represent cc. oxygen consumed by 5 worms per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. Solid line represents the average of three sets of worms fed once on July 20 (time of feeding indicated by the arrow). See table 9.

OXIDATIONS IN WORMS OF DIFFERENT SIZE

The rate of respiratory metabolism or heat production in higher animals is commonly said to decrease with increasing size and age of the body. It is obvious that a larger animal will use more oxygen, give off more carbon dioxide and liberate more heat than a smaller animal of the same species under the same conditions. In order to make any comparison of the rates of respiratory exchange or heat production in

the two animals, it is necessary to calculate the respiratory metabolism with reference to some common basis such as a unit of weight or surface area. It is understood that such comparisons should be made under standard conditions of *a*, fasting; *b*, uniform temperature; and *c*, muscular rest. But it should be distinctly kept in mind that when animals of different size and age are compared in this way a difference in the rates of respiratory metabolism *may or may not* mean that comparable

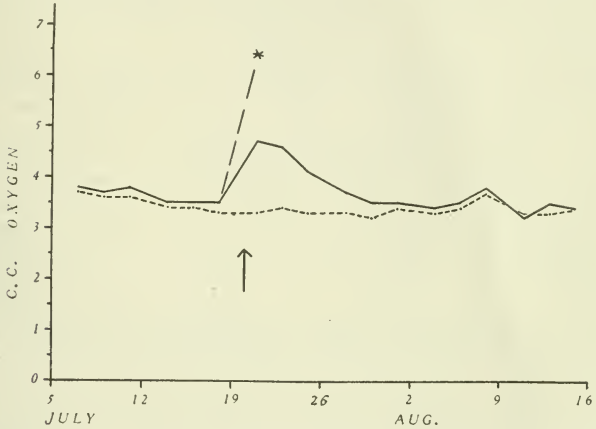

Fig. 7. Curves show constant rate of oxygen consumption by Planaria agilis during starvation and increase in rate of oxygen consumption after feeding. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. The solid line represents the average of three sets of worms fed once on July 20 (time of feeding indicated by the arrow). * represents the rate of oxygen consumption by the fed worms in cc. oxygen per gram empty body weight (weight of body if no food had been ingested) per 24 hours. See table 9 and figure 6.

cells in the two animals have inherently different rates of oxidation. Different opinions are held regarding the cellular rates of oxidation in these cases (see Benedict, 22). The observation is merely that the total amounts of heat produced are not proportional to the weights or surface areas of the animals.

It is well known that among warm-blood animals the smaller animal produces more heat in proportion to its weight than the larger individual.

Various authors since Rubner (21) have presented evidence that the respiratory metabolism of warm-blooded animals, at least, is proportional to the area of the surface of the body. The respiratory metabolism is often calculated per unit of body surface with the idea of eliminating size differences when other factors are being compared. Why the heat production should be proportional to the area of the skin is not entirely clear. In a general way, the proportionality between the rate of heat production and the area of the radiating surface is of adaptive value to animals which must maintain constant body temperature, but this consideration does not explain what mechanism regulates the rate of energy production in the individual. Apparently it is not simply an accommodation of the heat production to the rate of heat loss (see Krogh (13, pp. 133–140) and Lusk (10, p. 120)). Benedict (22), (23) has objected to the practice of computing metabolism in terms of the unit of surface and has collected data from a large number of normal controls in human metabolism experiments to show that metabolism, calculated per unit of area of the body surface, gives large variations. Some of the variation, may be traced, apparently, to errors in the prevailing method of estimating the surface area. The area of the surface of an animal is not easily measured. It is usually estimated from the weight by means of the formula of Meeh (24) which is based on the law that surfaces of similar solids are proportional to the two-thirds power of their volumes. The bodies of fat and thin persons are not geometrically similar, however, and this formula is known to give large errors in computing the skin areas in such cases. Upon the basis of careful measurements of the areas of the skin of individuals of very different proportions, Du Bois and Du Bois (25), (26), have derived a "linear" and a "height-weight" formula which give the surface area with a maximum error of only 5 per cent in contrast with the average error of 16 per cent and a maximum error of 36 per cent with the Meeh formula. The metabolism seems to be more nearly proportional to the skin area as computed with the new formula than with the older one (27). Among adult mammals, then, the individual of smaller size produces more heat in proportion to its weight than the larger individual, and whatever may be the reason, the amount of the difference seems to be in accordance with the "surface law." This is true of the persons of the same age.

When individuals of different ages are compared, the difference in the rate of heat production per unit of weight does not seem to follow the surface law so closely. The rate of heat production per square meter of surface is higher in youth and lower in old age than in middle life (28),

(29), (27) and (10, p. 128). On the other hand the metabolism of infants per square meter is less than that of older children. That of babies in the first month of life is lowest (12, p. 117); (30), (31), (32), (29, charts 1 to 3). But Bohr's (33) measurements of the respiratory exchanges of pregnant guinea pigs with circulation through the umbilical cord suspended, indicate a rate of oxidations in the embryo per unit of weight not far different from that of the parent. Bohr and Hasselbalch (34) found that the carbon dioxide production by the developing chick embryo from the ninth to the eighteenth days was closely proportional to the weight of the embryo. These observations upon infants and embryos serve to emphasize the fact that the interpretation of differences in rate of respiratory metabolism in warm-blooded animals in relation to size and age, whether calculated per unit of surface or of weight, involves questions of the constitution and functional conditions of the body. While the metabolism studies on warm-blooded animals are commonly cited to show that the rate of oxidations is higher in animals of smaller size and in younger animals, as a matter of fact the warm blooded animals are very unsuited to the critical study of these questions.

Turning to the cold-blooded animals, observations on the rate of respiratory metabolism in relation to size and age are very inadequate. It may be noted at the outset that there is no reason for supposing that metabolism in these animals, of widely different structural organization, should follow the "surface law." It has been shown definitely by Morgulis (36) that this relationship does not exist in the flounder. He finds, rather, that as size of the body increases, the oxygen consumption per unit of body surface increases, and that per unit of body weight decreases, while after removal of the fins the oxygen consumption is proportional to the mass. Buytendijk (quoted by Montuori (35, p. 216)) and Montuori (35, p. 216) both report that Scyllium of smaller size consume more oxygen per kilogram hour than those of larger size.

Observations on the rate of respiratory metabolism in invertebrates in relation to size and age are conflicting but this may be due to unsatisfactory experimental methods. Buytendijk (as quoted by Montuori (35, p. 216)) measured the oxygen consumption by Scorpaena, Octopus and Echinus in relation to body size, and Montuori (35) attempted a survey of these relations in 80 different kinds of marine animals belonging to the coelenterates, echinoderms, worms, crustaceans and fish. In the determinations on some species, the oxygen consump-

tion per unit of weight was greater in the animals of smaller size than in those of larger size. In other series the reverse relationship was recorded, and in still other series the results were highly variable. These results may mean that standard conditions were not maintained in all cases, that the individuals compared were not strictly comparable or that in some cases the forms chosen were unsuited in behavior and respiratory habits to the solution of the question. Each species would require more critical individual study to determine the reasons for the irregularities. The conclusion of Montuori that "the consumption of oxygen, calculated per unit of weight, is, in general, absolutely independent of the dimensions of the animal," hardly seems justified from such highly variable determinations. The subject requires much more careful quantitative work under more definitely controlled conditions than has hitherto been done, before any conclusions of general application can be drawn. Some forms of animals, because of inconstant behavior, are quite unsuited as experimental material.

Careful measurements of the rate of respiratory metabolism per unit of weight in lower cold-blooded animals have indicated, however, that there is a decrease in rate of oxidations as the size of the body increases. Bounhiol (37) found this relationship in the oxygen consumption of annelids of different size, and Miss Wolf, in this laboratory, has found it in the leech, crayfish, branchipus, May-fly nymph and Stonefly nymph, under very conclusive experimental conditions. The number of determinations, made by the writer, of the rate of oxygen consumption by Planaria in various kinds of experiments, makes it certain that consistent results can be obtained for this form with the methods used. Various incidental observations in the course of these experiments point to the fact that smaller worms absorb more oxygen in proportion to their weight than larger worms, and the following systematic experiments show it very clearly.

Table 10 gives comparative measurements of the oxygen consumption by P. agilis of five different sizes. Planaria differ considerably in body proportions, the region posterior to the pharynx being much longer in some individuals than in others. To make worms as nearly uniform as possible, all except the smallest, set I, were cut at the plane of normal fission, a short distance posterior to the mouth. This was done 21 days before the determinations of oxygen consumption were made. All worms were without food for 22 days previous to the experiment. At the time of the respiration test, the worms of set I averaged 6.08 mm. in length, worms of set II, 8.4 mm., those of set III,11.8,

TABLE 10

Showing decrease in the rate of oxygen consumption by Planaria agilis with increase in size of the body. Worms starving for 22 days preceding the experiment and during the experiment. Tails removed at the fission plane 21 days before the experiment of all sets except set I, the smallest worms. Test period of 24 hours' duration. Temperature 19.6°C. ± 0.01. Capacity of bottles 130 cc. Correction for 4 cc. of water displayed by rods and reagents, multiply by 1.032. One cubic centimeter thiosulphate = 0.1738 cc. oxygen at N. T. P. Worms weighed 6 hours after the test. See figure 8

PART	SET	BOTTLE	BLANKS									
			cc. thio.			*average*						
A	Blanks (no worms present)	1	4.17			4.18						
		2	4.18									
		3	4.20									
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	
			AVERAGE LENGTH OF WORMS	NUMBER OF WORMS IN EACH BOTTLE	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	OXYGEN CONSUMED PER 10 WORMS PER 24 HOURS		WEIGHT PER 10 WORMS		OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS		
			mm.	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*average*	*grams*	*average*	*cc. oxygen*	*average*	
B	I	1	6.03	20	0.33	0.0295		0.0069		4.3		
		2	6.13	20	0.44	0.0394	*0.0349*	0.0073	*0.0070*	5.4	*4.9*	
		3	6.08	20	0.39	0.0349		0.0069		5.1		
	II	1	8.4	15	0.68	0.0812		0.0178		4.6		
		2	8.4	15	0.68	0.0812	*0.0792*	0.0179	*0.0175*	4.5	*4.5*	
		3	8.4	15	0.63	0.0753		0.0168		4.4		
	III	1	11.7	10	0.89	0.1595		0.0437		3.6		
		2	11.7	10	0.97	0.1738	*0.1678*	0.0469	*0.0451*	3.7	*3.7*	
		3	11.9	10	0.95	0.1702		0.0448		3.8		
	IV	1	14.8	6	0.92	0.2748		0.0832		3.3		
		2	14.0	6	0.84	0.2509	*0.2559*	0.0772	*0.0769*	3.3	*3.3*	
		3	12.9	6	0.81	0.2419		0.0702		3.4		
	V	1	17.8	4	1.04	0.4659		0.1568		3.0		
		2	17.9	4	1.08	0.4838	*0.4674*	0.1603	*0.1551*	3.0	*3.0*	
		3	17.5	4	1.01	0.4525		0.1483		3.0		

those of a set IV, 13.9 mm. and those of set V, 17.7 mm., as shown in column 1. Length, however, is no satisfactory measure of size even though precautions are taken, as described above, to make the worms as nearly comparable as possible in body proportions. The weights, as given in columns 6 and 7, ranged from 0.0070 to 0.1551 gram per 10 worms. The largest weighed twenty-two times as much as the smallest. Because of this great difference in size, the number of individuals in each bottle was varied so as to make the amounts of oxygen absorbed in each bottle nearly equivalent. The average amount of oxygen consumed per gram body weight per 24 hours decreased from 4.9 cc. in the

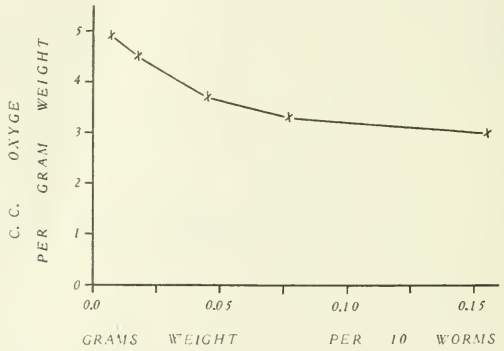

Fig. 8. Curve shows decrease in rate of oxygen consumption by Planaria agilis with increase in size of the body. Abscissas represent weight per 10 worms in grams. Ordinates represent rate of oxygen consumption in cc. oxygen per gram body weight per 24 hours. Each point on the curve represents the average of three sets of worms. See table 10.

smallest worms to 3.0 cc. in the largest. All worms were tested simultaneously under identical conditions. Independent determinations were made for three different bottles of worms of each size, and column 8 shows that, with one exception, there was no overlapping among the individual determinations. Set I, bottle 1, gave a very low value. The titration reading of thiosulphate, column 3, was only 0.33 cc. in this case. It should be remembered that a difference of one drop in titration would make a difference of 0.06 cc., and that titration values as small as this are less accurate than larger ones. This experiment shows, therefore, that the rate of oxidations decreased as the size of the

worms increased. These results are shown in figure 8 where the amount of oxygen consumed per gram body weight per 24 hours at 20°C. is plotted against body weight per 10 worms. This curve shows at a glance that the rate of oxidations decreases with increasing size, and that the decrease in oxidations is not directly proportional to the increase in weight. Among smaller worms, these differences are greater than among larger worms. Most of the difference was between worms less than 0.08 gram weight per 10 individuals. Worms of twice that size showed little further decrease in their oxidations.

It might be thought that the differences in the rates of oxidations among Planaria of different sizes, recorded in table 10, might be due to differences in activity. Was there a graduated difference in activity among the worms of different sizes during the respiration test? This question was tested by removing the heads of worms of different sizes so as to eliminate practically all locomotion. Worms of three different sizes were used, table 11. Those of each size were divided into two sets of three bottles each. A control test was made in which all worms were uncut and normal. Columns 5 and 6 show that, in each case, the two sets of worms of the same size had the same rate of oxygen consumption, and that the differences between the different sizes confirm the previous experiment, table 10. Before the second test, table 11, the heads were removed from the worms in one set of each size. In this test the normal worms, column 10, duplicated the rates of oxygen consumption given in the previous control test, while the headless worms in each case used less oxygen, 85 to 90 per cent of the normal. In comparing the headless worms of the three sizes, however, the size differences remain. The headless small worms used 3.9 cc. of oxygen per gram weight per 24 hours, the medium-sized worms, 3.3 cc., and the large worms, 2.8 cc. We conclude, therefore, that the rate of oxidations in Planaria decreases with increasing total body weight, and that this decrease is not accounted for by difference in locomotion.

The worms of sets II, III and V of table 10 were used as sets II a, III a and V a, respectively, of table 11. Since the worms were weighed for each test, these experiments constitute, in reality, three independent determinations of the normal rate of oxygen consumption by these worms, in relation to size. The three determinations gave the same results. The smaller worms used 4.5, 4.5 and 4.6 cc. of oxygen per gram body weight per 24 hours in the three determinations. The medium-sized worms used 3.7, 3.6 and 3.7 cc. of oxygen, and the larger worms 3.0, 3.1 and 3.1 cc. of oxygen, respectively, in the successive determinations.

TABLE 11

Showing that the difference in rate of oxygen consumption by Planaria agilis of different size is not due to differences in locomotion. Worms starving for 27 days preceding the experiment and during the experiment. Tails removed at the fission plane 26 days before the experiment. Worms of sets II, III and V are of the same sizes as sets II, III and V respectively of table 10. Duration of first test period 24.08 hours, of second test period 24 hours. Worms weighed the day before each test. Just before the beginning of the second test the heads were cut from the worms of sets Ib, IIb, and IIIb. Worms of sets Ia, IIa and IIIa were not cut. Temperature 19.8 C. ± 0.1. One cubic centimeter thisosulphate = 0.1738 cc. oxygen at N. T. P.

PART	SET	BOTTLE			FIRST TEST (CONTROL)				SECOND TEST (EXPERIMENT)				
					Blanks				Blanks				
					cc. thio.		*average*		*cc. thio.*		*average*		
A	Blanks (no worms present)	1			4.35		4.39		4.41		4.44		
		2			4.43				4.48				
		3			4.42				4.43				
		4			4.37				4.43				
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			WORMS OF SMALL, MEDIUM OR LARGE SIZE	NUMBER OF WORMS IN EACH BOTTLE	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS		OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS		WORMS NORMAL OR HEADLESS
					cc. thio.	*grams*	*cc. oxygen*	*average*	*cc. thio.*	*grams*	*cc. oxygen*	*average*	
B	II a	1	Small	15	0.54	0.0195	4.9	4.5	0.51	0.0184	5.0	4.6	Normal
		2		15	0.57	0.0237	4.3		0.53	0.0227	4.2		
		3		15	0.57	0.0233	4.4		0.54	0.0209	4.6		
	II b	1	Small	15	0.53	0.0209	4.5	*4.8*	0.41	0.0185	4.0	*3.9*	*Headless*
		2		15	0.61	0.0216	5.0		0.43	0.0192	4.0		
		3		15	0.54	0.0196	4.9		0.37	0.0174	3.8		

B	III	a	1	Medium	10	0.82	0.0391	3.7	3.6	0.84	0.0385	3.9	3.7	Normal
			2		10	0.86	0.0423	3.6		0.85	0.0408	3.5		
			3		10	0.82	0.0403	3.6		0.73	0.0353	3.7		
		b	1	Medium	10	0.85	0.0378	4.0	*3.8*	0.64	0.0317	3.6	*3.3*	*Headless*
			2		10	0.93	0.0444	3.7		0.65	0.0377	3.1		
			3		10	0.93	0.0444	3.7		0.68	0.0375	3.2		
	V	a	1	Large	4	0.96	0.0559	3.1	3.1	0.94	0.0537	3.1	3.1	Normal
			2		4	1.01	0.0580	3.1		0.96	0.0561	3.1		
			3		4	0.97	0.0548	3.2		0.92	0.0528	3.1		
		b	1	Large	4	0.95	0.0567	3.0	*3.0*	0.76	0.0513	2.7	*2.8*	*Headless*
			2		4	0.93	0.0556	3.0		0.81	0.0487	3.0		
			3		4	0.94	0.0580	2.9		0.78	0.0526	2.7		

While it is clear that larger Planaria use less oxygen in proportion to their weight than smaller ones, it is not certain that this difference is to be associated with age or senescence of protoplasm. In the case of man, it is commonly supposed that there is a decrease in the rate of oxidations, associated with age, apart from changes in the oxidations which may be associated with alterations in the size of the body. Such a distinction is not apparent in the data concerning the rate of respiratory metabolism in Planaria. It may be convenient to call a "full-grown" Planarian "old" in comparison with a "half-grown" individual, but it should be remembered that Planaria of the species dealt with in this discussion, after they have attained the more or less variable maximum size for the species, are not known to die of old age. Old age in man is not synonymous with the attainment of a maximum ("adult") body size. We have no evidence, as yet, that a Planarian of constant size becomes senescent or suffers a reduction in the rate of oxidations.

The statement that the rate of oxidations per unit of body weight is higher in small Planaria than in larger ones of the same species, assumes that the worms are compared in the same condition of nutrition, namely that they have been starved long enough to pass the accelerative effects of previous feedings, and that the differences in size are not due to differences in length of starvation. Within the limits of the experiments which have been performed on starvation we have concluded that the rate of oxidations continues practically constant after the early stages. In figure 3, starving Planaria weighed 0.0720 gram per 10 worms on July 12, by which time the accelerative effects of previous feeding had disappeared, and these same worms were reduced in weight to 0.0360 gram per 10 worms on August 16 at the end of the last respiration test. Figure 8 shows that worms of these two weights and of equal starvation differed in oxygen consumption by about 0.5 cc. oxygen per gram body weight per 24 hours. Such a change in the respiratory exchange of the starving worms was not found (see fig. 7). In this case the worms were reduced in weight to one-half of the original. The respiratory exchange in more extreme starvation, with reduction in size to a minute fraction of the original has not been studied.

OXIDATIONS DURING REGENERATION

The species of Planaria employed in these studies reproduce more commonly by agamic fission than by sexual processes. If the rate of oxidations decreases with increasing size of the body, as shown in the

preceding section, then it follows that it should increase with regeneration of small pieces of large worms into complete small worms. Natural fission results in two pieces of very unequal size and character. The anterior piece is much larger and retains the old head and body as far back as a level just posterior to the mouth. After regeneration it is essentially the original parent with a shorter tail but with little change in other proportions. On the other hand, the posterior piece is much smaller and undergoes profound reorganization into an animal with head and body of smaller proportions. Experiments show that the posterior piece undergoes considerable increase in rate of oxidations during regeneration while the anterior piece retains the original rate of oxidations of the parent practically unchanged.

Table 12 records the results of a preliminary experiment in which P. maculata were cut at the normal fission plane and allowed to regenerate, the oxygen consumption of the anterior pieces, the posterior pieces and uncut control worms being followed for 24 days after the operation. Column 4 gives the oxygen consumption by the normal controls (see also table 5). In column 11 the average rate of oxygen consumption by the anterior pieces is seen to be almost equal to that of the normal controls throughout the period of regeneration. The rate of oxygen consumption by the posterior pieces, however, is considerably higher in each determination than that by the controls or that by the anterior pieces. These results are shown also in figure 9.

In a second preliminary experiment, worms were cut just posterior to the auricles, and allowed to regenerate new heads. Under these conditions they were not much reduced in size during the regeneration of the new head. These worms were more comparable, therefore, to the anterior pieces of the preceding experiment than to the posterior pieces. Table 13 shows that the worms regenerating their heads maintained a rate of oxidation *practically equal* to that of control normal worms for the period of observation, 17 days. These results are shown also in figure 10.

Table 14 and figure 11 give a more complete series of determinations of the oxygen consumption by P. agilis during two weeks preceding and four weeks following experimental fission. The rate of oxygen consumption per gram body weight per 24 hours of normal worms ran very uniform throughout the experiment, column 4. These normal worms have been discussed in connection with table 7. The experimental worms duplicated very closely the rate of oxygen consumption by the controls during two weeks preceding the operation, table 14, column 11. After cutting the experimental worms in two at the normal fission plane, the

TABLE 12

Showing increase in the rate of oxygen consumption by Planaria maculata with regeneration. Worms 10 to 13 mm. in length January 8; starving for 15 days previous to the first test and during the experiment; 8 worms in each bottle. January 15 experimental worms cut in two a short distance posterior to the mouth. Control worms not cut. Weighed January 12, 14, 15, February 5 and 13. One cubic centimeter thiosulphate equals 0.1698 cc. oxygen at N. T. P. See figure 9. For further data on controls, see table 5

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			CONTROL	EXPERIMENT (WORMS CUT IN TWO JANUARY 15, AT NORMAL FISSION PLANE)						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours (cf. table 5)	Part of worm (whole, anterior or posterior)	Oxygen consumed by the worms in each bottle. Average	Average weight of worms in each bottle	Rate of oxygen consumption per gram body weight per 24 hours			
							Individual determinations			Average
	hours	*°C.*	*cc. oxygen*		*cc. thio.*	*gram*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
January 12–14	41.37	19.9–20.1	4.8	Whole	1.13	0.0247	4.7	4.6	4.5	*4.6*
January 15–17	42.66	20.0–	5.2	Anterior	0.71	0.0140	5.0	5.1	5.0	5.0
				Posterior	0.53	0.0077	7.1	7.1	6.5	*6.8*
January 18–20	43.23	19.9–20.1 (20.6)*	4.7	Anterior	0.63	0.0130	4.7	4.9	4.6	4.7
				Posterior	0.45	0.0071	5.8	6.2	6.4	*6.1*
January 20–22	46.48	19.9–20.1 (20.3)*	4.3	Anterior	0.62	0.0125	4.2	4.7	4.4	4.5
				Posterior	0.44	0.0068	6.0	5.7	5.9	*5.8*
January 22–24	42.45		4.1	Anterior	0.49	0.0117	4.1	4.3	4.0	4.1
				Posterior	0.34	0.0064	5.4	5.1	5.3	*5.3*

January 24–26	46.90	19.9–20.0 (19.0)*	4.0	Anterior	0.51	0.0111	4.3	4.1	4.1	4.2
				Posterior	0.36	0.0061	5.6	5.0	5.3	*5.3*
January 26–28	45.44		4.2	Anterior	0.48	0.0104	4.5	4.3	3.9	4.2
				Posterior	0.34	0.0057	5.6	5.6	5.4	*5.5*
February 5–9	94.77	16.7–22.6	4.6	Anterior	0.74	0.0067	5.1	4.9	4.7	4.9
				Posterior	0.48	0.0036	5.7	6.2	5.6	*5.9*

* For a short time.

anterior pieces continued for four weeks to absorb the same amounts of oxygen per gram body weight as the controls, columns 11 and 4. The rate of oxygen absorption by the posterior pieces, however, rose gradually above the controls during regeneration. These posterior pieces reorganized during this period to form complete worms of smaller size than the controls or than the anterior pieces. The following notes indicate the progress of regeneration of the tail pieces.

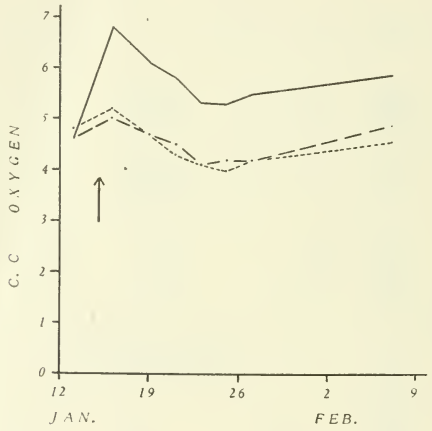

Fig. 9. Curves show increase in the rate of oxygen consumption by Planaria maculata during regeneration of posterior pieces from experimental fission, and no increase, within the limits of error of the experiment, in the rate of oxygen consumption in anterior pieces. Abscissas represent time in weeks, indicated by dates. Ordinates represent the rate of oxygen consumption in cc. oxygen per gram body weight per 24 hours. The broken line represents the control, an average of three sets of normal worms. The solid line represents the average of three sets of posterior pieces from experimental fission. The line of dashes represents the average of three sets of the anterior pieces from experimental fission of the same worms. Worms cut on January 15 (time of the operation indicated by the arrow). See table 12.

July 20. Cut experimental worms across body a short distance posterior to the mouth.

July 24. New head, wedge-shaped. Some locomotion after disturbance of dish.

July 26. Auricles and eyes. Worms elongating.

July 29. Auricles well developed but not full length. Heads appear white to the naked eye. Pigment appearing on head. Inactive till prodded. Locomotion rapid.

August 2. Still white-headed.

August 5. Pigmentation of head still incomplete. Worms more elongated.

TABLE 13

Showing no increase, within the limits of error of the experiment, in the rate of oxygen consumption by Planaria maculata during regeneration of the head. Worms 7 to 10 mm. long February 25; starving for 5 days preceding the first test and during the experiment; 10 worms in each bottle. Heads cut off from the experimental worms just posterior to the auricles March 12. Control worms not cut. Worms weighed March 2, 5, 11, 12, 18 and 30. One cubic centimeter thiosulphate = 0.1698 cc. oxygen at N. T. P. See figure 10. For further data on the controls, see table 6

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
			CONTROL	EXPERIMENT					
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours	Oxygen consumed by the worms in each bottle. Average	Weight of the worms in each bottle. Average	Rate of oxygen consumption per gram body weight per 24 hours			
						Individual determinations			Average
	hours	°C.	*cc. oxygen*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
March 2– 4	41.40	20.0–20.5	*5.0*	0.77	0.0163	5.0	5.1	4.4	*4.8*
March 5– 7	45.00	20.0–20.2	*4.8*	0.80	0.0149	4.9	5.2	4.8	*5.0*
March 7– 9	47.30	20.0–20.5	*4.8*	0.74	0.0138	4.7	5.1	4.5	*4.8*
March 9–11	45.65	20.0–20.4	*5.5*	0.72	0.0126	5.1	5.3	5.4	*5.3*
						Heads removed from experimental worms on March 12			
March 12–14	46.38	20.1–20.5	*5.2*	0.51	0.0092	4.9	5.4	4.9	*5.0*
March 14–16	46.16	20.0–20.5	*5.6*	0.52	0.0084	5.7	5.8	5.5	*5.6*
March 16–18	45.00	20.1–20.6	*5.9*	0.46	0.0077	5.4	6.1	5.5	*5.6*
March 19–21	42.90	20.0–	*4.8*	0.34	0.0067	5.2	4.9	4.5	*5.0*
March 21–29	191.09	20.0–20.5	*5.4*	1.39	0.0056	5.7	5.9	5.0	*5.5*

The smaller individuals in a population of Planaria usually result from fission and regeneration. This experiment shows that during the processes of regeneration the higher rate of oxidation characteristic of smaller worms is attained. If the respiration of these small worms during growth were followed, undoubtedly a progressive decline in rate

TABLE 14

Showing increase in the rate of oxygen consumption by Planaria agilis with regeneration in the posterior zoöid after artificial fission, and no increase in the rate of oxygen consumption, within the limits of error of the experiment, with regeneration in the anterior zoöid. Worms 17 to 18 mm. long July 5, starving for 7 days previous to the experiment and during the experiment; 5 worms in each bottle before the operation and 10 in each bottle after the operation. Experimental worms cut in two at a level a short distance posterior to the mouth on July 20. Control worms not cut. Worms weighed once each week, namely July 5, 13, 19, 20, 26, August 2, 9 and 16. One cubic centimeter thiosulphate equals 0.1768 cc. oxygen at N.T.P. from July 6 to July 19. One cubic centimeter thiosulphate equals 0.1751 cc. oxygen at N. T. P. from July 20 to August 16. See figure 11. For further data on controls, see table 7

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			CONTROL	EXPERIMENT						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours	Part of worm (whole, anterior or posterior)	Oxygen consumed by the worms in each bottle. Average	Average weight of worms in each bottle	Oxygen consumption per gram body weight per 24 hours (rate of oxygen consumption)			
							Individual determinations			Average
	hours	*°C.*	*cc. oxygen*		*cc. thio.*	*gram*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6–8	45.15	22.0–20.0	3.7	Whole	1.68	0.0476	3.4	3.6	3.4	*3.4*
July 8–10	43.08	20.0–19.6	3.6	Whole	1.50	0.0461	3.3	3.3	3.3	*3.3*
July 10–12	45.25	20.4–19.9	3.6	Whole	1.63	0.0456	3.5	3.4	3.4	*3.5*
July 13–15	45.75	20.2–19.4	3.4	Whole	1.48	0.0432	3.2	3.2	3.5	*3.3*
July 15–17	45.00	20.5–18.8	3.4	Whole	1.44	0.0417	3.3	3.3	3.4	*3.4*
July 17–19	45.00	20.4–19.2	3.3	Whole	1.37	0.0402	3.3	3.2	3.4	*3.3*
							Experimental worms cut in two at normal fission plane, July 20.			
July 20–22	42.25	19.6–20.0	3.3	Anterior	1.57	0.0504	3.2	3.2	3.2	3.2
				Posterior	0.49	0.0151	3.3	3.3	3.5	*3.3*

July 22–24	43.50	20.0–19.7	3.4	Anterior	1.54	0.0486	3.1	3.2	3.2	3.2
				Posterior	0.54	0.0142	3.6	3.7	4.2	*3.8*
July 24–26	42.00	19.7–20.0	3.3	Anterior	1.43	0.0468	3.1	3.1	3.3	3.1
				Posterior	0.49	0.0135	3.6	3.7	3.8	*3.7*
July 27–29	45.10	19.6–20.2	3.3	Anterior	1.48	0.0442	3.1	3.2	3.3	3.2
				Posterior	0.49	0.0122	3.8	3.7	3.9	*3.8*
July 29–31	45.00	19.6–19.8	3.2	Anterior	1.40	0.0426	3.1	3.1	3.2	3.2
				Posterior	0.49	0.0118	3.9	3.8	4.2	*4.0*
July 31–August 2	43.00	19.8–	3.4	Anterior	1.32	0.0410	3.2	3.2	3.4	3.3
				Posterior	0.46	0.0113	4.1	4.1	4.1	*4.1*
August 3–5	45.25	19.6–19.9	3.3	Anterior	1.33	0.0385	3.3	3.3	3.3	3.3
				Posterior	0.48	0.0106	4.3	4.1	4.6	*4.3*
August 5–7	45.07	19.3–20.0	3.4	Anterior	1.29	0.0367	3.4	3.3	3.5	3.4
				Posterior	0.46	0.0101	4.3	3.9	4.8	*4.4*
August 7–9	45.07	20.0–20.7	3.7	Anterior	1.33	0.0350	3.5	3.5	3.8	3.6
				Posterior	0.49	0.0097	4.8	4.7	5.2	*4.9*
August 10–12	45.42	19.8–19.6	3.3	Anterior	1.17	0.0329	3.3	3.4	3.5	3.4
				Posterior	0.41	0.0090	4.4	4.2	4.4	*4.4*
August 12–14	45.33		3.3	Anterior	1.15	0.0317	3.3	3.6	3.6	3.5
				Posterior	0.38	0.0086	4.4	3.7	4.6	*4.2*
August 14–16	45.00	19.6–19.7	3.4	Anterior	1.08	0.0304	3.3	3.4	3.6	3.4
				Posterior	0.37	0.0082	4.3	4.1	4.6	*4.3*

of oxygen consumption would be found as the animals became larger. It is not very convenient to follow the oxidations in the same worms during growth, but it seems unnecessary to do so. Worms of different sizes selected from a laboratory stock of the same history, except as regards reproduction, and tested simultaneously under identical conditions, give more certain results. Such experiments are described in the preceding section.

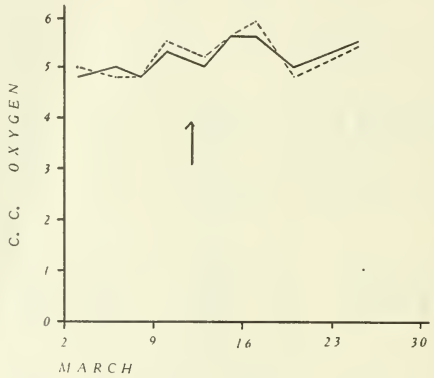

Fig. 10. Curves show no increase, within the limits of error of the experiment, in the rate of oxygen consumption by Planaria maculata during regeneration of the head. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. The broken line represents the control, an average of three sets of normal worms. The solid line represents the average of three sets of worms whose heads were cut off just posterior to the auricles on March 12 (time of the operation indicated by the arrow). See table 13.

It should be clearly understood that these experiments upon regeneration of Planaria are not advanced as evidence that the regeneration process, as such, or, more generally, that growth or morphogenesis, necessitates a higher rate of oxidation. In figure 11 the rate of oxygen consumption in the tails rose progressively during regeneration and *did not show a fall* with completion of the process. The higher rate at the end of the process is attributed to the new organization of the worm, i.e., to the result of the process, not to the process itself. At least the experimental data do not constitute a secure foundation for concluding otherwise.

SUSCEPTIBILITY AS A MEASURE OF OXIDATIONS

The susceptibility of Planaria and other animals to the toxic action of alcohol, potassium cyanide and other substances, in other words, the length of time the animals live in solutions of these substances, has been used extensively by Child, Hyman and others as a measure of the "rate of metabolism." This practice has been based upon inadequate knowledge either of the nature of the action of these toxic agents

Fig. 11. Curves show increase in the rate of oxygen consumption by Planaria agilis during regeneration of posterior pieces from experimental fission, and no increase, within the limits of error of the experiment, in the rate of oxygen consumption in the anterior pieces. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. The broken line represents the control, the average of three sets of normal worms. The solid line represents the average of three sets of experimental worms before the cutting operation on July 20, and of the posterior pieces after that date. The line of dashes represents the average of the three sets of anterior pieces from the operation. The time of the operation is indicated by the arrow. See table 14.

or of the parallelism between the susceptibility of animals and their "rate of metabolism." The substances first used for this purpose were anesthetics and potassium cyanide, sometimes classed as an anesthetic. It was supposed that these substances inhibit the oxidations, and they were spoken of as "depressing agents" (1, p. 571); (2, p. 150); (3, pp. 419, 420, 444); (4, p. 65); (8, p. 104). The effect of none of these substances upon the oxidations in Planaria or other animals upon which

they were used was determined. The writer (13) has shown that potassium cyanide, the agent most used for the susceptibility studies, does inhibit oxygen consumption in Planaria, but it does not follow from this fact that the worms with higher rate of oxidation must be more susceptible to the cyanide, nor that those that are more susceptible to cyanide must have a higher rate of oxidations. Furthermore, many other substances of very different chemical nature and probably of very different physiological action have been used for determining susceptibility. Before drawing conclusions regarding metabolism from the data of susceptibility, in the absence of knowledge of the nature of the action of the toxic agents used, it should be determined, at least, that there is a parallelism between susceptibility and rate of oxidations under all normal conditions. This has not been done excepting for the comparisons with the carbon dioxide production by Planaria in the Tashiro "biometer" previously mentioned. Upon the basis of the data presented in the present paper, it is possible to make certain comparisons between susceptibility of Planaria and their rate of oxidations. If the susceptibility is a measure of the oxidations, it must not vary independently of the rate of oxygen consumption.

Table 15 summarizes the facts regarding susceptibility and the rate of oxygen consumption per unit of body weight. Four normal conditions are considered; *a*, increase in the size of the body by growth; *b*, decrease in the size of the body by agamic reproduction; *c*, starvation; and *d*, feeding. Starvation is naturally divided into the early and the later stages, making five items in the table. In the so-called "indirect susceptibility method," less susceptibility, or, conversely, greater *resistance*, is supposed to indicate a higher "rate of metabolism," while in the "direct method" greater *susceptibility* is supposed to indicate a higher rate of metabolism or rate of oxidations. The letters in the table refer to the following quotations and references to the literature on susceptibility:

A. (1) pages 544 to 547. Figures 1 and 2.

(4) page 83 and figure 4.

B. (1) pages 555 to 564.

C. "In this connection it should be noted, again, that when well-nourished worms are kept without food, the resistance increases somewhat during the first three or four weeks, as the reserves disappear and after that decreases rapidly" (1, p. 549).

"If the resistance of the worms to anesthetics is in any degree a criterion of age, as it seems to be, then it is evident that while starvation alone does not

TABLE 15

Showing that susceptibility of Planaria to the toxic action of alcohol or potassium cyanide varies independently of the rate of oxygen consumption. Changes in susceptibility determined by Child. For references, see text pages 464 to 466 under letters A, B, C, etc., as given in table. Changes in rate of oxygen consumption determined by the writer

	(1)	(2)	(3)	(4)
	CONDITION OF PLANARIA	SUSCEPTIBILITY TO ALCOHOL AND POTASSIUM CYANIDE		RATE OF OXYGEN CONSUMPTION
		Resistance in "indirect method"	Susceptibility in "direct method"	
I	Increase in size by growth	Decreases A (⇔ decreased oxidations)	Decreases F (⇔ decreased oxidations)	Decreases
II	Decrease in size by regeneration of posterior piece of large worm	Increases B (⇔ increased oxidations)	Increases G (⇔ increased oxidations)	Increases
III	Starvation early stages (5–15 days)	*Increases* C (⇔ increased oxidations)	*Increases* H (⇔ increased oxidations)	*Decreases*
IV	Starvation later stages (5–8 weeks)	Increases D (⇔ increased oxidations) then *Decreases* (⇔ decreased oxidations)	*Increases* I (⇔ increased oxidations)	*Constant*
V	Feeding	*Increases* E after 48 hours (⇔ increased oxidations)	*Decreases* J (⇔ decreased oxidations)	*Increases* within 48 hours

rejuvenate these animals, *except to a slight extent in the early stages*,[3] yet feeding after a long period of starvation does accomplish this very effectively" (1, p. 555).

"During the first two or three weeks of starvation before any marked reduction occurs, the rate of metabolism increases but decreases after reduction begins (indirect method, meaning that resistance increases during first two or three weeks) (3, p. 437).

[3] Italics mine.

D. "I have performed numerous experiments with worms in various stages of starvation and always with the same result, viz., the reduced worms show even less resistance than before reduction" (1, p. 547).

E. (1) page 555, quoted above under C.

"The effect of feeding after starvation does not appear as increased resistance to any marked extent until at least 48 hours after feeding. Probably this period of time represents that necessary for the attainment of a certain stage and rate of the metabolic reactions after feeding" (1, p. 549).

F. (3) pages 423 to 427.

(4) pages 93 to 100.

G. (4) pages 105 to 110.

H. "Animals which have been without food for only a few days are distinctly more susceptible to depressing agents than animals which have been fed up to the beginning of the experiment" (3, p. 434).

(4) pages 156 to 160, especially table 11, page 157.

I. "In all several hundred individuals have been testd and always with the same result, viz., increase in susceptibility from the beginning of starvation on until the animals are reduced to a minute fraction of their original size and death occurs. If the method is valid this increase in susceptibility must mean that the rate of the metabolic processes increases as starvation and reduction proceed, a conclusion exactly the opposite of that reached from my earlier work with the indirect method alone" (3, p. 427).

(4) pages 156 to 160, especially table 11, page 157.

"But the results obtained in later investigations by the direct susceptibility method which have been briefly presented above, and the confirmation of these by the estimates of carbon dioxide production, force us to the conclusion that the rate of metabolism increases during starvation. This being the case, the decrease in capacity for acclimation in starved animals cannot be due to a low rate of metabolism, but must be associated with the nutritive condition in some way independent of the metabolic rate" (4, p. 165).

J. "Each feeding is followed by a distinct decrease in susceptibility, and later, as the animals begin to starve, the susceptibility increases again" (4, p. 169).

Inspection of this table shows at a glance in what cases susceptibility and rate of oxygen consumption per unit of body weight agree and in what cases they disagree. When the size of the body is increased by growth, part I, the resistance by the indirect method decreases, the susceptibility by the direct method decreases and the rate of oxygen consumption decreases. When the size of the body is decreased by experimental fission and regeneration, part II, these three items all increase. These facts are consistent with the theory of a relationship between susceptibility and rate of metabolism. But when the conditions of starvation and feeding are considered, the picture changes. During the first stages of starvaton the rate of oxygen consumption falls sharply, column 4, but the susceptibility by the direct method increases instead of decreases. This striking decrease in the oxidations occurs chiefly

during the first few days. Since the indirect method requires a number of days for making determinations, it is perhaps not well adapted for catching the conditions during this brief period. Statements were made, however, that the resistance by this method increased somewhat during the first three or four weeks without food, as contrasted with the decrease afterwards. During the later stages of starvation, i.e., after the first 10 days, approximately, the rate of oxygen consumption runs practically constant, while the susceptibility in the direct method continues to increase steadily. In the indirect method, the increase in resistance which begins in the early stages is reported to continue during a total of three or four weeks, after which it is followed by a decrease in resistance. It should be clearly understood that this table and discussion refer only to the first seven to nine weeks of starvation, and do not include later stages of more extreme starvation. What changes may occur in the respiratory metabolism, later on, do not concern the point under discussion, which is that the susceptibility of Planaria to potassium cyanide and alcohol, and the rate of respiratory metabolism per unit of total body weight vary independently of each other. Such independent variations are clearly shown within the periods which are considered in this discussion. The fall in rate of oxidations during the early stages of starvation is *large* and is not reflected in any decrease in susceptibility. On the other hand, the increase in susceptibility *during the period of starvation included in these experiments is large* (4, p. 157, table 11) and is not paralleled by an increase in rate of oxygen consumption. It may be noted, furthermore, that the discrepancies revealed in the table are too great to be altered materially by minor differences in the respiratory metabolism of different species of Planaria.

Feeding causes an enormous increase in the rate of oxygen consumption, but it is reported (4, p. 169) that "each feeding is followed by a distinct decrease in susceptibility, and later, as the animals begin to starve, the susceptibility increases again" (direct susceptibility method). In the indirect method, feeding results in an increased resistance as well as in an increased oxygen consumption but it may be noted that "the effect of feeding after starvation does not appear as increased resistance to any marked extent until at least 48 hours after feeding." The maximum effect of feeding in increasing the rate of oxygen consumption is within this 48-hour period. These comparisons show, therefore, that susceptibility in Planaria varies independently of rate of respiratory metabolism.

B. L. Lund (38) and E. J. Lund (11), (39) have shown a similar lack of agreement between susceptibility and rate of oxidations in Paramecium. Starving Paramecia are more susceptible to potassium cyanide than those that are well fed, but the starving Paramecia consume less oxygen and give off less carbon dioxide. Child (7, p. 217) has criticized Lund because "he has apparently failed to recognize an essential point, viz., that susceptibility as measured by progress of death and disintegration concerns primarily the ectoplasm or the body-surface and body-wall. Statements to this effect have been made repeatedly in the work on susceptibility." These statements, however, give no indication that the *"rate of metabolism" under discussion* "concerns primarily the ectoplasm or the body-surface and the body-wall." On the contrary, statements have been made repeatedly in the work on susceptibility to the effect that estimations of the carbon dioxide production by Planaria in the Tashiro "biometer" have confirmed results obtained by the susceptibility method (e.g., (3, pp. 422, 343); (4, pp. 73, 161, 202); (8, p. 104)). The following quotation may suffice:

> The rate of production of carbon dioxide in the starved, reduced animals is practically equal to that in the young, growing animal of the same size, and this rate is much higher per unit of body weight than that in large, old animals. The results obtained by the direct susceptibility method are thus fully confirmed by the estimations of the carbon dioxide production (in the "biometer") (4, p. 161).

But they are not confirmed by measurements of the oxygen consumption by Planaria made by the writer, nor by measurements of the oxygen consumption and carbon dioxide production by Paramecium made by Lund.

CONCLUSIONS

In conclusion it should be noted that the purpose of these studies is to contribute to the critical analysis of the rôle of rate of respiratory metabolism in the animal organism. It may be conceived to be the function of bold and suggestive theories such as those of Child regarding the "rate of metabolism" to serve as a basis for analysis of all the factors involved in the question. All facts should be welcome and should be examined with critical impartiality to place them in their proper relation to the general problem. As the analysis proceeds, new aspects appear and new need for discrimination between the various factors involved. The term "rate of metabolism" may be used in different senses. Attention has been confined in this paper to the rate of respiratory metabolism. Even this term is capable of meaning different things. What should seem to be the only strictly sound basis for

comparisons of the relative rates of respiratory metabolism in animals of different size and age are data that give us the rate of respiratory metabolism per unit of weight or volume of *living protoplasm* in the body of the animal. No other basis is really satisfactory. Material should be selected and methods devised which supply these conditions as nearly as possible. Warm-blooded animals are very unsatisfactory material for such work, and this fact should be considered before drawing conclusions of general application from the data of mammalian calorimetry. If the method of susceptibility to the toxic action of such substances as potassium cyanide, when applied to the lower invertebrates, could provide a means of approaching the ideal conditions in measuring the "rate of metabolism" or rate of oxidations, it would be of very great value in the analysis of these problems. This method has been compared in the present paper with measurements of the rate of oxygen consumption in proportion to the weight of the body, and it has been found that the susceptibility does not give a reliable index of the rate of oxidations as thus defined. Since we have no data regarding the rate of oxidations upon the basis of any other definition, we must conclude that, in the present state of our knowledge regarding the action of these substances, only direct methods are reliable for determining the rate of respiratory metabolism.

SUMMARY

1. The oxygen consumption by P. maculata and P. agilis decreases progressively during starvation at constant temperature, more rapidly at first and somewhat more slowly later. The body weight also decreases during starvation, and the worms become smaller in body dimensions.

2. The rate of oxygen consumption per unit of body weight in starving P. maculata and P. agilis decreases rapidly during the first few days, due to the decreasing accelerative effect of food residuum left from the previous feeding. The length of this period may vary with the temperature and the amounts and kinds of food previously ingested, but in the experiments reported it lasted as long as 10 to 14 days, although the accelerative effects were small after the first 7 days.

3. At the end of this period of the accelerative effects of food reserve from previous meals, the rate of oxidations reaches a constant level which is maintained for several weeks in P. agilis (5 to 8 weeks in the experiments reported). During this period of constant rate of oxidations, the starvation results in a decrease in the body weight of at least one-half of the original.

4. The ingestion of food by starving P. maculata and P. agilis results in a great increase in the oxygen consumption (80 to 140 per cent in the experiments reported). After this initial rapid rise, which reaches its maximum within the first 48 hours or less, the *rate of oxidations per unit of body weight* falls more slowly during 7 to 14 days to reach a constant level. When this constant level is reached after a single feeding, the rate of oxygen consumption per unit of body weight is the same, within the limits of error of the experiment, as it would have been if the animal had not been fed. However, after the rate of oxidations has returned to this constant level the animal weighs more and consumes more oxygen in absolute units than would have been the case if the animal had not been given a meal (see figs. 4 and 6).

5. The later period of starvation, after the accelerative effects of food reserves have disappeared, is the proper basis of reference in studying respiratory metabolism in Planaria.

6. Larger Planaria have a lower rate of oxidations per unit of total body weight than smaller worms of the same species when the latter do not owe their small size to starvation. The experiments reported do not permit making any general statement to cover all cases of differences in size due to starvation alone since the more extreme stages of starvation are not included in this study (see paragraph 3 above).

7. After fission of a large worm, the tail piece, which becomes reorganized into a small-sized worm, gains a higher rate of oxygen consumption per unit of body weight. With regeneration in the anterior piece, which does not involve important alterations in the body, the rate of oxygen consumption remains constant within the limits of accuracy of the method. It is not assumed that the increase in rate of oxidations in the posterior piece is due to the use of measurable amounts of energy in the processes of regeneration or morphogenesis as such.

8. The susceptibility of Planaria to the toxic action of potassium cyanide and alcohol, as reported by Child, varies independently of the rate of oxidations per unit of body weight, and is, therefore, not a reliable measure of the oxidations as thus defined. If the rate of oxidations or the "rate of metabolism" is defined differently, then it remains to be shown that susceptibility is a reliable measure of either.

APPENDIX

Since the above was written, three papers have appeared which deal with the respiratory metabolism in Planaria (40), (41) and (42). Hyman (41) has attempted to strengthen the theory that susceptibility is a

measure of rate of oxidations by showing that potassium cyanide inhibits the oxidations in Planaria dorotocephala; her results confirm those of the writer reported in the first paper of this series (13). The two studies supplement each other since the experiments of Hyman covered short periods of time while those of the writer covered longer periods. Hyman argues that "since cyanides decrease the rate of oxygen consumption, it follows that cells or organisms or parts of organisms which have the highest rate of respiratory exchange will be more susceptible to cyanide and will, therefore, die in lethal concentrations of cyanide faster than parts or organisms respiring less actively" (41, p. 353). The writer does not wish to discuss this argument further than to call attention to the facts observed in Paramecium and Planaria, namely, that fed animals have a higher rate of respiratory exchange but live longer in lethal concentrations of cyanides than starving animals.

Child (40) has compared the rates of carbon dioxide production by equal weights of Planaria dorotocephala of different sizes and in different stages of starvation, as determined by the phenolsulphonephthalein indicator method. The data do not permit a calculation of the actual quantities of carbon dioxide produced, but show that the worms in one tube caused a higher or lower hydrogen ion concentration than those in another tube. Child concludes from his experiments with this indicator method that the rate of carbon dioxide production is greater in small than in large worms, that it "decreases rapidly during the first stages of starvation and continues to decrease more slowly during several weeks, but that in advanced stages of starvation it increases" (40, p. 256). The advanced stages in which it is reported that the rate of oxidations increases are apparently later than the stages included in the experiments of the present writer, although it is hardly possible to compare the rates of starvation in two different species of Planaria under different conditions of observation. The data regarding differences in rate of oxidations in animals of different size and during the earliest stages of starvation are in agreement with those of the present paper.

These conclusions, it may be noted, are very different from either of the two former conclusions of Child which were based on *susceptibility* data. Child himself calls attention to these discrepancies and offers an explanation to preserve the theory that susceptibility is a measure of rate of oxidations. He recalls observations previously neglected, to the effect that during early starvation of Planaria, the susceptibility of the intestinal tract is different from that of the body wall, the former decreasing while the latter increases. The interpretation suggested is that

the ectoderm and body wall, which maintain their functional activity and subsist in part upon their own substance from the beginning, show a gradual increase in rate of oxidation during starvation, while the rate of oxidation in the alimentary tract undergoes rapid and marked decrease in the absence of food and probably still further decrease more slowly, as the reserves are used. Alimentary tract oxidation in well-fed animals is so large a part of the total oxidation that the decrease in activity of the alimentary tract in the absence of food brings about a decrease in total CO_2 production, in spite of the probable increase in CO_2 production in ectoderm and body well (40, p. 254).

In the absence of any experimental evidence that the oxidations in the body wall change with starvation and feeding, as Child suggests, rather than in accordance with the oxidations of the animal as a whole, the observed facts must be considered damaging to the theory that susceptibility is a measure of rate of oxidations. If the possibility that the oxidations in the body wall may change in a direction exactly opposite to the rate of oxidations of the whole animal is pressed, it must be borne in mind that the evidence which is cited in support of the theory, also, consists of measurements of the respiratory exchange of *whole animals*. Estimations of the total carbon dioxide production by Planaria in the Tashiro "biometer" have served as proof of the value of the susceptibility method. They were cited for this purpose in the last paper by Child (42, p. 381) although in the preceding paper it was reported that the biometer gave different results (as regards different conditions of nutrition) than the indicator method, so that "work with the biometer was discontinued because I came to believe that the method was not suitable for the material" (40, p. 250). However, whether reliance is placed upon the "biometer," the indicator method or the writer's Winkler method, the data obtained by these methods have given only the rate of respiratory metabolism of *whole animals*.

The distinction between the rate of oxidations in the animal as a whole and that in particular organs carries the question ultimately to the individual cells. Here we have the information that starving Paramecia are more susceptible to cyanide but have a lower rate of oxidations than fed Paramecia. The explanation suggested by Child is that during starvation in Paramecium, the ectoplasmic oxidations increase while the endoplasmic oxidations, which make up the major part of the whole, decrease. Is susceptibility in other animals such as Planaria to be attributed finally to conditions in the surface of the cell rather than to the oxidations of the cell as a whole? What does survival time tell us *with certainty* about oxidations?

BIBLIOGRAPHY

(1) Child: Arch. f. Entwicklungsmech., 1911, xxxi, 537.
(2) Child: Arch. f. Entwicklungsmech., 1913, xxxvii, 108.
(3) Child: Arch. f. Entwicklungsmech., 1914, xxxviii, 418.
(4) Child: Senescence and rejuvenescence, Chicago, 1915.
(5) Child: Individuality in organisms, Chicago, 1915.
(6) Child: Biol. Bull., 1918, xxxiv, 49.
(7) Child and Hyman: Biol. Bull., 1919, xxxvi, 183.
(8) Hyman: Journ. Exper. Zoöl., 1916, xx, 99.
(9) Hyman: Journ. Exper. Zoöl., 1917, xxiv, 55.
(10) Lusk: Science of nutrition, 3rd ed., 1917.
(11) Lund: This Journal, 1918, xlvii, 167.
(12) Krogh: Respiratory exchange of animals and man, London and New York, 1915.
(13) Allen: This Journal, 1919, xlviii, 93.
(14) Johansson, Landergren, Sonden and Tigerstadt: Skand. Arch. f. Physiol., 1897, vii, 29.
(15) Benedict: Carnegie Institution Pub. 77, 1907.
(16) Lehman, Mueller, Munk, Senator and Zuntz: Arch. f. path. Anat. u. Physiol., 1893, cxxxi, Suppl. 1.
(17) Benedict: Carnegie Institution Pub. 203, 1915.
(18) Zuntz: Biochem. Zeitschr., 1913, lv, 341.
(19) Hill: Journ. Physiol., 1911, xliii, 379.
(20) Brunow: Zeitschr. f. Allg. Physiol., 1911, xii, 215.
(21) Rubner: Zeitschr. f. Biol., 1883, xix, 535.
(22) Benedict: Journ. Biol. Chem., 1915, xx, 263.
(23) Benedict, Emmes, Roth and Smith: Journ. Biol. Chem., 1914, xviii, 139.
(24) Meeh: Zeitschr. f. Biol., 1879, xv, 425.
(25) Du Bois and Du Bois: Arch. Int. Med., 1915, xv, 868.
(26) Du Bois and Du Bois: Arch. Int. Med., 1916, xvii, 863.
(27) Gephart and Du Bois: Arch. Int. Med., 1916, xvii, 902.
(28) Magnus-Levy and Falk: Arch. f. Physiol., 1899, Suppl. 314.
(29) Du Bois: Arch. Int. Med., 1916, xvii, 887.
(30) Benedict and Talbot: Carnegie Institution Pub. 201, 1914.
(31) Benedict and Talbot: Carnegie Institution Pub. 233, 1915.
(32) Murlin and Hoobler: Amer. Journ. Diseases of Children, 1915, ix, 81.
(33) Bohr: Skand. Arch. f. Physiol., 1900, x, 413.
(34) Bohr and Hasselbalch: Skand. Arch. f. Physiol., 1900, x, 149.
(35) Montuori: Arch. Ital. de Biol., 1913, lix, 213.
(36) Morgulis: This Journal, 1915, xxxvi, 207.
(37) Bounhiol: Ann. des Sci. Naturelles, 1902, xvi, 1.
(38) Lund: Biol. Bull., 1918, xxxv, 211.
(39) Lund: This Journal, 1918, xlvii, 318.
(40) Child: This Journal, 1919, xlviii, 231.
(41) Hyman: This Journal, 1919, xlviii, 340.
(42) Child: This Journal, 1919, xlviii, 372.

EXPERIMENTAL STUDIES OF THE URETER

Y. SATANI

From the Physiological Laboratory of the Johns Hopkins University

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A large number of papers have been published dealing with the physiology of the ureter; the main conclusions may be summarized briefly.

Engelmann (1) making use of simple inspection described the peristalsis and anti-peristalsis proceeding with a certain velocity. On microscopic examination he found no ganglionic structures except in the lower part. He believed, therefore, that the contraction originates and travels in the muscle tissue itself without any relation to extrinsic or intrinsic nerves. Protopopow (2) also making use of ocular observation investigated the movements of the ureter in asphyxia and in different conditions of the circulation of the kidney and ureter. His experiments showed that an acceleration of the movements is caused by the distention of the ureter lumen, while a depression results from the ligation of the upper end. Concerning the innervation of the ureter he obtained a motor effect from the splanchnic nerve and a slight inhibitory result by stimulating the anastomosis between the inferior mesenteric ganglion and the plexus hypogastricus. In his work he tested the influence of a number of drugs upon the ureteral movements in situ. Atropin caused first an acceleration, later a depression; diuretin had no effect and caffeine stimulated the contractions in small doses, but depressed them in large doses.

Besides simple inspection by means of which Engelmann and Protopopow carried out their experiments, more recent investigators have studied the movements of the ureter either by recording intra-ureteral pressures or by using excised ureter strips.

Papers dealing with the pressure changes in the ureter lumen, by which its movements were studied, have been published by Fagge (3), Henderson (4), Berensnegowsky (5), Lucas (6), Sokoloff and Luchsinger (7).

Fagge found a motor effect of the hypogastric nerve upon the ureter;

Henderson and Berensnegowski separately concluded that a ureteral pressure does not depend entirely upon the contraction of the ureter. Lucas mentioned that the contractions are different in size and in number in various parts; the middle part showing relatively large and few contractions, while the upper part gives small and frequent waves of contractions. He also observed a complete prevention of the reflux of the bladder content into the ureter and described an action of the ureter for preventing by its peristalsis the accumulation of urine in the renal pelvis. Sokoloff and Luchsinger obtained contractions by the distention of the ureter lumen.

On the other hand some authors as Stern, Lucas (6), Roth (8) and Macht (9) made their experiments with ureter strips removed from the body.

Stern records some facts concerning the temperature relations and the proper nutrient solutions for the contractions of the excised ureter. Lucas tested the effects of various drugs; adrenalin, diuretin and barium chloride increased the tone and contraction, while chloroform, ether, chloral and magnesium sulfate exerted a final depressing effect preceded by a slight irritation. Roth stated that oxygenated Locke's solution of a certain composition is an excellent medium for the development of the contraction of the excised ureter. He observed two varieties of contractions occurring in a longitudinal strip, namely, large and small contractions. According to his experiments the small contractions predominated in the kidney third, while in the lower part large contractions were most frequently observed. Adrenalin and barium chloride caused an immediate increase in the tone and in the rate of the contraction, while apocodein depressed the activity of the ureter. From the effect of these drugs he assumed the presence of sympathetic nerve endings throughout the ureter.

Macht recently published a series of communications under the title of *On the pharmacology of the ureter*. He used the ureter ring preparation to study the behavior of the circular muscle coat. After suspending his preparation in normal Locke's solution he introduced several drugs such as adrenalin, ergotoxin, physostigmin, atropin, nicotin, morphin and various derivatives of morphin. From these experiments he obtained pharmacological proof that two sets of nerve endings are found in the ureter wall; one belonging to the sympathetics and the other to the parasympathetics. He also demonstrated the presence of ganglia throughout the ureter from the effect of nicotin.

1. CONTRACTION OF THE EXCISED URETER STRIP

a. Single strips

A number of observations on the contractions of the excised ureter in nutrient solutions at certain temperatures and effects of certain drugs upon their contractions have been described by such authors as Stern, Lucas, Roth, Macht and others. I have repeated these experiments under the same or different conditions.

Method. In this study I have generally used the excised ureter of the pig, because as Macht has stated, the pig's ureter has the most regular contraction and a longer vitality in artificial nutrient solutions. The material was obtained from a slaughterhouse in the neighborhood of the school. Pigs were bled to death and immediately after killing the ureters on both sides, connected with the kidneys and the bladder, were removed and placed in Locke's solution at room temperature. For longer preservation of their vitality, they were kept in Locke's solution on ice. Under these conditions striking contractions may be observed two days or more after removal. A very short strip (2 or 3 mm.) was cut off from the ureter to use as a ring after the manner of Macht, and also a longer section (10 to 20 mm.) as a longitudinal segment (circular fibers were opened). The former preparation registers the contraction of the circular muscle fibers and the latter represents that of the longitudinal fibers.

For the nutrient solution, Locke's solution of the following composition was employed almost exclusively in this work, because Roth found it the most efficient for the development of the spontaneous rhythm and the preservation of the vitality of the excised ureter of the dog.

Sodium chloride	9.0 gm.
Calcium chloride	0.24 gm.
Potassium chloride	0.24 gm.
Sodium bicarbonate	0.3 gm.
Dextrose	1.0 gm.
Distilled water	1000.0 cc.

The solution was made fresh for every experiment, and I could remove the sugar from the solution with very little deleterious effect upon the action of the ureter. Thus a ureter ring (or longitudinal strip) was placed in a bath of 20 cc. of Locke's solution, which was kept at a constant temperature of 38°C. The solution was also oxygenated by continual bubbles of oxygen gas. The ureter ring was

suspended between two hooks, of which the one was fixed at the base of the bath and the other was connected with the arm of a writing lever.

Character of the contraction of the ureter To demonstrate the character of the ureteral movements, the ureter rings were employed chiefly because the circular layer is mainly concerned in causing the peristaltic contractions. Every part of the ureter is capable of spontaneous contraction when placed in oxygenated Locke's solution at a temperature of 38°C.

The beginning of spontaneous contractions of the ureter ring after being placed in Locke's solution varies in the first place with the part of the ureter from which the ring is taken, and in the second place it depends upon the length of time after its removal from the body. The initial contraction starts immediately in the fresh ureter, while the ureter kept in the refrigerator for twenty-four or more hours begins to contract thirty minutes or even one hour after being placed in the bath.

The curve of contraction exhibits a steep ascending part rising almost vertically on a slowly revolving drum. The descending limb falls less steeply so that the contraction curve approaches gradually to the base line. Each contraction is followed by a pause represented as a horizontal line for a certain length. Sometimes the contractions follow one another closely, separated by no pause. But as a rule they do not fuse together, the descending limb reaching the level of the base line during the pause. Occasionally the contractions followed so closely, that there was partial fusion into a compound contraction. Finally there was observed a different kind of contraction in which there was a "plateau" at the summit of the curve. Such a curve has the form of an inverted U.

In spite of the different types of the contraction curves described above, there is noticed a striking similarity in each tracing made with a single ureter ring, placed in normal Locke's solution. In other words each ureter ring has only one kind of contraction curve from beginning to end.

The height and width of the curves also are the same within certain limits of time except when the contractions become gradually weaker due to fatigue.

The rate of the contractions varies in the various segments. Generally they are infrequent at first and then show a gradual increase to a certain rate, which is maintained for many hours even though becoming weaker in amplitude.

The amplitude of the contractions of the several segments also varies. This variation seems to depend to a large extent upon the structure of the muscle layer of the different parts as described elsewhere.[1]

Effects of certain drugs. The above description applies to the contraction of the ureter ring under normal physiological conditions. An attempt was made to study the action of various drugs upon the contraction of the ureter rings.

The definite finding that nerve plexuses and ganglia exist throughout the ureter wall has been described in the anatomical part of my work. Previous investigators are not in accord as to the innervation of the ureter; some maintaining a supply only from the thoracico-lumbar autonomies, the so called "true sympathetic," and others finding two sets of nerve fibers, namely the sympathetic and the autonomic nerve supply of sacral root origin, the so-called "parasympathetic."

For the purpose of investigating these nerve supplies, adrenalin and ergotoxin on the one hand and physostigmin and atropin on the other hand were employed, following the general belief that the first two act upon the sympathetic nerve endings while the latter two are excellent agents for revealing the presence of the parasympathetic nerve endings.

Experiments with these drugs were carried out according to the following schema.

1. Adrenalin (in small to large doses).
2. Ergotoxin (in small to large doses).
3. Adrenalin (in large doses) and then ergotoxin (in large doses).
4. Ergotoxin (in large doses) and then adrenalin (in large doses).
5. Physostigmin (in small to large doses).
6. Atropin (in small to large doses).
7. Physostigmin (in large doses) and then atropin (in large doses).
8. Atropin (in large doses) and then physostigmin (in large doses).
9. Atropin (in large doses) and then adrenalin (in large doses).
10. Ergotoxin (in large doses) and then physostigmin (in large doses).
11. Nicotin (in small to large doses).

Adrenalin even in a small dose stimulates the contraction of the ureter.

When a minute quantity of adrenalin (1 drop of 1: 10,000 solution) was added to the bath consisting of 20 cc. Locke's solution and in which a ureteral ring preparation had been suspended, a marked effect was soon noticed. The spontaneous contractions of the ring showed

[1] To appear shortly in the Journal of Urology.

a striking increase in rate, although no change in tonus and amplitude was obtained with such a small amount of adrenalin. A ureter which had been kept on ice in Locke's solution for one day or more and had shown no contractions when suspended at room temperature, began to contract rhythmically.

The larger doses (3 to 8 drops of 1: 10,000 solution in 20 cc. Locke's) caused an immediate increase of the rate, together with a slight increase in the tone. The amplitude of the contractions showed also a very slight increase. Thus adrenalin exerts an influence on the ureter ring in rate, tonus and amplitude in proportion to the increase in concentration.

Ergotoxin in relatively small doses (0.5 to 1 mgm. in 20 cc. Locke's solution) caused an increase in the tone and in the rate of contraction.

FIG. 1. Portion of graphic record obtained in experiment of November 6, 1918, with a ureter ring made from the middle of the pig's ureter in 20 cc. Locke's solution. *1*, spontaneous contractions; *2*, the effect of addition of 1 drop of adrenalin (1: 10,000); *3*, the effect of addition of 2 drops of adrenalin (1: 10,000); *4*, the effect of addition of 3 drops of adrenalin (1: 10,000); *5*, return to Locke's solution.

This effect may be regarded as a primary stage of stimulation. With increasing doses the tonus increased correspondingly, but the rhythmic contractions disappeared after a certain period of time, so that the ureter ring passed into a condition of tonic contracture. I did not find how long and how far this contracture would go on, as I put the ring again in the normal Locke's solution before observing a relaxation. But there was no relaxation throughout a rather long interval of time while under the influence of ergotoxin.

These experiments with adrenalin and ergotoxin indicate the presence of sympathetic nerve endings in the ureter wall. The effects of these drugs are distinctly different in several parts of the ureter, as will be described later.

Physostigmin in small and large doses (1.0 to 15 mgm. in 20 cc. Locke's solution) and pilocarpin in small doses (30 mgm. in 75 cc.

Locke's) increased the contractions in rate and also in tonus. Atropin in small doses exhibited sometimes a primary stage of stimulation, so that the ureteral contractions increased in rate. But the amplitude of the contractions decreased gradually in every case, and after a longer or shorter interval movements ceased entirely. There was found, however, no sudden disappearance of the contractions of the ureter even after the introduction of a rather large amount of atropin (15 mgm. in 20 cc. Locke's).

Fig. 2. Experiment of November 6, 1918, on a ureteral ring from the middle third of a pig's ureter. *1*, the effect of addition of 0.5 mgm. ergotoxin; *2*, the effect of addition of 1.0 mgm. ergotoxin; *3*, the effect of addition of 5.0 mgm. ergotoxin; *4*, return to Locke's solution.

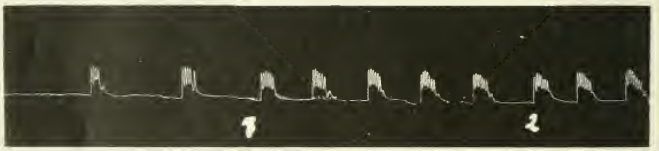

Fig. 3. Portion of graphic record obtained in experiment of November 8, 1918, with a ring from the lower part of the middle third of the pig's ureter in 20 cc. Locke's solution. *1*, the effect of addition of 1.0 mgm. physostigmin; *2*, the effect of addition of 3.0 mgm. physostigmin.

Inasmuch as physostigmin and atropin are regarded as exercising a selective action on the parasympathetic nerve endings, these latter results seem to furnish pharmacological evidence for the presence of sacral autonomic fibers. This conclusion is corroborated by the results obtained from direct stimulation of the nerves, as will be described below.

The effect of combinations of these drugs upon the excised ureter is of interest as throwing more light on the question of its innervation.

As I have shown above, ergotoxin antagonized the action of adrenalin, but had no effect on the stimulating effect of physostigmin. In other words the pressor action of physostigmin was not affected by previous administration of ergotoxin, while adrenalin after ergotoxin was not able to cause contractions of the ureter ring. Moreover in experiments in which atropin and adrenalin were introduced successively after a certain interval of time, it was found that atropin did not interfere with the action of adrenalin although it inhibited entirely the effects of physostigmin.

Fig. 4. Portion of graphic representation traced by a ring from the middle of the pig's ureter in experiment of November 7, 1918. Preparation was placed in 20 cc. Locke's solution, and shows the final inhibitory action of atropin. *1*, the effect of addition of 1.0 mgm. atropin; *2*, the effect of addition of 5.0 mgm. atropin; *3*, the effect of addition of 7.0 mgm. atropin; *4*, the effect of three drops of adrenalin (1: 10,000) after atropin.

Fig. 5. Portion of graphic record obtained in experiment of November 6, 1918, with a ring preparation from the middle of the pig's ureter in 20 cc. Locke's solution. *1*, no effect of addition of 3 drops of adrenalin (1: 10,000) after ergotoxin; *2*, the effect of addition of 5.0 mgm. physostigmin after ergotoxin.

These facts may be accepted as showing that these drugs exert their action on different structures in the ureter wall, in accordance with the view outlined above.

In addition it was thought desirable to examine the action of nicotin upon the ureter ring, since it is one of the accepted agents used to paralyze peripheral ganglion cells. Its action is to stimulate first and then paralyze. Small doses of nicotin (3 drops of a 1: 100 solution in 20 cc. Locke's) caused an increase in the contractions, especially in

amplitude, and medium doses (8 drops of a 1: 100 solution in 20 cc. Locke's) exerted the same effect in a stronger measure, but caused a little decrease in the rate of contractions.

Large doses (1 drop of the undiluted solution in 20 cc. Locke's) paralyzed the contractions immediately.

Thus the effect of nicotin upon the excised ureter indicates the existence of ganglion cells in the ureter itself. It also points to the presence of ganglionic structure throughout this organ, since experiments with all parts of the ureter gave positive results of the kind described.

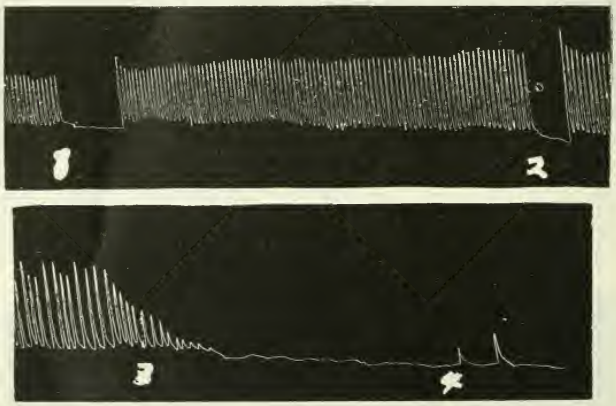

Fig. 6. Portion of graphic record obtained in experiment of November 7, 1918, with a ring from the middle part of the pig's ureter in 20 cc. Locke's solution, showing the action of nicotin in increasing doses. *1*, three drops of nicotin (1: 100); *2*, eight drops of nicotin (1: 100); *3*, one drop of concentrated nicotin; *4*, return to Locke's solution.

b. The use of three strips to compare the contractions of three different parts

As pointed out above, the contraction of the ureter ring is markedly different in the several parts of the organ. These differences were observed not only under normal physiological condition, but also after the introduction of drugs in the bathing solution. In the present experiments three ureter rings made from the upper, the lower and the

middle portions were selected for the purpose of comparing their contractions, since in these regions the greatest difference in the structure of the muscle coat is found. They were placed in a glass beaker containing 75 cc. oxygenated Locke's solution. Three straw levers, which were of the same length and of the same weight, were connected with the rings. Tracings of these levers were obtained on a revolving drum. In this way curves of the contractions of the three parts under similar conditions can be obtained simultaneously. It should be stated that the ureters used in these experiments were not perfectly fresh but had been kept for some hours (3 to 24) in cold Locke's solution.

The three rings did not begin to contract at the same time. The upper ring (the ring made from the upper part of the ureter) began to contract usually immediately after placing it in the bath, and the middle ring followed after a longer or shorter period of time, while the lower ring was quiescent in most of the experiments in the normal Locke's solution for a number of hours.

This finding indicates that the irritability of the ureter varies in different parts; the upper end being the most susceptible to stimulation, and the lower parts showing a gradual decrease in irritability.

Further there were noticed marked differences in the amplitude of the contractions. The upper ring exhibited small rhythmic contractions, while the middle ring gave much larger curves, so that the height of the contractions was many times greater than that of the upper ring. The spontaneous contractions of the lower ring, on the contrary, were very small, much smaller than those of the upper ring. This difference in amplitude of the contractions depends upon the amount and the course of the muscle fibers. In other words, the amplitude of the contraction is proportional to the quantity of the circular muscle fibers.

The rate of the contractions also varied definitely in different parts of the ureter. The highest rate was found in the upper ring, while in the middle and the lower ring the rate was slower to about the same degree.

The difference in the effects of certain drugs on these three rings is most interesting. Adrenalin caused an increase in the contractions of the upper and middle rings in both rate and amplitude; its effect being stronger on the upper ring. On the lower ring, on the contrary, adrenalin had only a very small effect, causing a minute increase in the rate or sometimes no reaction at all, even in large doses. The re-

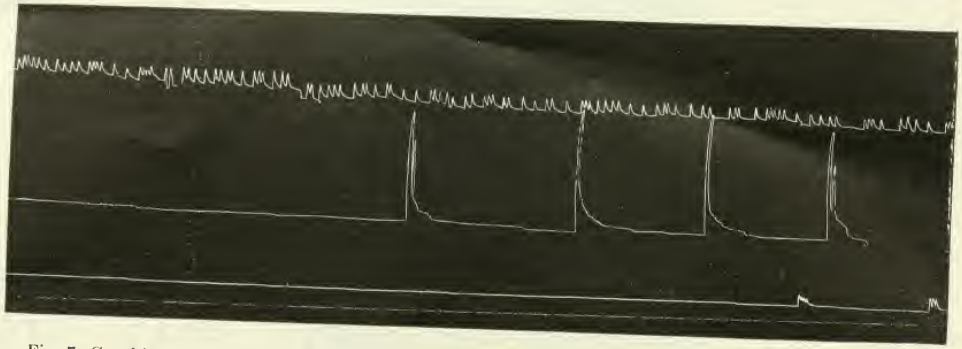

Fig. 7. Graphic record obtained in experiment of November 22, 1918. Spontaneous contractions of three rings of a pig's ureter, which had been kept for hours in a cold Locke's solution. Upper record, contractions of a ring from the upper part; second record, those of a middle ring; third record, movements of a lower ring; fourth record, base line.

sponse of the middle and lower rings to adrenalin started immediately after introducing the drug, while the upper ring began to react after a certain period of time. I cannot find a probable explanation for this result.

Physostigmin exerted its effect on the middle and lower ring, producing an increase in the rate and amplitude. Vigorous contractions were evoked in a quiescent lower ring, which had remained motionless for many hours after being placed in Locke's solution. In the upper ring, however, physostigmin had either a slight effect or no pressor action at all. In regard to the beginning of the contraction there was found a

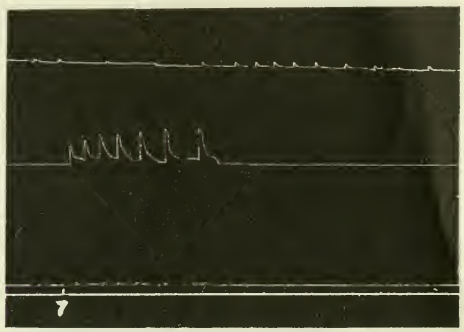

Fig. 8. Portion of graphic tracing obtained in experiment of November 21, 1918. Three rings were suspended in 75 cc. Locke's solution, to which adrenalin was added (8 drops of 1: 10,000 solution). Upper record, the contractions of the upper ring; second record those of a ring from the middle part; third record, movements of the lower ring; forth record, base line. *1*, addition of adrenalin.

reversed order compared with that of adrenalin. Contraction developed immediately, if at all, in the upper ring while in the middle and lower rings contractions appeared only after a certain interval.

Comparing the findings of the experiments carried out with three rings, it is seen that adrenalin has a stronger influence at the upper part of the ureter, while physostigmin exerts a more marked effect on the lower part.

The middle part reacts to these two drugs almost in the same degree.

These facts indicate that the upper part has a nerve supply from the sympathetics chiefly, while the larger part of the nervous element in

the lower part belongs to the parasympathetic system. In the middle part there exist both sets of nerve fibers, seemingly in the same quantity.

Comparison of the effects of adrenalin and physostigmin on the tonus of the three rings develops an interesting difference. Adrenalin caused an increase of the tonus in the upper and middle rings only to a slight degree. On the contrary, physostigmin produced tonic contractions in the middle and lower rings much more striking than the tonus of the upper and middle rings produced by adrenalin. This and other facts seem to indicate a difference between these two sets of nerves in the ureter in respect to their physiological functions; the action of the

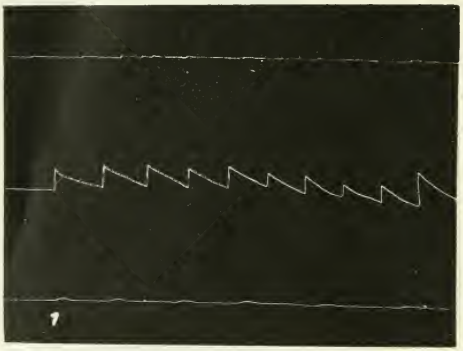

Fig. 9. Portion of graphic tracing obtained in experiment of November 21, 1918. Three rings of a pig's ureter were placed in 75 cc. Locke's solution, to which 15 mgm. physostigmin was introduced, *1*. Upper record, the contractions of the upper ring; middle record, those of the middle ring; lower record, movement of the lower ring.

sympathetics has an evident relation to the rhythmic contractions of the ureter, while the parasympathetics may act rather as a pressor nerve for heightening the tonus.

2. EXPERIMENTS UPON STIMULATION OF THE EXTRINSIC NERVES

After administration of 1 grain of morphin a dog of medium size was fixed on the holder. The animal having been anesthetized by ether and tracheotomized, the abdominal cavity was opened along the linea alba from the xyphoid process to the symphysis pubis. For the pur-

pose of making a sufficient space to find the left splanchnic nerve in the region above the suprarenal gland, another incision was made on the left abdominal wall along the lower border of the last rib. The intestines were drawn to the right side and the viscera, as well as the rest of the abdominal wall, were covered with warm towels. In this way the left ureter could be seen completely from the kidney to the bladder. For a little while after opening the abdominal cavity the regular peristaltic movements were noticed at the rate of 3 to 5 per minute. They disappeared, as a rule, in a few minutes but in some cases might continue for hours. In some dogs the ureters remained motionless even immediately after opening the abdomen.

The method employed in obtaining graphic records of the ureteral movements consisted in connecting the lumen of the ureter with a water manometer, the undulations of the column of water being then transmitted by means of a piston recorder to a revolving drum.

The connection with the ureter was made by the following method. After opening the bladder cavity by a long sagittal incision, a small cannula with a thin tip was introduced into the orifice of the left ureter. To avoid losing the cannula from the orifice and to keep the connection water-tight, four weak rubber bands were used, one end being hooked to the mucosa of the bladder in the neighborhood of the orifice and the other end being tied to the glass cannula, some distance from the orifice.

By this method the ureter was retained in its normal connection with the bladder, and no injury was inflicted upon the nerve and blood supplies which ascend up the ureter from the bladder. To prevent an increase of the intra-ureteral pressure due to the urine secretion of the kidney, the upper end of the ureter was squeezed with a weak artery-clamp. When all preliminary arrangements had been made the intra-ureteral pressure was raised by injecting Locke's solution through the cannula into the ureter lumen, which was thereby distended to a certain degree.

PROTOCOLS OF TYPICAL EXPERIMENTS

All of the experiments of the present series were made on dogs. The results of three typical experiments are reported.

Experiment 3. December 11, 1918. The preliminary operation described above was made. After tying the trunk of the left splanchnic nerve in the region above the suprarenal gland, the nerve was cut and the distal stump of the nerve was stimulated with induction currents for half a minute. As a result

of this stimulation there occurred after a quarter of a minute three contractions in the previous quiescent ureter. The stimulation was repeated with the result of one contraction for each stimulation. But the interval between the stimulation and the appearance of the contraction became gradually longer, as the stimulations were repeated. After the seventh stimulation there was no response owing probably to a fatigue of the nervous mechanism. By pulling the splanchnic nerve also there occurred a series of contractions (mechanical stimulation).

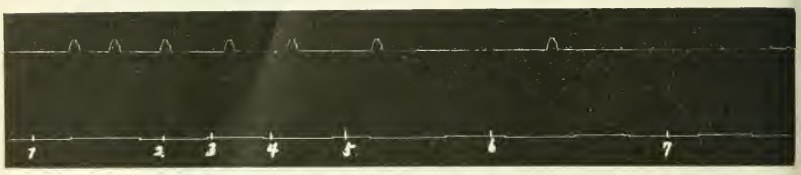

Fig. 10. Portion of graphic record obtained in experiment of December 11, 1918. Upper record, contractions of the ureter from stimulating the splanchnic nerve; lower record, time record in minutes. *1, 2, 3, 4, 5, 6, 7*, stimulations of the nerve.

The left pelvic nerve was dissected out in the pelvic cavity and was then ligated and cut. On the distal side of the nerve many branches making up a plexus spread in the form of a fan over the side of the bladder. The uppermost one of these branches, which seemed to be larger than the other fibers, was stimulated electrically. There was observed, however, no contraction of the ureter. But when the electrodes were brought under the nerve plexus and touched a certain branch, there appeared suddenly contractions, which continued rhythmically for a short time, giving a series of contraction curves.

An interesting event, which indicated clearly that mechanical stimulation is able to cause contractions of the ureter, happened during this experiment.

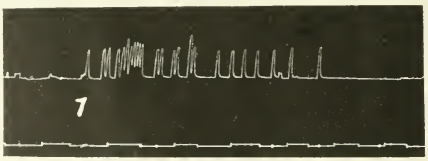

Fig. 11. Portion of graphic record obtained in experiment of December 11, 1918. Upper tracing, contraction of the ureter from mechanical stimulation (squeezing) of the pelvic nerve, *1*. Lower record, time record.

I lost the distal stump of the pelvic nerve, as its end escaped from the ligature. When I found it after a little while and squeezed it with an artery forceps, there occurred a series of contractions. The contractions followed so closely, especially in the middle section of the series, that they fused into an incomplete compound contraction, showing an increase of the tonus of the ureter.

Experiment 6. December 18, 1918. The operation and the arrangement of the apparatus were the same as in the preceding experiment. At first the ureter remained quiescent. But electrical and mechanical stimulation of the splanchnic nerve caused a contraction or a series of contractions immediately.

After a few stimulations of the splanchnic nerve the ureter showed somewhat irregular spontaneous contractions, which continued for hours. Stimulation of the splanchnic nerve had an effect of making the rhythm more regular.

Stimulation of the pelvic nerve gave also contractions of the ureter. The curves of contraction, however, were lower and wider than in the case of the contractions due to stimulation of the splanchnics.

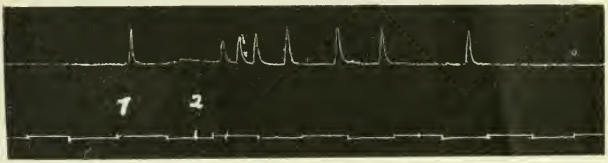

Fig. 12. Portion of graphic record obtained in experiment of December 18, 1918. Upper tracing, contractions of the ureter from stimulating the splanchnic nerve mechanically, *1*, and by induction current, *2*. Lower record, time record.

In regard to the number of contractions following a single stimulation of either the splanchnic or pelvic nerves the result varied within wide limits, sometimes only a single contraction appearing either immediately or after a short time, or sometimes a number of contractions succeeding each other.

Comparing the contractions caused by stimulating the splanchnic and pelvic nerves, a difference was observed. The contractions caused by the stimulation of the splanchnic nerve were quicker and stronger, while the contractions caused by stimulating the pelvic nerve were slower and weaker. This difference seems to indicate that the two sets of nerves may have different actions upon the ureteral movements.

Further there was noticed a number of vigorous contractions by stimulating a nerve trunk, which was found under the left kidney. It was situated 2 or 3 cm. below the origin of the renal artery, over the ventral side of the aorta.

The contractions continued for several minutes at the rate of 4 to 8 per minute. But the nerve was not dissected out to find its origin.

Experiment 11. January 8, 1919. The ureteral movements caused by stimulating the splanchnic and pelvic nerves were just the same as in the previous experiments. In this experiment a very delicate branch from the inferior mes-

enteric nerve was dissected out. It had its origin from a point of the inferior mesenteric nerve in the neighborhood of the inferior mesenteric ganglion, and divided soon after leaving the inferior mesenteric nerve into two or more branches, the upper one ascending somewhat and the lower branch taking a more descending direction. These final branches evidently had their terminations in the ureter wall. Stimulation of this branch of the inferior mesenteric nerve with induction currents caused an immediate and marked diminution in the rate of a series of spontaneous contractions which the ureter had been giving.

After cessation of the stimulus the rate of contractions increased markedly, so that it became one and a half times more than that during the spontaneous contractions.

In regard to the amplitude of the contractions, however, there was observed almost no change during and after stimulation of this nerve branch. From this finding the small branch from the inferior mesenteric nerve may be regarded as an inhibitory nerve to the movements of the ureter. Protopopow states

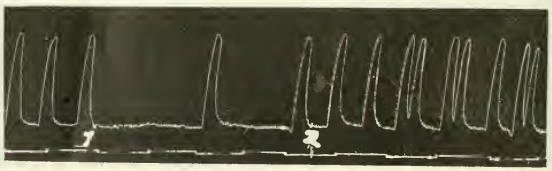

Fig. 13. Portion of graphic tracing obtained in experiment of January 8, 1919. Upper record, inhibition of the contractions of the ureter from stimulating the ureteral branches of the inferior mesenteric ganglion. *1*, point of beginning of stimulation; *2*, end of stimulation. Lower record, time record in minutes.

that stimulation of the anastomosis between the inferior mesenteric ganglion and the hypogastric plexus caused usually no acceleration, but sometimes rather a slowing of the ureteral contractions. When the hypogastric nerve was stimulated electrically, there was a very weak inhibitory result.

This nerve was cut at the middle and the distal stump was stimulated, causing no change in the movements of the ureter. On the other hand, stimulation of the proximal stump caused clearly an inhibitory effect of the ureteral movements though in a slight degree. These facts accord with Protopopow's findings and seem to indicate that the electrical stimulation reached the inferior mesenteric ganglion through the hypogastric nerve and presumably as an axon reflex influenced the ureteral contractions through the nerve branches passing from the inferior mesenteric nerve to the ureter. Sometimes there occurred evidence of tonic contractions after stimulation of the branch was stopped.

Painting and intra-ureteral injection of adrenalin, atropin and other drugs caused their respective effects in the same manner on the ureter in situ as in ring preparations, but to a slighter degree.

As a result of these three typical experiments and others which gave similar results light is thrown upon the important question of the innervation of the ureter. The motor effect of stimulation of the splanchnic nerve indicates evidently that there are nerve endings of the thoracico-lumbar (sympathetic) outflow in the ureter. As this fact has been stated by many different observers we may assume that it can be accepted without question. In almost all of my experiments (nos. 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 15) this result was obtained.

In regard to the innervation of the ureter by the pelvic nerve, in other words the presence of parasympathetic nerve endings, there has been some difference of opinion. In most of the present experiments (nos. 3, 4, 6, 8, 9, 11, 12, 14) definite evidence was obtained of the existence of the parasympathetic motor neurones, which come up to the ureter through the connector nerve fibers of the sacral outflow, i.e., the pelvic nerve. Some objections may be made against the experiments on the ground that the electrodes must be brought so near to the ureter that the currents might escape into the ureter through the tissues which accompany the fibers of the pelvic nerve.

But the fact that mechanical stimulation such as squeezing the nerve, as in experiment 3, also caused a series of vigorous contractions, can be regarded as indisputable evidence that the pelvic nerve carries motor fibers to the ureter. This result accords also with the results which were obtained pharmacologically with the ring preparations of the excised ureter, as described above.

The method of experimentation described in this section did not throw any light upon the relative distribution of the sympathetic and parasympathetic fibers. The conclusion in regard to this point rests upon the pharmacological results already described.

DISCUSSION

On the peristalsis of the ureter. All smooth muscle tissues possess the power to contract, but they need to be in close continuity with nerve elements for the development of the contraction. The ureter has a well developed muscle coat, and within the ureter wall there is found a complete nervous system composed of a network of fibers with many ganglion cells as shown by many authors and confirmed in my study of the histology of this organ. According to my finding there are two nerve plexuses situated in the outer fibrous coat and submucosa immediately embracing the muscle layer over its outer

and inner sides. From the close situation of these nerve plexuses to the muscle layer we may infer that the contractions of the ureter are due to this intrinsic nervous system. That the ureter is capable of contracting spontaneously without the action of extrinsic nerves, is demonstrated by the spontaneous contractions of excised pieces when kept in Locke's solution at a temperature of 38°C.

In regard to the direction of the contraction wave of the ureter, which begins at the upper end and proceeds toward the bladder in the form of a peristalsis, the author finds an immediate explanation in the experiment in which three ring preparations made from different parts of the ureter were observed to contract after different periods of time; the upper end being most responsive to a stimulus and the responsiveness decreasing gradually toward the lower end. In the normal condition in situ the contraction wave develops at the upper end on account of the greater irritability at that end, favored perhaps also by the mechanical pressure of the secretion.

On the innervation of the ureter. The innervation of the ureter has been investigated by many authors, whose statements are quite divergent. Langley and Anderson (10) dissected out three ureteral branches from the hypogastric nerve in the cat. Protopopow suggested that the splanchnic nerve augmented the peristalsis of the ureter in the dog. Fagge and Stern observed an accelerating effect of the hypogastric nerve. Elliot (11) also described a slight movement of the ureter by stimulating the hypogastric nerve.

Gaskell (12) in his monograph on *The involuntary nervous system* mentions that the segmental ducts, namely the uterus, the ureter and the vas deferens, possess both motor and inhibitory fibers, which arise from nerve cells belonging to the thoracico-lumbar outflow, but adds that there is no evidence that these structures are connected in the slightest degree with the pelvic nerve. He states also that the nerve cells with which the sympathetic nerve fibers are connected are placed in the ureter itself. Gaskell's argument for the innervation of the ureter by the sympathetics only rests largely upon embryological considerations.

In my experiments I found pharmacologically, on the one hand, the presence of both the sympathetic and parasympathetic nerve endings. On the other hand, I confirmed by stimulation the innervation of this organ by both the splanchnic and the pelvic nerves. There is, therefore, no doubt of the existence of sympathetic and parasympathetic nerve endings in the ureter wall, connected with the preganglionic fibers of

the thoracico-lumbar and sacral outflow (i.e., the splanchnic and pelvic nerves) respectively. In two cases small nerve filaments were followed anatomically from the pelvic nerve to the ureter.

Delicate filaments given off from the inferior mesenteric nerve and going to the ureter were found on stimulation to exert an inhibitory effect.

On the prevention of the reflux of the bladder content into the ureter lumen. It is an important problem as to whether or not the bladder content can flow back into the ureter under normal conditions. As a matter of fact various clinicians describe an ascending nephritis, which follows infection of the bladder. If such is the case there is some doubt as to whether the deleterious condition in the bladder may have an effect on the kidney by direct transmission through the ureter, or in some indirect way.

Some urologists as Petit, Hallé, Sappy, Zuckerkandle and Tuchman, and the physiologist, Sigmund Mayer, have stated that the normal closure of the ureter is competent to prevent a reflux. Harrison, from his clinical observation, believed that regurgitation can only be brought about by very gradual means, not by a sudden flow into the ureter. Guyon and Albarran obtained the same result as Harrison in their experimental work. On the other hand, most of the experiments of Lewin and Goldschmidt (13) have indicated the possibility of a reflux even in normal conditions, but Lucas claims that the normal ureterovesicular valves can wholly prevent the reflux of the urine.

As a result of my experiments I find that normally there is no reflux into the ureter, although from my anatomical findings and those of many authors there is found no special structure of the nature of a sphincter at the end of the ureter.

In some of my experiments on dogs and pigs the bladders were removed with a small adjacent part of the ureters and urethras. The bladder was gradually distended by salt solution to the greatest degree, but there was no drop of leakage from the ureter stump; and the bladder thus distended could be kept for days. This fact indicates that the bladder content may not flow back into the ureter lumen even with fullest distention of the bladder. After destroying all muscles of the bladder wall, which cover the intraparietal part of the ureter, a slow leakage was produced through the ureter. The prevention of a reflux is referable to a physical mechanism to a large extent. The distended bladder muscles over the ureter press the ureter together closely and moreover the slit-like opening of the ureter tends to

close the opening so that even after destruction of the bladder musculature the leakage back into the ureter is very slow.

In addition to these physical mechanisms the prevention of a reflux is probably aided by a more distinctly physiological activity. A layer of longitudinal muscle is found on the outer side of its intraparietal part, while the Waldeyer's ureter sheath covers the medial side of the small section just above the intraparietal part. The contraction of the former tends to close the orifice, and the contraction of the latter causes the lip-shaped upper rim to protrude. These mechanisms thus assist in preventing the reflux of urine into the ureter.

CONCLUSIONS

1. The excised ureter gives spontaneous contractions in oxygenated Locke's solution. The contractions vary in rate and character with different individuals.

2. The amplitude and rate of the spontaneous contractions vary also in different parts of the ureter. The contractions of the middle part are large and slow, those of the kidney end are lower and faster, while at the bladder end the contractions are very small in size and slow in rate. These differences so far as size is concerned are correlated with the different thickness of the circular muscular coat which has its largest development in the middle section.

3. Adrenalin and physostigmin stimulate the movements of the ureter.

Adrenalin affects the upper portion of the ureter more strongly while physostigmin gives a more distinct reaction on the lower portion.

4. Ergotoxin in large doses antagonizes the action of adrenalin but has no influence upon the action of physostigmin. With atropin in large doses the opposite reaction holds; it antagonizes the action of physostigmin but not of adrenalin. In accordance with the usual interpretation, these reactions indicate the presence in the ureter of sympathetic and parasympathetic fibers.

5. Nicotin in large doses paralyzes the movements of all parts of the ureter, thus corroborating the histological finding of ganglion cells along the whole length of the ureter.

6. The irritability of the ureter as displayed by the rhythmic contractions of isolated rings in Locke's solution is greatest at the upper end and diminishes gradually toward the bladder.

7. The action of adrenalin and ergotoxin is most marked at the upper end of the ureter, while the characteristic effects of physostigmin

and atropin are displayed especially by rings taken from the lower end. The middle section reacts somewhat equally to the two sets of drugs. It is inferred from these facts that the upper end is innervated chiefly by sympathetic fibers, and the lower end by parasympathetic fibers, while in the middle there is an approximately equal distribution of the two sets of fibers.

8. Stimulation of the peripheral end of the splanchnic nerve causes the development of contractions in a quiescent ureter. Stimulation of the peripheral stump of the pelvic nerve also causes the development of contractions in the ureter.

9. The ureteral muscles receive inhibitory fibers by way of the communicating branch to the inferior mesenteric ganglion. The action of these fibers is to slow the rhythm of the ureteral contractions.

10. The prevention of a reflux of the bladder content into the ureter is complete under normal conditions. The closure is effected mainly by a physical mechanism, but is assisted by the physiological activity of the musculature at the intraparietal portion of the ureter.

BIBLIOGRAPHY

(1) Engelmann: Arch. f. d. gesammt. Physiol., 1869, ii, 243.
(2) Protopopow: Arch. f. d. gesammt. Physiol., 1897, lxvi, 1.
(3) Fagge: Journ. Physiol., 1902, xxviii, 306.
(4) Henderson: Journ. Physiol., 1905, xxxiii, 175.
(5) Berensnegowsky: Centralbl. f. Physiol., 1908, xxii, 461.
(6) Lucas: This Journal, 1906, xvii, 392; N. Y. Med. Journ., 1907, xxxvi, 254; This Journal, 1908, xxii, 245.
(7) Sokoloff und Luchsinger: Arch. f. d. gesammt. Physiol., 1881, xxvi, 464.
(8) Roth: This Journal, 1917, xliv, 275.
(9) Macht: Journ. Pharm. Exper. Therap., 1916, viii, 155, 261; 1916, ix, 197, 287, 427; 1918, xii, 255; Journ. Urology, 1917, i, 97.
(10) Langley and Anderson: Journ. Physiol., 1895, xix, 73.
Langley: Schaefer's Textbook of physiology.
(11) Elliott: Journ. Physiol., 1906, xxxv, 73.
(12) Gaskell: Monograph on the involuntary nervous system, 1916.
(13) Lewin und Goldschmidt: Arch. f. path. Anat. u. Physiol., 1893, cxxxiv, 33.

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A QUANTITATIVE STUDY OF THE EFFECTS PRODUCED BY SALTS OF SODIUM, POTASSIUM, RUBIDIUM AND CALCIUM ON MOTOR NERVE OF FROG

ESTHER GREISHEIMER

From the Hull Laboratories of Physiological Chemistry and Pharmacology, University of Chicago

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INTRODUCTION

Mathews (1) in the course of his investigations on the nature of chemical and electrical stimulation found the chloride of rubidium to be the most powerful stimulating agent for motor nerve of frog. Owing to conflicting reports in the literature on the subject, further investigation seemed desirable.

A brief review of the physiological effects of the rubidium salts and the related sodium and potassium salts will be given. Bunsen discovered rubidium in 1861 and knew from the chemical behavior of its compounds that it belonged in the group of alkali metals. In most of its peculiarities rubidium exhibits the greatest similarity to potassium, which it follows in the periodic system. Grandeau (2), however, as early as 1864 concluded that rubidium was more nearly related to sodium than to potassium. He injected 1 gram of the salt dissolved in 15 cc. of water into dogs. If the dog died the salt was considered toxic, but if the animal recovered sufficiently to run about the salt was considered ineffective. Grandeau, by these methods, found potassium alone to be toxic, and sodium and rubidium chlorides to be ineffective, in equal dosage.

Ringer (3) made a comparative study of these chlorides, using the ventricle of the frog's heart, in which he maintained an artificial cir-

culation. On using 1 cc. of a one and two hundred five thousandths (1.205) per cent solution of rubidium chloride in 100 cc. of normal saline solution, he observed the ventricular contractility to grow less and less until it had disappeared. On addition of small amounts of either strontium or calcium chloride (3.5 to 5 cc. of 1 per cent sol. to 100 cc. of the circulating fluid) fair contractions speedily returned. A calcium salt in saline broadened the beat, rounded its top and greatly retarded dilatation. Rubidium, like potassium, obviated these effects, and restored to the beat its normal character.

Blake (4) criticised the work of Ringer because it had been done on the isolated organs of a dead animal. He believed that the physiological action of a salt should be determined by introducing it directly into the veins or arteries of a living animal, so that it could, with the least disturbance possible, be brought into direct contact with tissues whose reactions he wished to investigate. He injected the chlorides of rubidium, potassium and sodium into the veins or arteries of frogs in order to test the effects on the heart. His results differed from those of Ringer in that he found sodium and rubidium to behave similarly and potassium to be exceptional in its behavior, but he failed to give the data obtained.

Harnack and Dietrick (5) injected rubidium chloride subcutaneously into frogs, and compared the results with those following the injection of potassium chloride. These salts did not essentially affect the heart muscle when given in doses of about thirty milligrams. From the results obtained they concluded that the differences between potassium and rubidium salts were quantitative, potassium being the stronger of the two. A few investigations were carried out in which isolated muscle was placed in rubidium or potassium chloride, and a rapid decrease in height of contraction following stimulation followed in either salt. These investigators gave no data from experiments on motor nerve of frog, but simply stated that rubidium salts appeared to affect motor nerve to a slight degree only.

Brunton and Cash (6) observed that "motor nerve was not paralyzed by rubidium except in very large doses." The height of contraction of muscle was increased by rubidium and potassium alike. The lethal activity on frogs of the chlorides used placed the metals in the following series, potassium being the strongest: potassium > rubidium > barium > caesium > lithium > strontium > sodium > calcium.

Richet (7) studied the effects of both subcutaneous and intravenous injections of rubidium and potassium chlorides on fish, frogs, guinea

pigs, pigeons and rabbits. The results were similar on the various animals, rubidium chloride being slightly less toxic than potassium chloride.

Botkin (8) injected these chlorides into dogs and found that rubidium chloride exerted effects similar to smaller doses of potassium chloride.

Grützner (9) has, as had Ranke (10) and Biedermann (11) before him, found the chloride of potassium, even in dilute solution, intensely toxic. In stimulating action, caesium was found most active, rubidium next, and potassium had no noticeable stimulating action at all. But in toxicity potassium stood highest, rubidium next and caesium lowest. Calcium also was found to be toxic to the nerve.

Many investigations on the antagonisms of these salts have been carried out. Loeb (12) found pure sodium chloride a strong poison for many (if not all) marine animals. He considered the poisonous effects due to the sodium ion. Equimolecular solutions of calcium and potassium chlorides were also found poisonous, but a combination of the three ions was not poisonous. Loeb and Cattell (13) showed that eggs of Fundulus poisoned with potassium chloride were unable to recover when placed in distilled water or in a saccharose solution, while they recovered when placed in an $\frac{M}{8}$ salt solution, or in acidified water. The relative efficiency of the salts for inducing the recovery of the heart beat increases, first with the concentration of the salt in the solution, then with the valency of the anion of the salt, the valency effect apparently following Hardy's rule. Loeb and Ewald (14) studied the inhibitory effect of calcium on motor nerve of frog. A portion of nerve immersed in an $\frac{M}{4}$ sodium chloride solution results in twitching of the attached muscle. They found that 2 cc. of an $\frac{M}{8}$ calcium chloride solution per 100 cc. of a $\frac{M}{4}$ sodium chloride are required to suppress these twitchings. On adding a quantity of calcium not quite high enough to inhibit entirely the effect of the stimulating salt, the latent period of stimulation was considerably increased. This fact harmonized with Loeb's assumption that the inhibitory effect of calcium is due to a prevention or a retardation of the diffusion of the stimulating salt into the nerve.

Burridge (15) perfused isotonic sodium chloride through the frog's heart. Stoppage in the dilated condition and loss of electrical irritability followed. Calcium opposed these effects.

Zoethout (16) found that gastrocnemii of frogs were thrown into contraction on immersion in $\frac{M}{8}$ potassium chloride. He showed that

treatment with sodium chloride previously, or the simultaneous introduction of sodium and potassium chlorides, hindered the development of the potassium contraction. A similar treatment with calcium chloride reduces the extent of the potassium contraction, and lengthens the latent period. He used 1 to 10 cc. $\frac{M}{8}$ $CaCl_2$ and 10 cc. of 6 per cent cane sugar solution followed by 1 cc. $\frac{M}{8}$ KCl in 10 cc. 6 per cent cane sugar.

Lillie (17) found that Arenicola larvae lost muscular contractility gradually in pure isotonic solutions of non-electrolytes, such as cane sugar and dextrose. Pure solutions of sodium salts induced well-marked contractions in such larvae, and the addition of calcium chloride (as low as $\frac{M}{25600}$) to sodium chloride solutions, increased the ability of the latter to restore normal contractility. Potassium and rubidium exerted a more vigorous effect than sodium.

Joseph and Meltzer (18) used the gastrocnemius and the sciatic. The muscle in sodium chloride alone stopped contracting after an hour and a half or two hours, while if the solution contained also some calcium, the contractions continued from six to eight hours. In nine out of ten experiments the nerve in calcium chloride lost its conductivity in two or three hours, but sodium chloride caused no loss of conductivity in the sciatic even after many hours.

Groves (19) stated that the various salts of potassium killed the nerve so rapidly that the results were not clear and uniform. Osterhout (20) used young plants of a fresh water alga, Vaucheria sessilis, which can live three to four weeks in distilled water. These plants were killed in a few minutes by $\frac{3M}{32}$ sodium chloride, and in a few days by $\frac{M}{1000}$ sodium chloride. The toxicty of a $\frac{3M}{32}$ solution of sodium chloride was inhibited by the addition of calcium chloride in the proportion of one part of calcium to one hundred parts of sodium. Potassium chloride was also able to neutralize the poisonous effect of the pure sodium chloride.

Burnett (21) continued the work of producing glycosuria in rabbits by the injection of sodium chloride. The usual glycosuria was 0.25 per cent in rabbits with injection of pure sodium chloride, but this was reduced, varying from a trace to 0.03 per cent on injecting $\frac{M}{6}$ sodium chloride to which had been added some potassium. Burnett added 2.2 molecules of potassium chloride per 100 molecules of sodium chloride. Calcium chloride was found equally effective in preventing glycosuria.

Miyake (22) found 25 cc. of $\frac{N}{10}$ sodium chloride plus 5 cc. $\frac{N}{10}$ calcium chloride the most favorable mixture for the growth of rice seedlings. A similar mixture, using potassium chloride in place of calcium chloride was found favorable.

Cramer (23) studied the effects of sodium and calcium salts on the growth of cells of a transplantable mouse carcinoma. The cells were immersed for an hour or two in isosmotic solutions of sodium and calcium chlorides. The subsequent transplantation showed a very distinct inhibition of growth on the part of the cells which had been suspended in calcium chloride. The growth of the cells which had been suspended in sodium chloride showed slight, if any, inhibition. The inhibition of growth produced by the calcium ions can be neutralized by a subsequent suspension of the cells in sodium chloride solution.

Mathews' (1) observations on nerve were more thorough than any of the preceding. He found that all the sodium salts stimulated the motor nerve, and likewise potassium chloride in one-fourth molecular solution or stronger, while solutions weaker than this never stimulated, but very rapidly depressed the nerve. Rubidium chloride was found to be the most powerful stimulant of the chlorides, and the irritability of nerves in this solution was lost very rapidly. In this observation Mathews (1) differed from Grützner (9) who found caesium a more powerful stimulant than rubidium, and from Zoethout (24) who found that nerves were not stimulated on being immersed in rubidium chloride.

Zwaardemaker and Feenstra (25) have made the most recent observations on these related salts. They have found that the potassium in Ringer's solution may be replaced by rubidium, uranium and thorium, and that the necessary quantities are proportional to their radioactivities. Their investigations were made on heart of frog.

Prof. A. P. Mathews suggested that further investigations be carried out on these salts, so the writer undertook a quantitative study of the toxic effects produced in each salt alone, and then in various combinations. These observations have necessarily been limited owing to the great difficulties in procuring rubidium salts. The purpose of this paper is not to set forth an exhaustive discussion on the nature of the nerve impulse, nor on the manner in which salts act, but simply to state the results observed on studying these salts in a thoroughly quantitative manner. The statements that "calcium rapidly depresses the nerve" and that "potassium is very toxic" must be reduced to quantitative terms. It is hoped that these results may be a helpful stepping stone to the next investigator in this line of work.

METHODS

The sciatic nerve and the gastrocnemius muscle of the leopard frog were used. The sciatic was not cut, the whole sacral plexus with an attached bit of spinal column being left intact. It was believed that more constant results could be obtained if nerves were kept as nearly uninjured as possible. The frogs were brought from the tank and the preparation rapidly made, scarcely five minutes elapsing between the pithing of the frog and the mounting of the preparations in the moist chambers. The fact that frogs are in varying conditions of health is a great uncontrollable source of error. Sex appears to make no difference in the results, but the writer believes that humidity will in the future be shown to be an influential factor in nerve work.

A 4 cm. length of nerve nearest the muscle was immersed in a paraffin cup containing 5 cc. of the solution whose effects were to be studied. The nerve as it emerged from the solution was led across electrodes. At first platinum electrodes were used, but it was found that copper electrodes gave results identical with those obtained from the platinum electrodes, hence the use of copper ones was continued. This is contrary to general opinion, but control nerves remaining in Ringer's solution showed practically no decrease in irritability, even after twenty hours, so injury from copper electrodes did not occur to any extent. The remaining portion of the nerve with the attached bit of spinal column was placed in a second paraffin cup containing Ringer's solution. The entire nerve varied from 7 to 10 cm. in length. The cover of the moist chamber was not removed during the experiment, which was often continued for sixteen hours. Every known precaution was taken for keeping the conditions as favorable as possible for the nerve, and as a result the controls in Ringer's solution, run with the daily series of observations, showed very little change, if any, in irritability, even when the experiments were continued over a space as great as eighteen or twenty hours.

Two Edison primary batteries attached in parallel, and renewed from time to time, furnished the current for the primary coil. The coil used at the University of Chicago was of the small type for laboratory use, put out by C. H. Stoelting Company, Chicago. The greatest distance between coils on this type was 21.4 cm. The coil used at the University of Minnesota was put out by W. Oehmke, Berlin. The coils could be separated as much as 75 cm. with this type. Various types of keys were tried out, and the one most suitable was an ordinary wall-switch, conveniently mounted, which apparently gave a constant

break shock. The greatest distance between the coils just effecting stimulation was determined at regular intervals. The increase in the strength of shock, as thus determined, was assumed to be a measure of the toxicity of the salt. The writer recognizes the criticisms of such an assumption, but knows of no improved method at present.

The salts were repeatedly recrystallized and the purity carefully tested. Redistilled water from a block tin condenser was used in making up the solutions. The data for purification are given in detail. The calcium chloride, C. P., was not recrystallized. About 45 grams were dissolved and made up to 250 cc. Then 10 cc. of this solution were diluted to 250 cc., and 50 cc. portions were evaporated and dried to constant weight in Jena dishes. Two samples weighed 0.3114 and 0.3116 gram respectively. This material was then redissolved, heated, and ammonium hydroxide and ammonium oxalate added. The material was kept on the steam bath over night. After filtering and washing, the precipitate was ignited to CaO in platinum crucibles. On drying to constant weight, the CaO weighed 0.1569 and 0.1568 respectively. Then 111 : 56.1 :: X : 0.1568 X = 0.3102 gram $CaCl_2$. The actual weight of $CaCl_2$ was 0.3114, the calculated weight 0.3102 gram, therefore, the impurity was 0.3 per cent.

The potassium chloride had been recrystallized by Miss M. Koch. About 38 grams were dissolved and made up to 250 cc. Of this, 10 cc. were diluted to 250 cc., and then 25 cc. portions taken for analysis. These were evaporated and dried to constant weight in Jena dishes, and two samples weighing respectively 0.1351 and 0.1352 gram were used. This material was then dissolved, potassium chromate added, and titrated with standard $AgNO_3$. The amounts of $AgNO_3$ required were 11.27 cc. and 11.28 cc. respectively. The factor of the $AgNO_3$ was 1.607. Then $11.27 \times 1.607 \times 0.00746 = 0.1351$ gram; $11.28 \times 1.607 \times 0.00746 = 0.1352$ gram. As these were identical with the actual weight, the salt was 100 per cent pure.

The sodium chloride was recrystallized four times. Five cubic centimeters of the stock solution were diluted to 250 cc., and 25 cc. samples were taken. The two samples were evaporated in Jena dishes and dried to constant weight. They weighed 0.0891 gram. This material was dissolved and titrated with $AgNO_3$, using potassium chromate as an indicator. The amount of $AgNO_3$ required was 9.58 cc. Then $9.58 \times 1.607 \times 0.00585 = 0.0900$ gram. The actual weight was 0.0891 gram, hence there was 1.0 per cent impurity.

The sodium bromide was recrystallized four times. About 28 grams

were dissolved and made up to 250 cc. Of this stock solution 10 cc. were diluted to 250 cc., and samples of 25 cc. were taken. The samples weighed 0.1093 gram, on evaporating and drying to constant weight. The material was dissolved and titrated with $AgNO_3$, 6.79 cc. being required for the titrations. $6.79 \times 1.607 \times 0.0103 = 0.1123$ gram. The actual weight was 0.1090, hence the impurity was 2 per cent.

The sodium sulphate was recrystallized four times. About 75 grams were dissolved and made up to 500 cc. Of this, 10 cc. were diluted to 250 cc., and 25 cc. were evaporated and dried to constant weight. The samples weighed 0.1417 gram. This was dissolved, heated and precipitated by adding $BaCl_2$. The precipitate was filtered, washed and ignited to constant weight. The actual weight of $BaSO_4$ was 0.2335 gram. The calculated weight $-(142.16 : 233.46 :: 0.1417 : X)$ was 0.2327. The impurity was therefore equal to 0.3 per cent.

The rubidium chloride was made from rubidium carbonate. After several recrystallizations, about 32 grams were dissolved and made up to 250 cc. Of this 1 cc. portions were evaporated and dried to constant weight. The samples weighed 0.1261 gram. This was dissolved and titrated with $AgNO_3$; 10.59 cc. were required for the titration. The factor for this $AgNO_3$ was 0.985. $10.59 \times 0.985 \times 0.012095 = 0.1261$ gram hence the salt was 100 per cent pure.

The rubidium bromide was recrystallized three times. About 45.5 grams were dissolved and made up to 250 cc. Of this 1 cc. portions evaporated and dried to constant weight. The samples weighed 0.1787 gram. This material was dissolved and titrated with $AgNO_3$, using 11.27 cc. in the titration. $11.27 \times 0.985 \times 0.016546 = 0.1836$ gram. Hence the impurity of the rubidium bromide was 2.7 per cent.

The rubidium sulphate was recrystallized three times. Five cubic centimeter portions of the stock solution were taken, evaporated and dried to constant weight. The samples weighed 0.1807 gram. This material was dissolved and precipitated with $BaCl_2$. The precipitate, after standing on the steam bath for twenty-four hours, was filtered off, washed and ignited to constant weight. The $BaSO_4$ weighed 0.1568 gram. The theoretical weight was $(267.06 : 233.46 :: 0.1807 : X)$ 0.1579 gram. Hence the impurity of the salt was 0.7 per cent. This is given in detail because the writer feels that physiologists generally do not realize the importance of having pure salts to start with.

The salts most extensively studied were the chlorides of sodium, calcium, rubidium and potassium. Fewer observations were made on sodium and rubidium sulphates and sodium and rubidium bromides. The sulphates of sodium and rubidium and the chloride of calcium were

used in twelfth-molar concentration, but all others were used in an eighth-molar concentration, unless otherwise stated. Assuming that salts composed of a monovalent cation and a monovalent anion dissociate into two ions, and those composed of either a divalent cation or a divalent anion dissociate into three ions (as $CaCl_2$ or Na_2SO_4) an eighth-molar solution of the former is approximately isotonic with a twelfth-molar solution of the latter. The solutions must be isotonic rather than equimolecular, for work on nerve.

In the early period of the work records were kept of the muscular twitchings, but these were discontinued after a time, since they gave no additional information. The results of a large number of experiments were averaged. In spite of the current opinion that there is nothing constant about nerve work, the writer believes that a fair degree of constancy may be obtained if reasonable precautions are taken.

RESULTS

1. Isotonic salt solutions

Sodium salts. With $\frac{M}{8}$ sodium chloride the latent period was two to three hours, and the period of contractions lasted about three hours. The contractions occurred as single twitches, and relaxation followed each contraction, with short periods of inactivity between the successive twitches. The irritability dropped considerably at first, then increased again, before the final fall. Fifty-two experiments were done for the three-hour period. As these were done at the University of Chicago, they were tested by the small induction coil, which was arranged to read at a maximum of only 21.4 cm. between the coils. Of course, no decrease in irritability was shown by this coil, so a straight line may represent the results, as shown by curve A, figure 1.

Quite a different picture is obtained when a larger coil is used. The coil used for the experiments carried out at the University of Minnesota was arranged to allow a maximum distance of 75 cm. between the coils. Twenty-five experiments were carried out with this coil, and table 1 shows the results for a period of twenty-two hours:

TABLE 1

A	0	½	1	2	3	5	7	8	9	13	22
B	63.3	31.8	24.7	27.6	39.4	43.9	61.7	40.5	39.1	31.7	12.3
C	68.0	62.5	26.5	33.5	68.0	73.0	71.0	72.0	75.0	39.2	31.0
D	57.5	22.0	21.0	20.5	15.0	13.5	45.7	8.3	7.5	22.7	5.0

As it is impossible to give all the readings for all the nerves, only the average, the maximum and the minimum readings are given in the table.

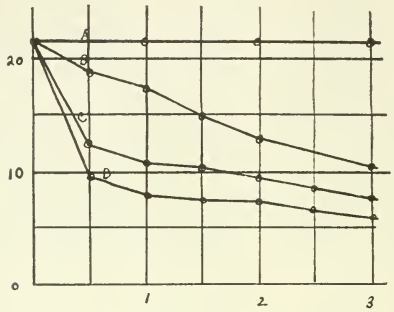

Fig. 1. $A = \frac{M}{8}$ NaCl; $B = \frac{M}{12}$ $CaCl_2$, table 4; $C = \frac{M}{8}$ KCl, table 6; $D = \frac{M}{8}$ RbCl, table 8. Abscissae—time in hours. Ordinates—distance in cms. between coils.

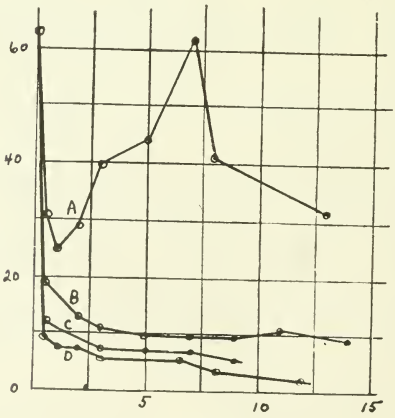

Fig. 2. $A = \frac{M}{8}$ NaCl, table 1; $B = \frac{M}{12}$ $CaCl_2$, table 5; $C = \frac{M}{8}$ KCl, table 7; $D = \frac{M}{8}$ RbCl, table 9.

A indicates the time in hours; B, the average readings of the twenty-five experiments; C, the maximum reading of the nerve showing the greatest irritability; D, the minimum reading of the nerve showing the least irritability. The extremes are variable, as shown by the table, but it must be borne in mind that the majority of the nerves were near the average degree of irritability. Of the twenty-five nerves, two had become non-irritable within twenty-two hours. Curve A, figure 2, indicates the results obtained for the longer period. The abscissae represent the time in minutes or in hours, as specified, and the ordinates indicate the distance in centimeters between the primary and secondary coils.

With $\frac{M}{8}$ sodium bromide the stimulation was less than with $\frac{M}{8}$ sodium chloride. No experiments with sodium bromide were done on the large coil, but the average for those done on the small coil is shown in table 2.

TABLE 2

	TIME								
	0	½	1	2	3	4	5	7	12
Average	21.4	21.4	21.4	21.4	21.4	21.4	19.6	17.6	12.8
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4	21.4	21.4
Minimum	21.4	21.4	21.4	21.4	21.4	21.4	5.7	5.1	2.2

Only nine experiments were carried out with this salt. No comparisons can be made with the other sodium salts respecting the original drop in irritability, since the small coil did not register this. The bromide is evidently more toxic than the chloride, as shown in curve B, figure 3.

Sodium sulphate in $\frac{M}{1/2}$ concentration was more toxic than either the chloride or the bromide. The latent period of stimulation was from ten to twenty minutes, then prolonged low tetanus continued from one to three hours. The decrease in irritability was more rapid and greater than in the other sodium salts. The results of thirty-two experiments with the small coil are shown in table 3.

The greater toxicity of the sulphate is believed to be due to the sulphate ion, rather than to the higher sodium ion concentration. In the Hofmeister series sulphate is more effective than chloride, in many physical systems, so we expect it to behave as it does in physiological systems. Its toxicity is shown in curve C, figure 3.

TABLE 3

	TIME									
	0	½	1	1½	2	2½	3	5	7	12
Average	21.4	20.9	20.9	20.5	19.4	18.2	15.1	7.6	5.2	2.7
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4	20.0	19.2	14.5
Minimum	21.4	8.5	7.7	7.0	5.8	1.8	1.0	1.0	1.0	1.0

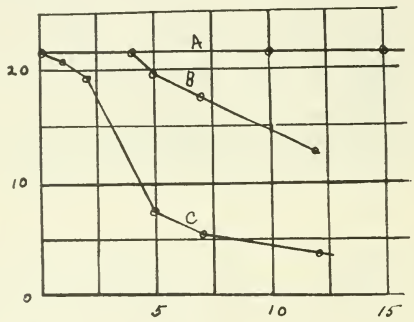

Fig. 3. A = $\frac{M}{8}$ NaCl; B = $\frac{M}{8}$ NaBr, table 2; C = $\frac{M}{12}$ Na_2SO_4, table 3

Calcium salts. The chloride was the only calcium salt used. In $\frac{M}{1\frac{1}{2}}$ concentration it did not stimulate the nerve, but it did produce a medium decrease in irritability. Mathews (28) states that sodium salts are necessary for the maintenance of irritability of the nerve. The author found the nerves still irritable after being immersed in calcium chloride for sixteen hours. Calcium chloride occupies a position between sodium on the one hand, and rubidium and potassium on the other, in respect to its depressing effect. Sixty-six experiments were carried out with the small coil for three hour periods. The results are shown in table 4, also in curve B, figure 1.

TABLE 4

	TIME						
	0	50	60	90	120	150	180
Average	21.4	18.8	17.1	14.8	12.6	11.7	10.4
Maximum	21.4	21.4	21.4	21.2	21.4	21.4	18.8
Minimum	21.4	7.3	6.0	6.3	6.0	3.0	3.0

Twenty experiments were carried out with the large coil for long periods. The results are shown in table 5, and in curve B, figure 2.

TABLE 5

	TIME													
	0	½	1	2	3	4	5	7	9	10	11	12	14	18
Average	63.3	18.8	17.1	12.6	10.4	9.7	9.6	9.7	9.4	10.8	10.5	10.3	8.9	7.3
Maximum	75.0	21.4	21.4	21.4	18.8	18.1	15.6	9.0	17.1	16.4	15.9	15.0	14.0	14.0
Minimum	59.0	7.3	6.0	6.0	3.0	3.6	2.9	2.5	3.0	3.0	3.0	3.0	2.0	1.0

Thirteen out of twenty were still living at the end of fourteen hours, and three out of six were living at the end of eighteen hours.

Potassium salts. The chloride was the only potassium salt studied. An $\frac{M}{8}$ solution of potassium chloride was found to be a stimulant of the nerve, contrary to the observation made by Mathews (1). Eighty-seven experiments were carried out on this salt alone, and in about 80 per cent of the cases, stimulation occurred. The latent period was from ten seconds to one minute, and the contractions continued from two to four minutes, rarely longer. This stimulation may have been overlooked by previous investigators, but that it was due to the potassium chloride is shown by the fact that it occurred with no other salt. The decrease in irritability was rapid, and the results certainly show that rubidium and potassium chloride are similar in their toxicity effects (consult curves C and D, figs. 1 and 2).

Table 6 shows the results for three-hour periods, of eighty-seven experiments.

TABLE 6

	TIME						
	0	30	60	90	120	150	180
Average	21.4	12.3	10.7	10.2	9.4	8.4	7.4
Maximum	21.4	21.4	20.2	21.2	19.8	18.2	15.4
Minimum	21.4	5.8	2.5	3.0	3.0	2.5	1.1

Only twenty experiments were carried out on the large coil for longer periods. The results are shown in table 7.

TABLE 7

	TIME									
	0	½	1	2	3	4	5	6	7	8
Average	63.3	12.3	10.7	9.4	7.4	7.1	7.5	7.4	6.4	5.3
Maximum	75.0	21.4	20.2	19.8	15.4	13.0	12.0	9.8	8.6	7.4
Minimum	59.5	5.8	2.5	3.0	1.1	3.0	3.0	5.2	4.0	3.3

Rubidium salts. Rubidium chloride is undoubtedly a strong stimulant for nerve fiber. The latent period was found to be from ten seconds to two minutes. Twitching began, followed soon by tetanus, which continued from ten to twelve minutes. After a few single twitches the muscle relaxed and remained quiet during the remainder of the experiment. A rapid decrease in irritability was found, but a complete loss of irritability did not occur, even in twelve hours. This observation does not confirm that of Mathews, but may be attributed to a difference in technique.

Table 8 shows the results of thirty-eight experiments, on the small coil, for three hour periods.

TABLE 8

Curve D, fig. 1

	TIME						
	0	30	60	90	120	150	180
Average	21.4	9.9	7.8	7.4	7.1	6.1	5.8
Maximum	21.4	16.0	16.5	16.2	16.2	14.0	14.0
Minimum	21.4	5.5	5.2	3.0	2.5	2.0	1.0

Twenty experiments were carried out for long periods on the large coil. The results are shown in table 9, and likewise in curve D, figure 2.

TABLE 9

	TIME								
	0	½	1	2	3	4	6	8	12
Average	63.3	9.9	7.8	7.1	5.8	4.7	5.0	3.4	1.7
Maximum	75.0	16.0	16.5	16.2	14.0	6.1	8.3	7.6	8.0
Minimum	57.5	5.5	5.2	2.5	1.0	3.0	2.0	0.5	0.4

Rubidium bromide in $\frac{M}{8}$ concentration is less stimulating than rubidium chloride. It always stimulates in an $\frac{M}{4}$ solution, and occasionally in an $\frac{M}{8}$ solution. The decrease in irritability was less than with

the chloride. The following table shows the results of fifteen experiments, conducted with the small coil, for long periods.

TABLE 10

Curve B, fig. 4

	TIME										
	0	½	1	1½	2	2½	3	5	8	10	12
Average	21.4	9.6	9.4	8.2	7.5	7.2	6.6	5.6	5.4	5.6	2.5
Maximum	21.4	13.3	13.0	11.0	10.2	10.4	9.4	8.5	9.5	9.2	5.6
Minimum	21.4	6.8	6.5	5.2	3.8	5.2	4.4	2.6	1.7	2.5	0.8

Rubidium sulphate is a stronger stimulant than the chloride, and the toxicity is greater. The action of the rubidium sulphate bears a relation to the actions of rubidium chloride and rubidium bromide similar to the relation between sodium sulphate and the chloride and bromide. Table 11 gives the average for eight experiments.

TABLE 11

Curve C, fig. 4

	TIME									
	0	½	1	1½	2	2½	3	6	7	8
Average	21.4	10.2	8.5	6.3	6.1	4.9	4.3	4.4	3.2	2.4
Maximum	21.4	18.7	17.1	13.8	10.3	9.4	7.3	5.0	4.2	3.6
Minimum	21.4	6.6	6.1	4.8	2.9	1.2	1.2	3.4	2.0	1.0

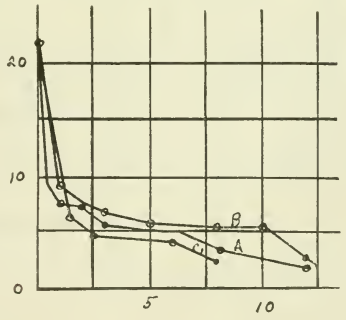

Fig. 4. A = $\frac{M}{8}$ RbCl, table 9; B = $\frac{M}{8}$ RbBr, table 10; C = $\frac{M}{12}$ Rb_2SO_4, table 11

2. Combinations of three salts

Investigations were carried out in which rubidium was used instead of potassium in Ringer's solution. The Ringer's solution used as a control contained 7 grams of sodium chloride plus 1 cc. of 2.5 M KCl plus 1 cc. of 2.5 M $CaCl_2$ per liter. The modified Ringer's solution contained the same amounts of sodium and calcium chlorides, and 1 cc. 2.5 M RbCl per liter, instead of the same amount of KCl. The following table shows the results of twenty experiments, conducted with the large coil, for long periods:

TABLE 12

Curve B, fig. 5

	TIME								
	0	½	1	1½	2	2½	3	7	18
Average	63.3	55.8	54.8	53.5	52.7	52.7	53.1	55.0	35.9
Maximum	75.0	78.5	71.5	62.5	63.0	63.0	63.0	65.0	48.5
Minimum	45.0	45.0	45.0	45.0	44.0	44.0	44.0	13.5	13.5

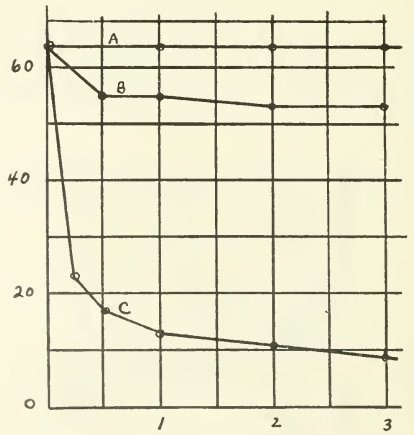

Fig. 5. A = Controls in Ringer; B = Ringer—K substituted by Rb, table 12; C = Ringer—Na substituted by Rb, table 13.

This shows that rubidium is certainly closely related to potassium, since the nerves lose very little in irritability within several hours, in a solution containing rubidium in place of potassium.

Another series of experiments was carried out in which rubidium was used instead of sodium in Ringer's solution. This solution contained 119.6 cc. of $\frac{M}{1}$ RbCl per liter, 1 cc. of 2.5 M $CaCl_2$, and 1 cc. of 2.5 M KCl. This modified Ringer was found very toxic, as shown in table 13, and again in curve C, figure 5, where the results of twenty-four experiments are given. Certainly rubidium is not similar in its action to sodium, else it could replace it in a balanced solution.

TABLE 13

	TIME							
	0	¼	½	1	1½	2	2½	3
Average	63.3	23.7	16.7	12.3	11.7	10.2	9.7	8.4
Maximum	75.0	50.0	30.0	23.5	22.0	19.0	18.0	16.0
Minimum	57.5	10.5	6.0	6.0	5.5	5.0	5.5	5.5

Curve A, figure 5, represents the controls in Ringer's solution. As these were run with daily observations a large number was obtained, but the curve shown may be considered to represent twenty. With frogs in good condition there was seldom any loss in irritability for twenty hours, as before stated. From these experiments alone, rubidium is shown to be most similar to potassium in its physiological behavior.

3. Hypotonic solutions

A series of experiments was performed with varying concentrations of rubidium salts. In one series a concentration of twentieth or fortieth molecular was used, knowing that the solution was hypotonic. In the other series a similar quantity of rubidium salt was used, but a final osmotic pressure equivalent to an eight-molar solution was obtained by the addition of the proper amount of saccharose. The results for $\frac{M}{20}$ RbCl in saccharose are shown in table 14 and in curve A, figure 6. Twenty experiments were performed.

TABLE 14

	TIME						
	0	30	60	90	120	150	180
Average	21.4	19.9	18.7	16.3	14.3	12.4	11.1
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4
Minimum	21.4	11.0	12.0	9.4	8.4	8.0	6.8

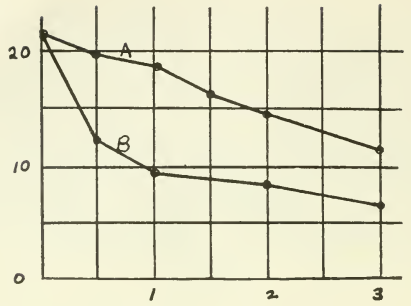

Fig. 6. A = $\frac{M}{20}$ RbCl in sacc., table 14; B = $\frac{M}{20}$ RbCl in H_2O, table 15

Eight experiments were carried out with $\frac{M}{20}$ RbCl in water, and table 15, also curve B, figure 6, show the effects of the hypotonic solution. Osmotic pressure appears to have some effect.

TABLE 15

	TIME						
	0	30	60	90	120	150	180
Average	21.4	12.1	9.3	8.4	8.1	7.8	6.5
Maximum	21.4	21.4	14.3	16.7	12.6	12.7	12.1
Minimum	21.4	6.0	6.0	3.6	3.8	3.6	1.2

The results of thirteen experiments with $\frac{M}{20}$ Rb_2SO_4 in saccharose are shown in table 16, and again in curve A, figure 7.

TABLE 16

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.9	14.7	10.7	10.3	9.1	8.8
Maximum	21.4	21.4	21.4	21.4	20.3	19.0	18.0
Minimum	21.4	9.5	8.8	8.2	6.1	4.8	1.1

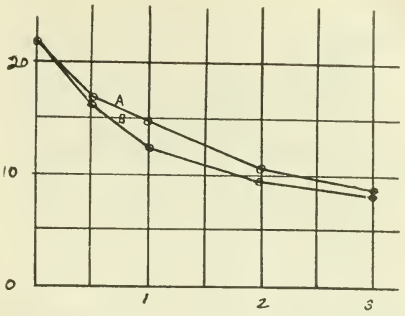

Fig. 7. A = $\frac{M}{20}$ Rb_2SO_4 in sacc., table 16; B = $\frac{M}{20}$ Rb_2SO_4 in H_2O, table 17

The results of six experiments with $\frac{M}{20}$ Rb_2SO_4 in water are shown in table 17, and in curve B, figure 7.

TABLE 17

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.2	12.3	10.6	9.3	8.7	8.2
Maximum	21.4	21.4	18.7	15.5	15.0	14.7	14.2
Minimum	21.4	11.1	9.9	8.7	5.9	6.5	5.8

This again shows a slightly higher irritability with the isotonic solution.

A very few experiments were done with $\frac{M}{40}$ Rb_2SO_4 in saccharose, the results of which are shown in table 18 and in curve A, figure 8. The corresponding nerves in $\frac{M}{40}$ Rb_2SO_4 in water gave lower results, as shown in table 19 and in curve B, figure 8.

TABLE 18

	TIME						
	0	30	60	90	120	150	180
Average	21.4	18.2	17.6	15.5	11.9	11.5	10.7
Maximum	21.4	21.4	21.4	21.4	18.2	18.8	18.1
Minimum	21.4	11.8	11.4	6.5	6.5	6.8	6.2

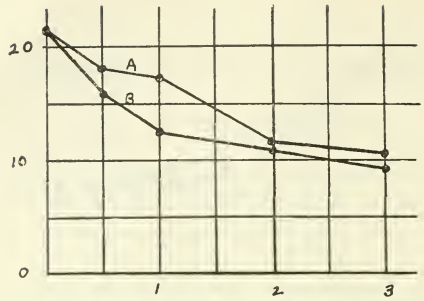

Fig. 8. A = $\frac{M}{40}$ Rb_2SO_4 in sacc., table 18; B = $\frac{M}{40}$ Rb_2SO_4 in H_2O, table 19

TABLE 19

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.0	12.6	11.7	11.3	10.3	9.1
Maximum	21.4	21.4	15.7	13.7	13.9	12.7	11.3
Minimum	21.4	10.6	8.7	8.7	9.7	8.6	7.5

This shows that the isotonic solution preserves a slightly higher irritability than the hypotonic solution. These experiments are by no means exhaustive, yet they indicate that isotonic solutions are less toxic than hypotonic, or that osmotic pressure is a possible factor in maintaining the irritability of nerves in various solutions.

4. Salt antagonisms

A series of investigations was made with combinations of two salts, for the purpose of testing the so-called antagonistic action. For these investigations 1 part of $\frac{M}{8}$ calcium chloride and 99 parts of $\frac{M}{8}$ sodium, potassium or rubidium chloride were used throughout. Twenty-one long experiments were carried out with the mixture of calcium and sodium. Undoubtedly the calcium chloride in this concentration had some antagonizing action, as shown in tables 1 and 20 and in curves A and B, figure 9.

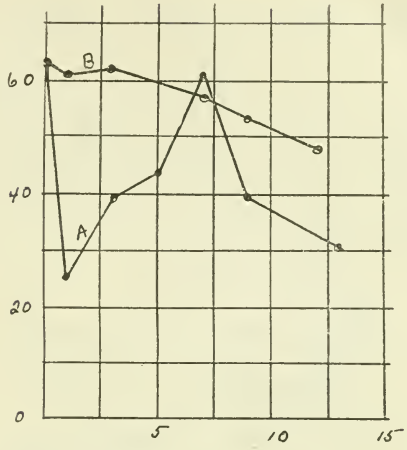

Fig. 9. A = $\frac{M}{8}$ NaCl, table 1; B = 99 parts $\frac{M}{8}$ NaCl, plus 1 part $\frac{M}{8}$ $CaCl_2$, table 20.

TABLE 20

	TIME										
	0	½	1	2	3	5	7	8	9	10	12
Average	63.3	60.9	60.8	61.4	62.0	59.8	57.1	56.9	53.0	49.9	48.0
Maximum	75.0	68.5	68.5	68.5	69.0	69.0	65.4	66.0	67.0	66.5	69.5
Minimum	52.5	52.5	53.0	53.0	55.0	51.0	44.0	35.0	25.5	9.0	4.2

The initial fall in irritability in $\frac{M}{8}$ sodium chloride is prevented by the addition of a small amount of calcium, and even after twelve hours the nerve in the mixture is much more irritable than the one in sodium chloride alone. This surely indicates a protective action of some kind on the part of calcium.

The picture obtained by using 1 part of $\frac{M}{8}$ calcium chloride and 99 parts of $\frac{M}{8}$ potassium chloride is quite different from the above picture. Twenty-three experiments were carried out with this combination. The calcium here played a minor rôle, and its protective action was not a decided one. The results are shown in tables 6 and 21, and in curves A and B of figure 10. The decreases in irritability of the

two series were similar, the addition of calcium being slightly beneficial.

TABLE 21

	TIME				
	0	½	1	2	3
Average	63.3	14.9	13.3	10.5	9.0
Maximum	75.0	28.0	23.0	19.5	17.5
Minimum	55.0	7.5	6.7	4.0	2.5

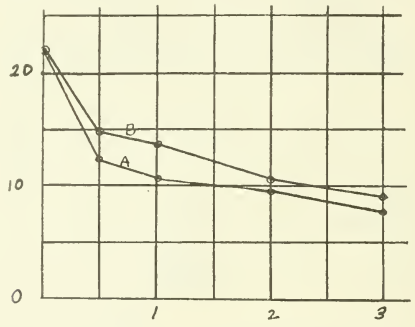

Fig. 10. A = $\frac{M}{8}$ KCl, table 6; B = 99 parts $\frac{M}{8}$ KCl plus 1 part $\frac{M}{8}$ $CaCl_2$, table 21.

Twenty-five experiments were carried out with a similar mixture of calcium and rubidium chlorides. The results were much like those obtained with the mixture of calcium and potassium chlorides. The calcium showed a slight protective action. The results are shown in tables 8 and 22 and in curves A and B of figure 11.

TABLE 22

	TIME				
	0	½	1	2	3
Average	63.3	13.1	10.8	9.3	7.9
Maximum	75.0	21.0	20.0	18.5	17.0
Minimum	55.0	5.0	4.0	2.5	2.0

It will be noticed that rubidium and potassium behave similarly in respect to the effects obtained when these are combined with a small amount of calcium. Neither is antagonized to a great extent, while in the case of sodium a decided antagonism exists.

The question of the part played by the radio-activity of rubidium has been considered by some authors. Henriot (26) states that rubidium emits a radiation giving a current of 3.64×10^{-13} amp. per sq. cm. of surface of rubidium sulphate, while in the same circumstance the radiation of potassium is only 2.74×10^{-13} amp. The rays are more intense and more penetrating than those of potassium. The radio-activities of the salts of potassium and rubidium probably serve to

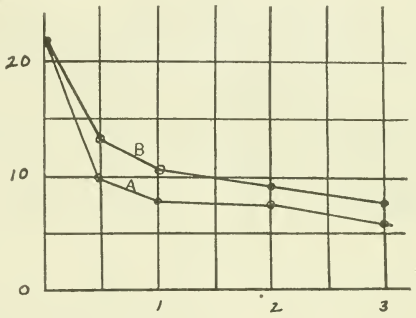

Fig. 11. A = $\frac{M}{8}$ RbCl, table 8; B = 99 parts $\frac{M}{8}$ RbCl plus 1 part $\frac{M}{8}$ $CaCl_2$, table 22.

differentiate them from the salts of sodium and calcium, in physiological effects, but facilities are not at hand for further investigation in this direction.

5. Additional observations

It appeared highly desirable to substantiate the physiological effects obtained above by results on non-living systems. A minor study of the comparative effects of sodium, potassium, and rubidium salts on the precipitation of lecithin and kephalin emulsions was carried out. Since the myelin sheaths of nerve fibers are composed largely of lecithin and kephalin and since the sodium and potassium contents vary considerably in lecithin and kephalin (27), it was supposed that

there would be a difference in the concentrations of the two cations required to precipitate emulsions. The lecithin and kephalin preparations used were made by the writer, from spinal cord of beef. On analysis, the lecithin yielded 1.74 per cent of N and 2. 7 per cent of P. The kephalin gave 1.92 per cent of N and 3.32 per cent of P. A small quantity (0.75 gram) was weighed out, soaked in water over night, and placed in the shaker for two and one-half or three hours, then made up to 250 cc., thus giving a 0.3 per cent emulsion. One cubic centimeter of the emulsion, a varying quantity of the salt solution and sufficient redistilled water to make a total volume of 10 cc., were measured off in each case.

Test tubes of 20 cc. capacity were used, into which the solutions were measured from burettes. The emulsion and water were mixed first, then the salt added. After thorough mixing the tubes were set aside in a cold place for 24 hours. At the end of this time the tubes were examined for precipitates. By this method the precipitation limit for each salt was readily determined. For an example, 1 cc. of 0.3 per cent emulsion + 4.5 cc. H_2O + 4.5 cc. $\frac{M}{100}$ $CaCl_2$ contained 0.03 per cent emulsion and 0.0045 M $CaCl_2$. The results, or precipitation limits, obtained by the above method, are shown in table 23.

TABLE 23

SALT	LECITHIN	KEPHALIN
$CaCl_2$	0.004–0.0035M	0.003–0.0025M
NaCl	0.250–0.225M	0.275–0.250M
KCl	0.350–0.325M	0.375–0.350M
RbCl	0.350–0.325M	0.350–0.325M
Na_2SO_4	0.150–0.125M	0.200–0.175M
NaBr	0.300–0.275M	0.300–0.275M
RbBr	0.375–0.350M	0.375–0.350M

Since in kephalin the potassium greatly exceeds the sodium, and in lecithin the reverse condition exists (27), it was expected that the precipitation limits would vary accordingly, but such was not the case. Since no striking differences were apparent between the sodium salts on one hand and those of rubidium and potassium on the other, this line of experimentation was discontinued.

SUMMARY

What happens when a nerve is placed in a salt solution? Mathews (28) long ago concluded that the stimulating or depressing effects exerted by electrolytes on the nervous system depend upon the relative efficiency of anions and cations. If in a given salt the anion markedly predominates, the salt stimulates; if the cation predominates, the salt depresses. The results above agree with this, since sodium, rubidium and potassium chlorides were stimulating agents, and calcium chloride was a depressing agent. In what manner does one salt stimulate and another depress? This question is as yet unanswered.

There is undoubtedly an antagonistic action between calcium chloride and sodium chloride as shown above; Clowes (29) and Mathews both consider such an antagonism attributable to a balance between cations on one hand and anions on the other. But why is not the antagonism between calcium chloride and rubidium or potassium chloride just as marked? In Clowes' emulsion systems sodium may be replaced by potassium; this does not hold for nerve work. Rubidium and potassium behave very much alike in physiological systems, but sodium differs from them. That rubidium and potassium are alike is shown by the similar effects on the irritability of the nerve in the pure solution, by the fact that rubidium can replace potassium in Ringer's solution, and by the failure of calcium, in the concentration used, to antagonize the toxic effects of either. The rate of diffusion, the solution tension and various other factors undoubtedly enter into the effects produced on physical and physiological systems, but the subject is still shrouded in darkness.

CONCLUSIONS

As a result of measuring the comparative rate of loss of irritability, the writer considers that the observations indicate that:

1. The effects of rubidium and potassium salts on motor nerve of frog are not so widely different as has formerly been supposed. The rate of loss of irritability appears to parallel the stimulating power of the salts studied (excepting calcium chloride, which does not stimulate, but does cause a loss of irritability).

2. An isotonic potassium chloride solution, $\frac{M}{8}$, does stimulate motor nerve of frog in at least 80 per cent of the cases.

3. Calcium chloride in isotonic solution, $\frac{M}{12}$, occupies a position between potassium and rubidium on one side and sodium on the other, in respect to its depressing effect on nerve.

4. Osmotic pressure plays a rôle, since hypotonic solutions were more toxic than isotonic solutions.

5. Rubidium is more nearly related to potassium than it is to sodium, and does not have an action on nerve peculiar to it alone.

6. Calcium chloride exerts a pronounced antagonistic effect on sodium chloride, and a scarcely perceptible one on rubidium and potassium chlorides.

7. Each salt has a specific action on nerve.

The author wishes to express her gratitude to Dr. F. C. Koch for his kindly interest in this work, the latter part of which he supervised.

BIBLIOGRAPHY

(1) Mathews: This Journal, 1904, xi, 455.
(2) Grandeau: Journ. de l'anat. at de la physiol., 1864, 378.
(3) Ringer: Journ. Physiol., 1884, iv, 370.
(4) Blake: Journ. Physiol., 1884, v. 124.
(5) Harnack and Dietrick: Arch. f. exper. Path. u. Pharm., 1884, xix, 153.
(6) Brunton and Cash: Proc. Royal Soc., 1883, xxxv, 324.
(7) Richet: Compt. Rend., 1885, ci, 667.
(8) Botkin: Centralbl. f. d. Med. Wissensch., 1885, xxiii, 749.
(9) Grützner: Arch. gesammt. Physiol., 1893, liii, 83.
(10) Ranke: Die Lebensbedingungen der Nerven, Leipzig, 1868.
(11) Biedermann: Ber. der k. Akad. der Wissensch., 1881, iii, Abt. 27.
(12) Loeb: This Journal, 1900, iii, 337.
(13) Loeb and Cattell: Journ. Biochem., 1915, xxiii, 41.
(14) Loeb and Ewald: Journ. Biol. Chem., 1916, xxv, 377.
(15) Burridge: Quart. Journ. Exper. Physiol., 1914, viii, 303.
(16) Zoethout: This Journal, 1908, xxiii, 374.
(17) Lillie: This Journal, 1909, xxiv, 459.
(18) Joseph and Meltzer: This Journal, 1911, xxix, 1.
(19) Groves: Journ. Physiol., 1893, xiv, 221.
(20) Osterhout: Journ. Biol. Chem., 1905, i, 363.
(21) Burnett: Journ. Biol. Chem., 1909, v, 351.
(22) Miyake: Journ. Biochem., 1913, xvi, 235.
(23) Cramer: Biochem. Journ., 1918, xii, 210.
(24) Zoethout: This Journal, 1904, x, 213.
(25) Zwaardemaker and Feenstra: Kon. Akad. von Wetanschappen, 1916, xxv, 517.
(26) Henriot: Chem. News, 1911, civ, 47.
(27) Koch: Zeitschr. f. Physiol. Chemie, 1902, xxxvi, 134.
(28) Mathews: This Journal, 1904, xi, 455.
(29) Clowes: Journ. Phys. Chem., 1916, xx, 407.

THE PRACTICABILITY OF FEEDING A SCIENTIFICALLY BALANCED RATION IN ARMY CAMPS

R. J. ANDERSON

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, U. S. Army

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The proper feeding of an army or of army divisions, such as were in training in numerous camps throughout the country during the past year, presents several important problems. First of all available food supplies must be considered. Those furnished by the Subsistence Branch of the Quartermaster's Corps constitute the Garrison Ration Articles. Certain supplementary articles may be obtainable in the local markets or adjacent territory. Equally important are the matters of storage, the prevention of spoilage and the cooking of the raw food products into edible and nutritious food. With ample food supplies available, those responsible for the feeding of the army must select such combinations of food as will safeguard and maintain the health of the troops and increase their vigor. In this selection it is necessary to consider the relative proportions of protein, fat and carbohydrate, and perhaps also the acid and basic elements contained in the food, together with so-called vitamines, which should be supplied for a properly balanced diet. In addition to the above a satisfactory diet must also contain a certain amount of roughage or indigestible residue.

It is self-evident that a group of men cannot subsist upon calories alone, but upon nutritious and wholesome food containing an adequate amount of nourishment for the bodily needs of repair and supplying sufficient fuel value in addition for the performance of such work as may be required. Unless these conditions are fulfilled the men will become dissatisfied, the morale will be lowered and the efficiency of the organization will be decreased.

In ordinary life people of ample means choose their food according to taste and appetite. The selections which are made are usually sufficiently varied to furnish an adequately balanced diet. It not infrequently happens, however, among the poor and ignorant that a

thoroughly inadequate diet is either selected or thrust upon them by necessity, with resultant serious disturbances in health and vigor.

In the army the men can not choose their own diet. The food is placed before them in the mess and they may either eat it or leave it. It is therefore of the utmost importance that the food supplied be properly and wisely selected and also that it be prepared so as to preserve or increase its nutritious and appetizing qualities.

In considering what constitutes a balanced diet we must bear in mind what was said above, that men do not live on calories alone. Frequent changes of menu and as great a variety of food as possible are not only desirable but necessary. It would be very easy to supply an adequate number of calories to any group of men if but little attention were given to variety, but it requires more care, experience and planning to furnish the necessary number of calories in a well-balanced diet upon which men can subsist to advantage for long periods.

All of the troops in the new armies in training camps in this country during the recent emergency were fed on the basis of the Garrison Ration and all of the messes enjoyed the privilege of "Ration Savings." In addition to the Garrison Ration articles extra-ration food supplies, such as fresh vegetables, fruits, eggs, etc., were usually purchased by the mess sergeants in the local markets and were paid for out of the ration savings. The Garrison Ration formed merely the basis upon which the cash value of the ration allowance was calculated, each organization receiving money credit for the number of men entitled to ration multiplied by the ration allowance. The money value of the ration allowance varied from month to month and from camp to camp, depending upon prevailing food prices. Against this money credit the organization drew at certain intervals such Garrison Ration articles as were needed during the month. The amount of the total food supplies thus drawn by each mess from the Quartermaster varied within rather wide limits (from about 65 to 90 per cent of the ration allowance) and it varied from camp to camp, depending upon local camp regulations as well as upon local market conditions. Under army regulations in effect April 1, 1919, all of the food used by troops must be purchased by, and supplied through, the Subsistence Branch of the Quartermaster's Corps. The ration savings privilege has been revoked, all savings reverting to the Government. However, 25 per cent of the ration allowance may be expended by the Camp Supply Officer in purchasing extra-ration articles. The success of this system will depend, of course, upon the ability of the Supply Officer to obtain

all of the articles called for and in sufficient quantity to fill the demand. Even under the old system a month seldom passed when the Subsistence, Branch was not out of certain products for a longer or a shorter period. Forced issues of various food products which were overstocked and therefore likely to spoil were also not uncommon. On the whole however, the new system has much to commend it. The purchase of all food supplies is centralized in one office, which should lead to much desired efficiency as well as economy in feeding the army. The outstanding disadvantage is that perishables can usually be purchased most advantageously on an opportunistic basis.

Under army regulations the company commander is responsible for the proper feeding of the men. In actual practice, however, it was frequently found that this duty was left largely, if not entirely, in the hands of the mess sergeants. Occasionally, however, mess officers and company commanders took a lively interest in the mess conditions of the men. In such cases results were more satisfactory than when the mess sergeants were largely left to their own devices. Often one could tell by mere inspection of the messes whether the commanding officers gave them their personal attention.

As has been indicated above, the amount of food consumed and the proportion of the different nutrients in the diet varied greatly in different messes in a camp. It could hardly be otherwise when we consider the enormous expansion of the army within a few months and the lack of training of the new soldiers in matters pertaining to dietetics and cooking. Mess sergeants and cooks frequently were "made" through necessity by orders of the company commanders. It is not to be wondered at, then, that many of the messes were run on a hit-or-miss plan, so far as a successful diet was concerned. Even after the mess force had had several months' experience it was not unusual to find that the average food consumption varied by 1000 or 1500 calories per man per day in adjacent companies in the same regiment. Good cooking and high food consumption appeared to stand in direct relation.

The activities of the Schools for Cooks and Bakers naturally helped to improve matters somewhat in the long run. The commanding officers of these schools were zealous and industrious but their knowledge of dietetics was very limited. Their instruction in mess accounting, on the other hand, was adequate. On the whole these schools succeeded in working marked improvements in mess conditions.

No matter how carefully the food material is selected and the menus calculated, a successful diet can not be maintained unless provision

is made for the proper preparation and cooking of the food. The finest raw material may be ruined by poor cooks. There is, therefore, urgent need to continue schools for cooks and bakers in the army. In order to turn out competent workmen, however, it will be necessary to give the students more thorough instruction in the art of cooking than was generally given in the schools during the past year. Many of the so-called graduate cooks who were turned out were fairly well instructed in the duties of the kitchen police, but the number who actually knew how to cook food was unfortunately small.

Personal observation in several camps during the past year showed plainly the need of a more centralized and effective control over mess conditions than existed at that time. From the standpoint of efficiency, dietetic as well as financial, the practically independent operation of some 200 to 250 messes in a camp cannot be considered successful. Some one with sufficient authority should have sufficient general supervision over mess conditions to insure that adequate variety of food be provided. The making of menus for the different organizations should also be supervised in order to insure the proper combination of food-stuffs in the diet. In the past the menus were frequently made out by the mess sergeants one day at a time and the foods thus supplied depended largely upon what was on hand or on what could be immediately obtained. Much better results would be secured if tentative menus were made out for a period of, say, ten days in advance; purchase could be made then in accordance with the requirement of the menus.

At the present time we possess sufficient knowledge regarding the composition of raw food material to calculate in advance any desired diet. We can also provide adequate variety of fresh vegetables and fruits, which will supply sufficient roughage and necessary basic elements as well as vitamines. Some of the latter should undoubtedly also be furnished in the form of fresh butter.

It may remain an open question just how much food should be furnished to each man, but the aim might well be to provide on an average about 3600 calories (net) per day for men doing moderately hard work. Some men will consume more and others less, depending upon bodily condition and appetite. Careful consideration should be given to the proportion of protein, fat and carbohydrate contained in the diet. The arbitrary selection of a standard may be of doubtful wisdom. On the other hand, standards itemized by means of statistical studies need not be taken as infallible guides. It has recently been

proposed by the Division of Food and Nutrition to aim at furnishing the following balance: Protein, 12.5 per cent, a quantity known to be adequate; fat, 25 per cent, the amount being intentionally restricted on economic grounds, and carbohydrate 62.5 per cent. Before definitely selecting any standard balance for the army it might be advisable to conduct careful experiments over relatively long periods of time in order to determine what diets actually give the most satisfaction to the men and which, from a physiological as well as an economical basis, are the most desirable. A diet containing only 25 per cent of fat might be less satisfying than one of higher content. Besides, if a reasonable amount of fresh butter is provided it is difficult to keep the fat content under 30 per cent.

In this connection it is interesting to note that all officers' messes, cadets' messes in aviation fields and student officers' messes in Camp Greenleaf, in which dietary studies were made, supplied 40 per cent or more of the total calories as fat. These messes appeared to furnish a diet which corresponded more closely to the usual civilian or family fare than was furnished to enlisted men. A considerable part of the fat in these officers' messes was derived from the butter which was used according to taste at the table. Enlisted men received, as a rule, very little butter. Food which is well cooked and which contains the higher percentage of fat is undoubtedly the most appetizing and satisfying.

We believe that it would be for the interest and welfare of the army if some officer trained in the science of nutrition and in practical dietetics were connected with each camp or army division. The officers selected for this purpose should be men of thorough practical training in the army and possessing, in addition, full knowledge of the composition of food and the science of nutrition. They should be particularly well acquainted with the practical side of nutrition and the problems involved in feeding large groups of men. An effort has been made in recent months to fill this want by appointing nutrition officers to serve in camps in this country. If nutrition officers are to form a permanent addition to our new army, men of the qualifications mentioned above should be selected. They should be at least coördinate in rank with division sanitary inspectors. Nutrition officers of subordinate rank will have a limited field of usefulness and they will with difficulty be able to accomplish much in the way of fundamental improvements.

Unless matters pertaining to nutrition be placed under the super-

vision and control of men thoroughly qualified on both the theoretical and the practical sides, one may well doubt the practicability of feeding a scientifically balanced ration in army camps. The only alternative would be to maintain a separate Department of Nutrition or Alimentation in connection with the War Department in Washington, with subordinate officers in the camps who could carry out orders from the Washington office.

In connection with the feeding of a balanced ration in view of the new system of supplying all food through the Subsistence Branch, one might suggest in the interest of good nutrition as well as to aid the camp supply officer, that a uniform menu be adopted for the whole camp. This should be done, however, only under the direction of competent authority for it is realized that a uniform menu, unless carefully prepared and supervised, might lead to very unfavorable conditions and a stereotyped form of diet. The menu should be made out from ten to fifteen days in advance in order to give the supply officer an opportunity to secure the necessary raw material. Unless some such scheme is adopted the men will have to subsist upon such food supplies as are available at the subsistence store from day to day. The menus should specify the amount in pounds of the different foodstuffs to be actually used each meal for a given number of men. When this is done, having due regard to the composition of the food material, a diet of any desired composition or balance can be provided. In this way a scientifically balanced ration can be furnished an entire camp almost as easily as for a squad of men in an investigational laboratory.

The results of the dietary studies in army messes made by Survey Parties of the Section of Food and Nutrition, to which reference has already been made, showed that numerous nutritional errors were committed which could easily be corrected if proper supervision over mess conditions were maintained. For example, we reproduce below the result of two dietary studies made early last fall. One mess consumed on an average the following quantities per man per day:

	GRAMS	PERCENTAGE OF DISTRIBUTION OF CALORIES
Protein	85	12
Fat	59	20
Carbohydrate	472	68

Total calories about 2830.

In an adjoining organization in the same camp and at the same time of the year where the men did less hard work, the following amounts were consumed:

	GRAMS	PERCENTAGE OF DISTRIBUTION OF CALORIES
Protein	131	13
Fat	145	33
Carbohydrates	540	54

Total calories about 4100.

Both of the above messes were well managed and the mess sergeants were above average ability and they had had considerable experience. In the latter mess the men were receiving more food than they required for the very light work which they were doing. In the former the men were decidedly underfed. But the men in both organizations were under the impression that they were well fed. In the first mess liberal quantities of cabbage, sauerkraut, onions, turnips and potatoes were supplied, which made up a large bulk of low fuel value. This is not an isolated instance but many messes were studied in which considerable degrees of disparity were observed.

What must be especially guarded against is the excessive use of canned goods instead of fresh vegetables and fruits, and the too frequent employment of the cheaper meat products, as was frequently noticed during the past summer. When one remonstrated against this form of diet the statement was always made, "Fresh material cannot be obtained." Under the new ration system the supply officer, in handling the large demand for an entire camp, will be in a position to draw on distant markets for many articles which are not obtainable locally. This would apply particularly to vegetables, fruits and fish.

As an aid and a step in the direction of correcting dietary errors, such as those mentioned above, the adoption of a uniform menu, prepared under the supervision of an expert in nutrition, seems worthy of trial. The menu might be prepared in a conference between the nutrition officer, mess officers, mess sergeants and the supply officer. Such conferences might be held every week or ten days. This arrangement would also serve to coördinate mess consumption with available food supplies.

The hearty coöperation of the nutrition officers, mess officers and other mess personnel, and all others concerned with handling the army ration, will be required to bring about the best nutritional conditions in the army. Some one with thorough training in nutrition and practical dietetics should be authorized to supervise the diet for the whole camp in order that a scientifically and correctly balanced ration may be provided.

AVERAGE FOOD CONSUMPTION IN THE TRAINING CAMPS OF THE UNITED STATES ARMY

JOHN R. MURLIN AND F. M. HILDEBRANDT[1]

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, United States Army

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One of the main objects of the Division[2] of Food and Nutrition of the Medical Department of the Army was to determine the actual food requirements of soldiers in active training. Beginning October 1, 1917, and continuing until December 1, 1918, nutritional surveys were made in 67 different camps including 49 divisional and other large concentration camps, 14 aviation fields, 3 war prison barracks, 1 recruiting station and 1 spruce production camp. Twenty-six of these camps were surveyed a second time and three of them a third time. The total of individual messes included was 458. Upon recalculation of results in the Washington office, errors were found sufficient to exclude from the final report 31 of these messes, leaving a total of 427 which could be accepted as exhibiting the necessary care and control.

From time to time as the statistical reports were received from the nutritional survey parties averages were compiled for 49, 68, 85, 143, 185, 213, 361 and finally 427 messes. Table 1 shows the average amount of food supplied, wasted and consumed on the man per day

[1] The following officers, as leaders of nutrititional survey parties, contributed to the results here summarized and vouch for the accuracy of the reports: Majors Walter H. Eddy, Frank C. Gephart, Roy G. Hoskins, H. A. Mattill, J. Garfield Riley and John P. Street, all Sanitary Corps, and Majors Don R. Joseph and Caspar W. Miller, Medical Corps; Captains R. J. Anderson, N. R. Blatherwick, J. F. Brewster, Henry R. Cates, Arthur W. Dox, Paul E. Howe, F. B. Kingsbury, M. G. Mastin, Arthur W. Thomas and Drury L. Weatherhead, all Sanitary Corps; and Lieut. T. A. Wayland, Medical Corps.

[2] The Division of Food and Nutrition was established as a separate Division by authority of General Order No. 67, W. D., dated July 15, 1918. After December 2, 1918, this order was tacitly disregarded and the Division became, for convenience in administration, a section of the Division of Sanitation.

TABLE 1

Nutrients and energy consumed in training camps

	FOOD PER MAN PER DAY				CONSUMED DISTR. OF FV	WASTED	PER MAN PER DAY	
	Nutrients	Supplied	Wasted	Consumed				
					per cent	*per cent*		
Averages 49 messes	Protein, grams	139	12	127	14	9	Supplied cost	41.39 cents
	Fat, grams	129	14	115	30	11	Waste cost	3.57 cents
	Carbohydrate, grams	539	40	499	56	7	Total waste	—— pound
	Fuel value, calories	3980	343	3637	100	9	Edible waste	—— pound
Averages 68 messes	Protein, grams	139	11	128	14	8	Supplied cost	40.82 cents
	Fat, grams	127	13	114	29	10	Waste cost	3.51 cents
	Carbohydrate, grams	537	37	500	57	7	Total waste	0.82 pound
	Fuel value, calories	3853	318	3635	100	8	Edible waste	0.45 pound
Averages 85 messes	Protein, grams	139	10	129	14	7	Supplied cost	41.58 cents
	Fat, grams	130	12	118	30	9	Waste cost	3.41 cents
	Carbohydrate, grams	536	35	501	56	7	Total waste	0.82 pound
	Fuel value, calories	3977	297	3680	100	7	Edible waste	0.43 pound
Averages 143 messes	Protein, grams	139	10	129	14	7	Supplied cost	42.55 cents
	Fat, grams	132	12	120	30	9	Waste cost	3.10 cents
	Carbohydrate, grams	534	33	501	56	6	Total waste	0.85 pound
	Fuel value, calories	3987	288	3699	100	7	Edible waste	0.44 pound
Averages 185 messes	Protein, grams	138	9	129	14	7	Supplied cost	42.17 cents
	Fat, grams	130	12	118	30	9	Waste cost	2.99 cents
	Carbohydrate, grams	529	32	497	56	6	Total waste	0.82 pound
	Fuel value, calories	3944	280	3664	100	7	Edible waste	0.42 pound

Averages 213 messes	Protein, grams	138	9	129	14	7	Supplied cost	42.75 cents
	Fat, grams	133	12	121	31	9	Waste cost	3.00 cents
	Carbohydrate, grams	527	31	496	55	6	Total waste	0.83 pound
	Fuel value, calories	3963	276	3687	100	7	Edible waste	0.42 pound
Averages 361 messes	Protein, grams	133	9	124	14	7	Supplied cost	43.35 cents
	Fat, grams	130	15	115	30	12	Waste cost,	3.46 cents
	Carbohydrate, grams	513	32	481	56	6	Total waste	0.86 pound
	Fuel value, calories	3858	308	3550	100	8	Edible waste,	0.40 pound
Averages 427 messes	Protein, grams	131	9	122	14	7	Supplied cost	44.06 cents
	Fat, grams	134	11	123	31	8	Waste cost	3.20 cents
	Carbohydrate, grams	516	31	485	55	6	Total waste	0.80 pound
	Fuel value, calories	3899	266	3633	100	7	Edible waste	0.38 pound

basis; the distribution of fuel value in the food consumed; the percentage of each class of food wasted as well as the percentage of the total fuel value wasted; the cost of the food supplied per man per day; the value of the food wasted; and the average amount of total waste and edible waste, for these numbers of messes.

It will be seen that the average amount of food consumed has not changed materially with the increase in the number of messes. This is significant as showing that the number of messes surveyed is actually representative of the whole army in training. The slight decrease shown with 361 messes is due to the fact that the messes added after the average for 213 messes was made up were surveyed chiefly during the summer months. This will be referred to later in connection with the seasonal variation of food consumption.

The figure for total food consumption (3633 calories) represents an average of averages for the different messes. It was important to determine whether a weighted average would be in agreement with this result. Accordingly the food consumption per man per day for each of the 427 messes was multiplied by the average attendance and the sum of these products divided by the total number of rations daily, viz., 134,879. The average consumption by this method proved to be 3625 calories. From this result it was unnecessary to make weighted averages of food supplied and the food wasted. It was found at Camps Grant, Dodge, Pike and Devens that the average net weight after four to five months of training was 146.5 pounds. The average food consumption from the mess, therefore, may be stated as 24.6 calories per pound of body weight or 52.1 calories per kilogram.

Chart 1 exhibits a distribution curve of the food consumption for the entire 427 messes. It will be observed that this ranges from 2100 to 5700 calories per man per day. The mean of the entire number falls between 3600 and 3700, as already noted. The curve is essentially symmetrical, showing that the man per day consumption within this range is quite a matter of chance. In other words, the curve presents the features of the typical variation curve for biological phenomena. The same sort of curve representing distances of shots from the bull's eye would be obtained by firing at a small target, or again a curve representing the weights of individuals would show a similar distribution. The number of organizations, however, consuming more than 4500 calories or less than 3000 when compared with the total number is practically negligible. For all practical purposes, therefore

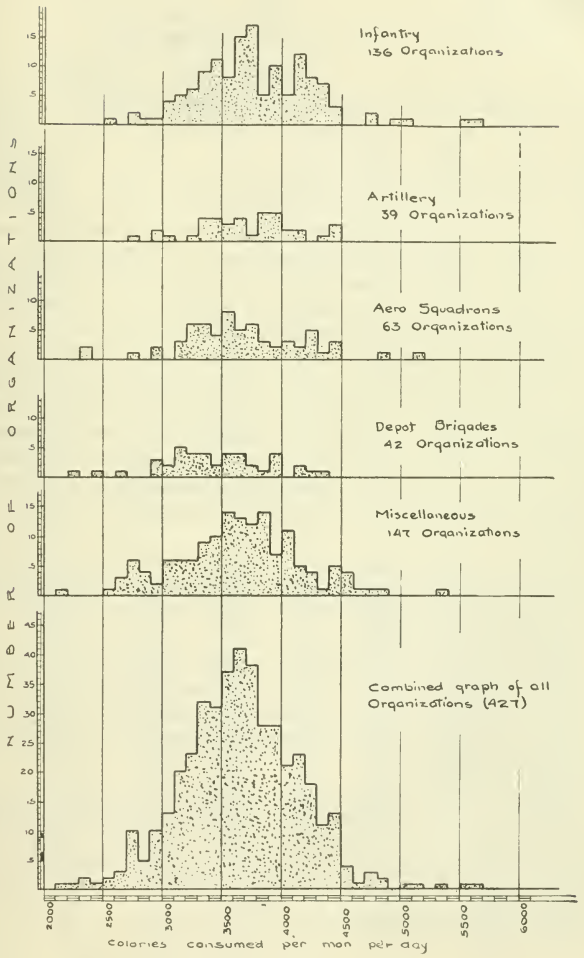

TOTAL CONSUMPTION
Total calories consumed in various types of organizations shown as distribution graphs.
Abscissas represent number of calories consumed; ordinates number of organizations.
Infantry
136 Organizations
Artillery
39 Organizations
Aero Squadrons
63 Organizations
Depot Briqades
42 Organizations
Miscellaneous
147 Organizations
Combined graph of all Organizations (427)
NUMBER OF ORGANIZATIONS
Calories consumed per man per day
2000
2500
3000
3500
4000
4500
5000
5500
6000

Chart 1

it might be stated that the range of food consumption in army messes lies between 3000 and 4500 calories with the average very close to the mean between these two extremes.

FOOD CONSUMPTION BY ORGANIZATIONS

Chart 1 exhibits also an attempt to arrange a distribution curve for the different kinds of organizations, infantry, artillery, aero squadrons, depot brigade organizations, and others grouped together under the heading "miscellaneous." The chart shows that the number of organizations of the separate kinds, with the possible exception of infantry, is not sufficient to give a satisfactory distribution curve. The actual average consumption for the artillery messes, for example, is no more representative than a number chosen at random. To practically the same extent this is true of the depot brigade organizations, including machine gun battalions, field hospital and field ambulance companies, medical detachment messes, etc.

The tendency exhibited in the infantry group, the aero squadron group and the miscellaneous group is, clearly, to duplicate the curve for the entire number of organizations. In other words, the mean, if a sufficient number of messes of the different sorts had been studied, would doubtless lie very close to that obtained from the total number. The same may be said of the range of food consumption. The number of messes outside 3000 on the one hand and 4500 on the other is quite negligible.

From the considerations just rehearsed it is evident that average figures for the food consumption in organizations of different kinds are less valuable than would be the case if some 400 messes of each sort could have been studied.

Chart 2 exhibits the average as actually determined. The only deviation worthy of note is that of the recruit organizations where the average total consumption is some 200 calories less than the grand average of all messes. The conditions responsible for this deviation will be discussed later.

Returning to table 1 it may be seen that the total consumption of nutrients for the average soldier is 122 grams protein, 123 grams fat and 485 grams carbohydrate. Before commenting on this average in comparison with other dietaries, it is necessary to consider *a*, the effect of season; *b*, the effect of the length of time in camp; and *c*, the effect of canteen purchases.

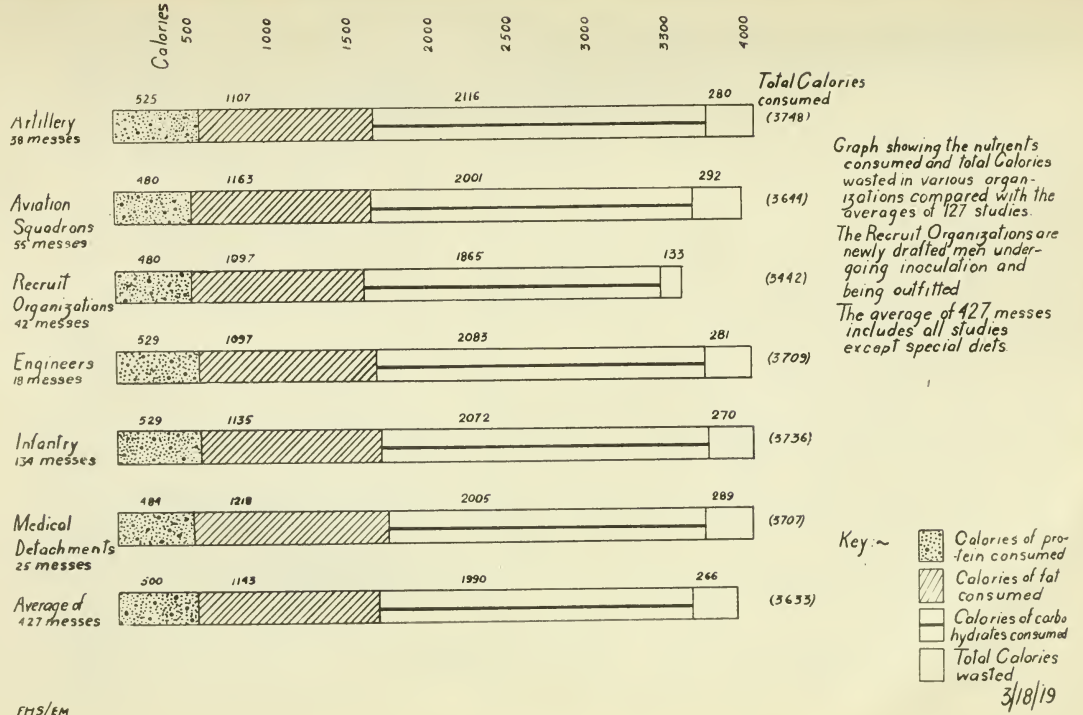

Calories
500
1000
1500
2000
2500
3000
3300
4000
Total Calories consumed
Artillery 38 messes
525
1107
2116
280
(3748)
Aviation Squadrons 55 messes
480
1163
2001
292
(3644)
Recruit Organizations 42 messes
480
1097
1865
133
(3442)
Engineers 18 messes
529
1097
2083
281
(3709)
Infantry 134 messes
529
1135
2072
270
(3736)
Medical Detachments 25 messes
484
1218
2005
289
(3707)
Average of 427 messes
500
1143
1990
266
(3633)
Graph showing the nutrients consumed and total Calories wasted in various organizations compared with the averages of 127 studies.
The Recruit Organizations are newly drafted men undergoing inoculation and being outfitted
The average of 427 messes includes all studies except special diets.
Key:~
Calories of protein consumed
Calories of fat consumed
Calories of carbohydrates consumed
Total Calories wasted
FHS/EM
3/18/19

Chart 2

THE EFFECT OF SEASON ON FOOD CONSUMPTION

It is a familiar fact that one eats more food in winter time than in summer and particularly more of the foods which stimulate heat production most, viz., meats. Few dietary studies have been made in a carefully controlled manner, however, which exhibit with certainty this seasonal variation, and so far as known to the writers, none at all made on bodies of men engaged in the same form of physical exercise. Chart 3 exhibits the average food consumption month by month from October, 1917 to December, 1918 as determined by nutritional surveys in the training camps. The number of messes included in each of these months, however, is significant only from the month of November, 1917 to the month of September, 1918. It will be noted that there is a gradual increase in food consumption from November to March, after which there is a sharp decline which continues throughout the summer. Attempts have been made to correlate these variations with the average temperatures prevailing in the camps at the time of the surveys. This has not been wholly successful, probably for the reason that there is no known factor which will account for the cooling effect of winds upon the human body. In other words, food consumption does not vary inversely with the temperature in any simple relationship, for the effect upon heat loss, and therefore upon heat production indirectly, of a windy day in summer may be as great or even greater than that of a still atmosphere in winter.

Wind velocities have been reported by the weather bureau stations in the vicinity of camps and attempts have been made to find some mathematical expression of the influence of winds upon food consumption as reported by the nutritional survey parties. The inherent error in the methods of a nutritional survey and the variability in food consumption depending probably upon the character of the cooking and service, or upon what may in short be called appetizingness of the food, are too great to exhibit the effects of the individual climatic factors. There remains, however, the seasonal variation shown in the chart which probably represents the combined effect of the various climatic factors. Thus on a priori grounds alone, one would expect a summation effect of low temperature, high wind velocity, high humidity and possibly high barometer. Of these, the variations in temperature doubtless are the most important, after which would probably come wind velocity and humidity, with the factor of barometric pressure occupying a doubtful position.

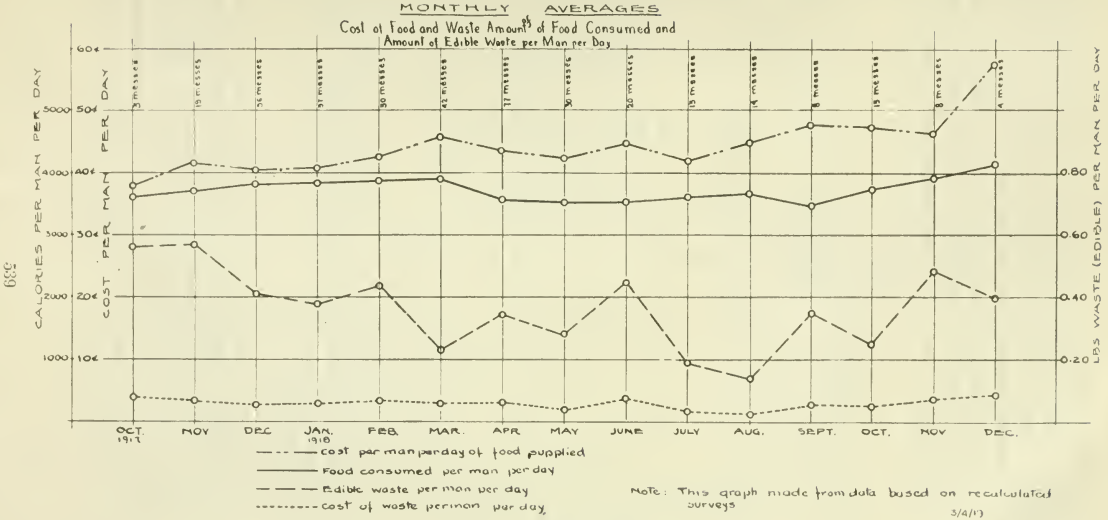

MONTHLY AVERAGES
Cost of Food and Waste Amounts of Food Consumed and Amount of Edible Waste per Man per Day
CALORIES PER MAN PER DAY
COST PER MAN PER DAY
LBS WASTE (EDIBLE) PER MAN PER DAY
5000
4000
3000
2000
1000
60¢
50¢
40¢
30¢
20¢
10¢
0.80
0.60
0.40
0.20
3 messes
19 messes
56 messes
91 messes
30 messes
42 messes
17 messes
36 messes
20 messes
15 messes
14 messes
8 messes
15 messes
8 messes
4 messes
OCT. 1917
NOV
DEC
JAN. 1918
FEB.
MAR.
APR
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV
DEC.
cost per man per day of food supplied
Food consumed per man per day
Edible waste per man per day
cost of waste per man per day
Note: This graph made from data based on recalculated surveys
3/4/19

Chart 3

How much of the seasonal variations in food consumption is due to the greater stimulus of atmospheric conditions to muscular work in winter time than in summer cannot be stated. In other words, the variation shown in chart 3 is certainly not a simple effect of temperature, but is complicated still further by the indirect effect of temperature upon muscular "willingness," if one may coin such a term. Certain it is that one works with greater ease under conditions

TABLE 2

Average by months per man per day

MONTH	NUMBER OF MESSES	COST SUPPLIED	FUEL VALUE CONSUMED	EDIBLE WASTE	EDIBLE WASTE COST
				pounds	
October, 1917	3	37.72	3606	0.56	3.82
November, 1917	19	41.57	3706	0.57	3.31
December, 1917	36	40.47	3819	0.41	2.58
January, 1918	37	40.85	3827	0.38	2.64
February, 1918	30	42.41	3864	0.43	3.24
March, 1918	42	45.61	3894	0.23	2.99
April, 1918	77	43.48	3545	0.34	3.02
May, 1918	30	42.15	3514	0.28	1.85
June, 1918	20	44.86	3517	0.44	3.62
July, 1918	13	41.89	3609	0.19	1.66
August, 1918	14	44.92	3658	0.14	1.14
September, 1918	8	47.64	3487	0.35	2.85
October, 1918	13	47.33	3727	0.23	2.42
November, 1918	7	46.14	3918	0.48	3.41
December, 1918	5	57.56	4145	0.40	4.04
Total	354				
Average		44.30	3722	0.36	2.84

Above information recalculated from nutritional survey reports. All base hospitals, officers', cadets' and prisoners' messes excluded. Cost supplied is average cost of food supplied per man per day.

of low temperature, low humidity and high barometer; the effort of muscular work is less, consequently there are probably many gratuitous muscular motions in the performance of any given piece of work. The tendency to frolic amongst soldiers is highest at such a time. All of these extra motions obviously would of themselves increase the heat production quite apart from the simple cooling effect on the skin, and would correspondingly increase the desire for food.

In conclusion of this subject of seasonable variation it may be noted from chart 3 that the maximum difference between the average for the winter and the summer months is not more than 300 to 400 calories. The average soldier, weighing 146 pounds (after four or five months' training) will consume, therefore, not to exceed 400 calories more daily throughout the winter months than in the summer months. Warmer clothing, heated barracks and warm bedding each play their part in keeping down heat loss.

Chart 3 is based upon table 2. In addition to seasonal variation in food consumption this chart and table exhibit also the variation from month to month in the cost of food supplied per man per day; likewise the variation in the amount of edible waste and its value. One fact only of these additional curves will be mentioned at this place, viz., the influence of the armistice, which is apparent in the averages for food waste for the months of November and December, 1918.

EFFECT OF LENGTH OF TIME IN CAMP

A number of studies of food consumption in recruit organizations have been made. These are in agreement as showing that in the first few weeks of camp life the recruit does not consume as much food from the mess as he does after becoming thoroughly habituated to camp conditions. In illustration may be cited chart 4, which represents a study made upon a recruit company at Camp Pike during the months of July and August, 1918. For three weeks the entire company was kept together, after which the men were separated into Class "A" and Class "B" men. It will be noted that the food consumption beginning at about 2700 calories per man per day in the first week mounts successively to 3000 and 3100 in the second and third weeks respectively and by the end of the fifth week has reached the average for all messes of about 3600.

The first two weeks in camp cover the period of inoculation for typhoid and paratyphoid. This, together with the strangeness of surroundings, particularly of the mess, menus, cookery, style of service, etc., and probably lack of digestive capacity, serves to keep down food consumption and is responsible for the noticeable loss in weight the first week. The weight is promptly recovered, however, by the end of the third week.

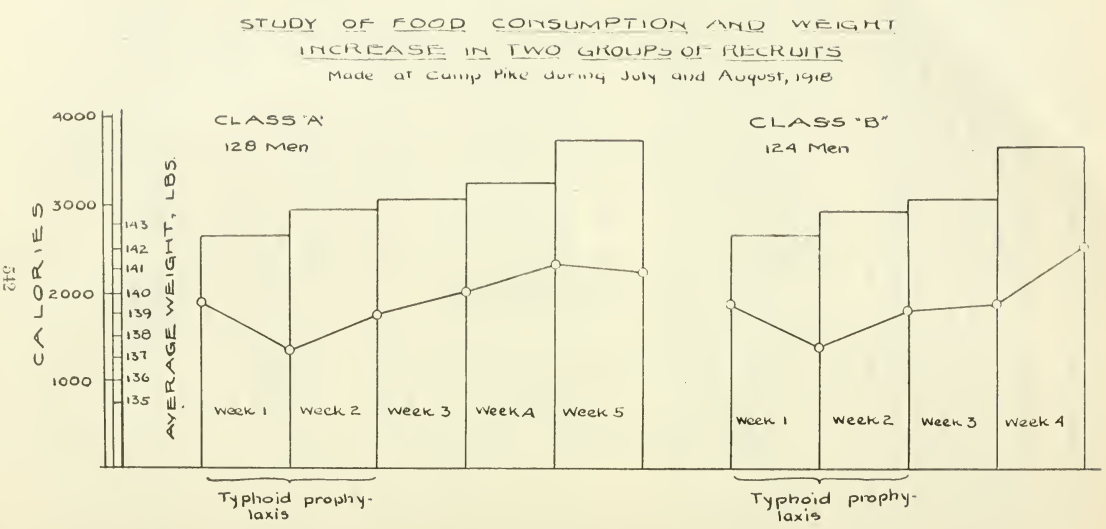

STUDY OF FOOD CONSUMPTION AND WEIGHT
INCREASE IN TWO GROUPS OF RECRUITS
Made at Camp Pike during July and August, 1918
CLASS "A"
128 Men
CLASS "B"
124 Men
CALORIES
4000
3000
2000
1000
AVERAGE WEIGHT, LBS.
143
142
141
140
139
138
137
136
135
Week 1
Week 2
Week 3
Week 4
Week 5
Typhoid prophy-laxis
Typhoid prophy-laxis

Chart 4

FOOD CONSUMPTION AT THE CANTEEN

The canteen, also called the "post exchange" or regimental "exchange," is partly a social institution and partly a supplementary mess. Regulations governing the exchange in the training camps have been on the whole very satisfactory as regards sanitation and the inspection of food materials sold therein.

It is naturally very difficult to keep a record on the man per day basis of food sold to a given organization at the exchange. The difficulty arises from several causes. In the first place, even if the organization has its own canteen, men from other organizations will make purchases there; also a single man may make purchases in the canteen for himself and a number of others. These things taken together make it difficult to pro-rate the total sales for any period. Again, the general experience of field officers has been that the post exchange is patronized much more by the recruits than by soldiers who have become habituated to the mess. Consumption may thus vary from that of the raw recruit, who in many cases will live almost entirely on candy, ice cream and cake purchased at the post exchange, to that of the old regular army man, who limits his canteen purchases largely to tobacco and toilet articles. An average of sales from a single canteen without due account of these causes would be of little value. The average of a large number of such surveys, however, may be accepted as approaching the true average consumption of food from these sources.

Altogether nutritional survey parties have reported 261 studies of food consumption from the exchange. Chart 5 represents for 27 such studies an attempt to learn whether the consumption in the exchange bears any relation to the amount of food consumed from the mess of the same organization. The canteen consumptions are arranged in the order of magnitude beginning with the largest on the left, and, for comparative purposes, the mess consumption is placed beside the canteen consumption. It will be noted that there is no definite relationship between the two. One cannot say, therefore, that soldiers purchase a large amount of food from the canteen because the mess does not supply a sufficiency of total energy. The average consumption for these 27 different canteens is 405 calories per man per day; the average for the total 261 studies is 365 calories per man per day.

POST EXCHANGE STUDY I
CALORIES
4000
3000
2000
1000
0
40¢
30¢
20¢
10¢
Co. M, 1st Inf. McCLELLAN
CRANE & Gen'l Mdse.
Co. K, 163 Inf. McCLELLAN
Area Organizations CHAFFEE FIELD
Det. E, 301 FA DIX
Co. D, 141 M.G. Bn. DEAVENWORTH
1359 Infantry COOK
303 FA DEVENS
102 Area Squad SCOTT FIELD
221 Area Squad SCOTT FIELD
125 Field Art. COOK
Co. C, 103d Eng. DIX
Hdq. Troop COOK
Det. D, 301 FA. DIX
134th Field Hosp. COOK
Hdq. Co. 302 FA. DEVENS
Co. K, 321 Inf. DIX
Co. A, 331 Inf. CUSTER
Co. A, 301 FS Bn. DEVENS
Co. L, 312 Inf. DIX
Co. A, 303 Eng. DIX
Co. A, 356 Inf. DOGGE
Co. M, 312 Inf. DIX
Amphibious Cos. COOK
Mch. Gn. Co. 302 Inf. - DEVENS
Co. K, 321 Inf. CUSTER
Hdq. Co. 351 Inf. DOGGE
In upper graph, solid block represents number of calories consumed in Post Exchange, hollow block represents number of calories consumed in mess.
Cost per 1000 Calories - Post Exchange
Amount spent in exchange per man per day
Cost per 1000 Calories - Mess
Chart 5

On chart 6 is shown a frequency curve representing the caloric consumption per man per day in all the exchanges studied, and a frequency curve representing the cost per thousand calories. The noteworthy feature of this study is that the frequency curve for the

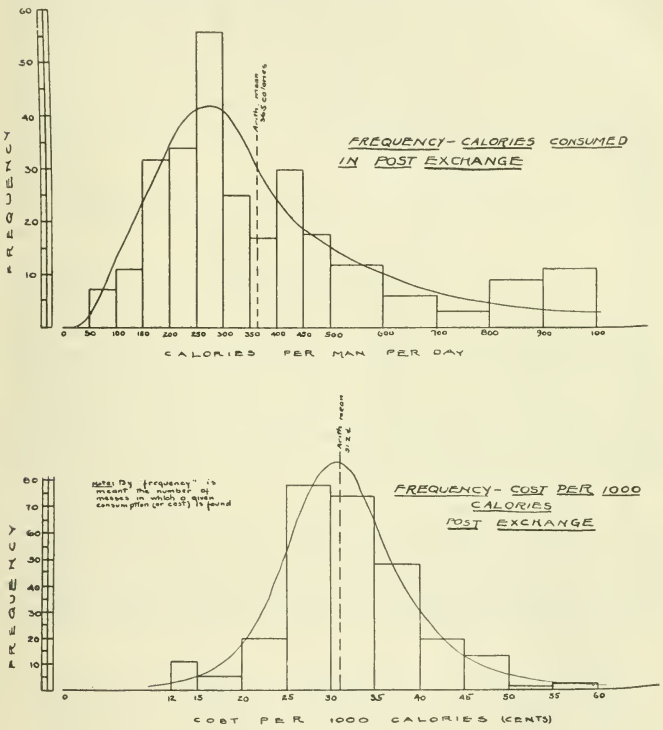


Chart 6

consumption is a skew curve, while the frequency curve of cost per thousand calories is symmetrical about the mean of all the observations. It is evident from the first curve that the amount of food purchased by the average soldier is not a mere matter of chance. To some extent the form of this curve is explained by the value in calories

of the 5, 10, 15, 20 and 25 cent purchases; to some extent also probably by the fact that in many exchanges restaurant sales were made, while in others what might be called confections only were sold.

The average consumption in the canteen does not, however, represent the average consumption outside the mess, for in many camps adjacent to cities, the soldiers had passes after the evening meal at the mess, and much supplementary nourishment was purchased in the eating houses in town. Several attempts were made by the nutritional survey parties to estimate the quantity of food consumed in this way. In some organizations men were required by order of the company commander to report what had been eaten outside the camp. In some instances there is every reason to believe that these reports were truthfully made but the terms in which they were reported, as, for example, "two pieces of candy," "a piece of pie," "ham and eggs," etc., were too indefinite for computation.

If a camp distant from any town is selected and a survey of all the camp exchanges is made, and the sales pro-rated for the entire camp population, a fairly accurate estimate of the food consumption outside the mess may be obtained. At Camp McClellan it was found possible to undertake just such a study. This camp is situated at a sufficient distance from Anniston, Alabama, to bring the consumption and purchase of food outside of camp to a low value. Also less outside food was received at this camp at the time of study than in most of the other large camps.

The study was made by Nutritional Survey Party No. 8 under the leadership of Captain H. A Mattill in March, 1918, several months before the departure of the 29th Division for overseas. The summary of this canteen survey shows that the expenditure per man per day at Camp McClellan during the week of the study was 21.9 cents, distributed as follows:

Candy	0.143 pound	6.17 cents	28 per cent
Drinks	0.921 bottle	5.05 cents	23 per cent
Cakes, cookies	0.214 pound	6.58 cents	30 per cent
Fruit	0.085 pound	1.06 cents	5 per cent
Ice cream	0.012 gallon	2.00 cents	9 per cent
Sandwiches, etc	0.044 pound	1.06 cents	5 per cent
		21.92 cents	100 per cent

The following notes quoted from the report summarize the observations of the party making the survey on the canteen situation at this camp.

The soldier buys food at the exchange probably for three reasons: (1) as a pastime and for sociability; (2) to secure sweets that take the place of alcohol, in the case of those who have been accustomed to its use; (3) because the desire for sugar, physiological or habitual, is not met by the army ration. General observation gives most weight to the last mentioned cause.

For his candy the soldier pays at the rate of 44 cents a pound; for his pastry 31 cents per pound; for his ice cream at the rate of $1.60 per gallon; and for sugar in the various drinks over $3 per pound.

Of the total expenditure 30 per cent is for cakes and box crackers, 29 per cent is for candy, 22 per cent for soft drinks, and 9 per cent for ice cream, a total of 90 per cent for sugar and starch preparations, leaving 10 per cent for fruit and miscellaneous items.

Using an average analysis for the various classes there is furnished (approximately) by

	Calories
Candy	305
Soft drinks (coca cola, etc.)	72
Fruit, 0.085 pounds	18
Cake, 0.21 pounds	124
Ice cream, 0.012 gallons	37
Miscellaneous (sandwich), 0.044 pounds	107
A total of	633

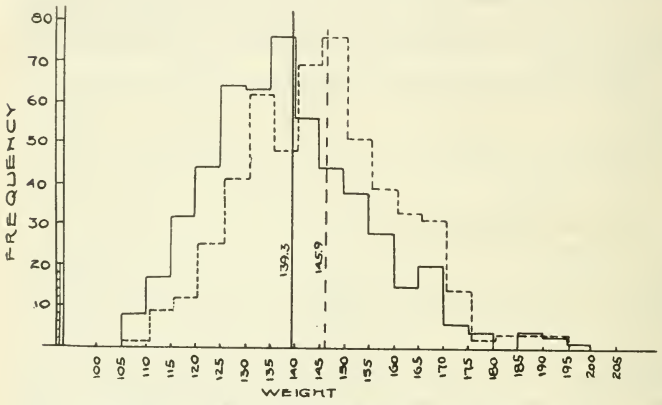
at a cost of 33 cents per 1000, which is about three times as great as the cost of a similar amount of food in the army ration.

The average net consumption in the four messes surveyed at Camp McClellan in the early part of March, 1918, was 3827 calories per man per day. Adding to this the 633 calories which represent the average consumption from all the canteens, gives the surprisingly high total of 4460 calories.

The average total consumption in the camps is found by adding to the average for all messes studied (427) the average for all canteens (261). Thus 3633 plus 365 equals 3998; the result in round numbers is 4000 calories per capita per day. Lusk[3] has estimated that it requires 4100 calories in order to maintain body weight and to supply the necessary energy for a seasoned soldier weighing 70 kilograms (154 pounds) to do a forced march of 30 miles in 10 hours carrying a pack weighing 44 pounds. Of these 1767 calories are for basal metabolism, that is, the mere maintenance of the body temperature at muscular rest. Standing alone requires 118 calories, walking 30 miles in 10 hours without pack requires 1705 calories, with pack 484 calories additional, making a total of 4704 calories for maintenance metabolism and the muscular effort.

[3] From an unpublished lecture on the *Dynamics of Nutrition*, written for the Division of Food and Nutrition as a part of the training of nutrition officers.

We have already seen that the average weight of the soldier of the first draft after four or five months' training in the camp is 146 pounds. For this weight the theoretical metabolism according to Lusk is as follows: Basal metabolism 1695, standing 113, walking 30 miles in 10 hours 1606, carrying equipment 44 pounds 484, total 3898. It may be seen therefore that the average soldier receiving 4000 calories



STUDY OF INCREASE IN WEIGHT OF 523 MEN IN THE 303 F.A., CAMP DEVENS

The weights were divided into groups differing by 5 pounds, and the number of individuals in each group counted. The group between 110-115 includes individuals weighing from 111 to 115, inclusive, the group between 115 and 120, individuals weighing from 116 to 120, inclusive, etc. In the above graph, the abscissas give the weights, the ordinates, the number of individuals in each weight group. The solid line shows the distribution at the time of enlistment, the dotted line, the distribution 5 months later. Averages are shown in a similar manner.

Chart 7

daily has energy supplied in sufficient quantity to do this maximum amount of work every 24 hours. If the work is not done, it is obvious that the soldier will take on weight and the universal testimony throughout the camps is that the recruit has gained anywhere from 3 to 9 pounds in the course of four or five months' training. At Camp Devens 523 men of the 303 Field Artillery made an average gain, which was nearly uniform for men of all sizes, of 6.6 pounds (chart 7).

It is certain, therefore, that the average food consumption of 4000 calories is sufficient for the training period. Making an average allowance of 7 per cent for waste (see table 1), a total food supply of 4280 calories would amply supply all needs, including the canteen. This, it should be remembered, covers the needs for men of all sizes and under all conditions of muscular work and climate.

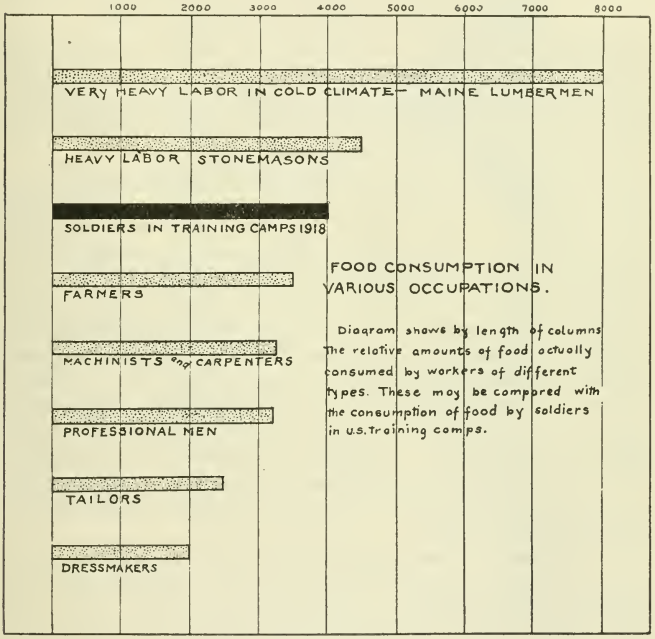


Chart 8

FOOD CONSUMPTION IN THE ARMY COMPARED WITH OTHER OCCUPATIONS

In chart 8 is shown diagrammatically the relation of average food consumption in a training camp in comparison with lumbermen, stone masons, farmers, machinists, professional men, tailors and dressmakers.

This diagram is based upon statistical observations made by well-known authorities and summarized in Lusk's *Science of Nutrition* (Second Edition, p. 348). The physical work of the soldier may be characterized as somewhat heavier than that of the American farmer, and not quite so heavy as that of a stone mason, who works steadily at lifting and hammering his materials for a period of eight hours.

DISTRIBUTION OF NUTRIENTS IN THE ARMY DIET

By reference to table 1, it may be seen that the average distribution of calories derived from protein, fat and carbohydrate consumed in the mess is 14 per cent, 31 per cent and 55 per cent respectively. The distribution for the different number of messes averaged in that table is seen to vary but little from this standard.

Chart 9 shows the range of distribution of calories to the several organic constituents of the diet. The percentage of protein calories varies from 11 to 17, fat calories from 20 to 40 and carbohydrate calories from 45 to 65 calories. Throughout these ranges the distributions are fairly symmetrical about the means. If they were perfectly symmetrical, the means would obviously coincide with the averages. Stated somewhat differently, it is obvious from the study of this chart that any given recruit cast into a camp and passing through the mill mechanically, as a ball on a roulette board, would stand an exactly equal chance of obtaining a diet which would contain 25 per cent of fat calories as one containing 37 per cent, that is, an equal distance from the mean. Similarly with reference to carbohydrate, he would stand an exactly equal chance of obtaining a diet containing 50 per cent as one containing 60 per cent of carbohydrate calories. So also with the protein.

When the canteen consumption is taken into account, the distribution is slightly changed. Examination of the report from Camp McClellan already referred to shows that the average canteen purchases contain 8 per cent protein, 16 per cent fat and 76 per cent carbohydrate. When the 365 calories are distributed according to these percentages and added to the consumption obtained in the mess, it is found that the distribution of protein, fat and carbohydrate for the total consumption is 13 per cent, 31 per cent and 56 per cent respectively. The predominance of carbohydrate in the canteen consumption serves to lower slightly the percentage of protein, to raise slightly the percentage of carbohydrate and to leave unaffected the percentage of fat.

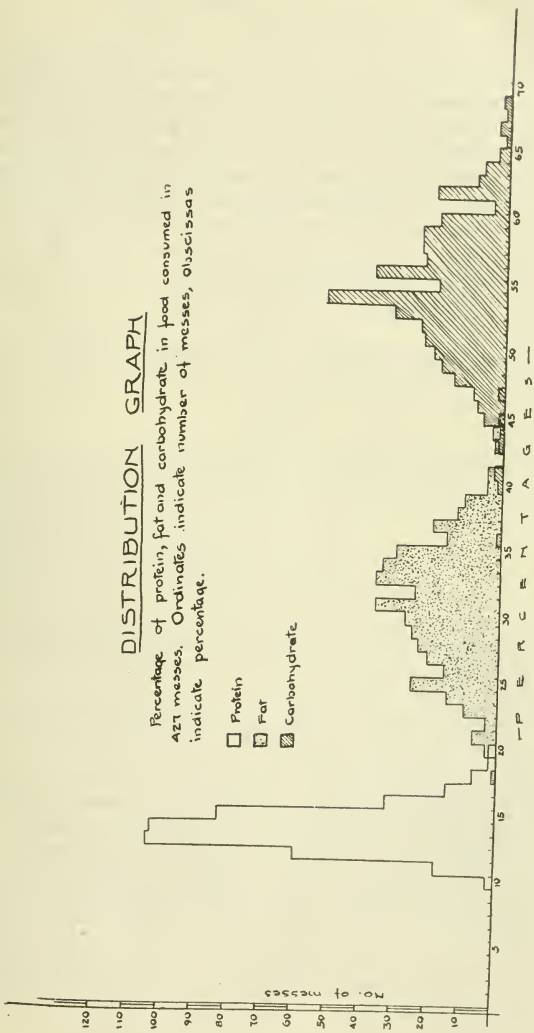

DISTRIBUTION GRAPH
Percentage of protein, fat and carbohydrate in food consumed in 421 messes. Ordinates indicate number of messes, abscissas indicate percentage.
Protein
Fat
Carbohydrate
No. of messes
PERCENTAGES

Chart 9

COMPARISON WITH OTHER DIETARIES

It is a matter of interest to compare this distribution of nutrients in the army diet with the distribution in certain standard diets well known in the literature of the science of nutrition. It is somewhat surprising to find that the percentage of protein is considerably lower in the diet of the soldier than in that of Voit's old ration for hard labor which has always been regarded as a moderate one from the standpoint of protein. The impression that the consumption of meat in the army training camp is excessively high is not borne out by this comparison. Protein is lower even than in the diet of the Finnish peasant as reported by Sundström in 1908 and in that of the American farmer at hard labor as estimated by Atwater some twenty years ago.

The total energy consumption of the American soldier in training approaches the Atwater standard for the American farmer at hard labor.

TABLE 3

Comparison of the soldier's diet in United States army training camps with other standard diets for hard muscular work

	GRAMS			CALORIES			
	Protein	Fat	Carbohydrates	Total	Percentages		
					Protein	Fat	Carbohydrates
Sundström (Finnish peasant)....	136	83	523	3474	16.1	22.2	61.7
Voit (hard labor).....	145	100	500	3574	16.6	26.0	57.4
United States soldier (hard training).....	129	136	545	3998	13.2	31.6	55.8
Atwater (farmer, hard labor)....	150	125	580	4150	14.8	28.0	57.2

Likewise in comparison with the rations prescribed for the training period of the other allied armies, as may be seen from the following table, the percentage of protein in the United States Army is lower than that of the British, Canadian, French or Italian Armies. The percentage of fat consumed is lower than the amount prescribed for the British and Canadian Armies and higher than that of the French and Italian Armies.

The total energy consumption, however, shows how difficult it would be for the American soldier to live on the training ration of either of the other armies.

PERCENTAGE OF CALORIES SUPPLIED BY CHIEF COMPONENTS OF THE RATION

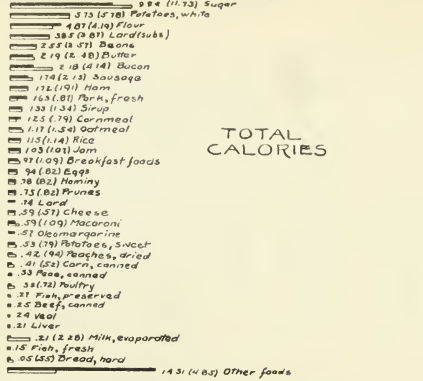
In chart 10 is shown the percentage of total calories supplied by each of the more important constituents of the army diet; likewise the percentage of protein calories, fat calories and carbohydrate calories furnished by each of the more important constituents. This study is based upon the first 87 messes and the first 213 messes surveyed. The importance of bread, beef, potatoes and sugar stands out prominently in a comparison of this sort. The chief difference observed between the

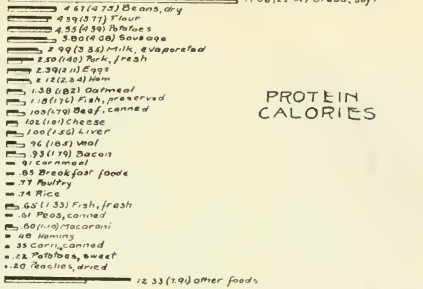
TABLE 4

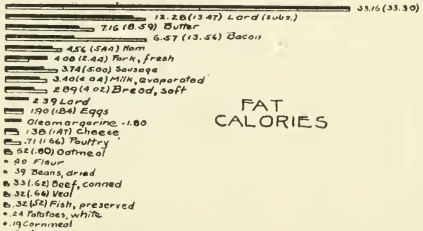
Training rations of the different allied armies

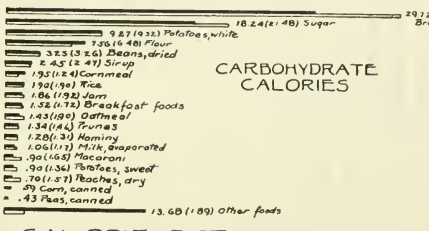
	GRAMS			CALORIES			
					Percentages		
	Protein	Fat	Carbo-hy-drates	Total	Protein	Fat	Carbo-dy-drates
British Home ration, May, 1918..	124	136	419	3483	14.6	36.4	49.0
Canadian, July, 1918.....	107	118	344	2946	14.9	37.2	47.9
French, Normal, March 29, 1918	138	98	467	3604	15.7	25.3	59.0
Italian territorial, February 1, 1917.....	127	38	469	2797	18.6	12.6	68.8
United States Garrison ration A. R. 1221.....	147	174	643	4859	12.5	33.3	54.2
Consumed in United States training camps 427 messes and canteen purchases.....	129	136	545	3998	13.0	31.0	56.0

two studies lies in the fact that there is in the second a considerable increase in the percentage of total energy as well as the percentage of each of the three nutrients derived from foods other than the principal staples included on the food list. In other words, from the time when the average for 87 messes was compiled to the time when the average for 213 messes was compiled, a period of two or three months, the number of different articles of food used in significant amount increased considerably. This reflects greater freedom and more intelligence on the part of the mess sergeant in purchasing food from outside sources.









CALORIE DISTRIBUTION

Showing relative importance of the most frequently used foods as sources of energy, protein, fats, and carbohydrates. Figures show amount of total energy, protein, fat, and carbohydrate furnished by individual foods and are expressed as percentages of total calories, total protein calories, total fat calories, and total carbohydrate calories respectively. The first figure, and the solid block represent in the case of each food the percentage as determined from the average consumption in 213 army messes. The figure in parentheses and the hollow block show the percentage as determined from the average consumption in 51 of the 213 messes.

The 51 messes were surveyed between October 1, 1917 and February 15, 1918

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Chart 10

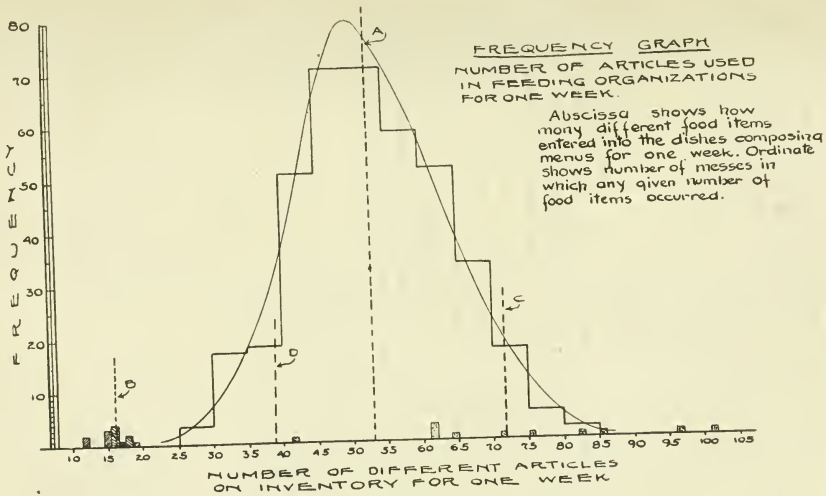
FREQUENCY GRAPH
NUMBER OF ARTICLES USED IN FEEDING ORGANIZATIONS FOR ONE WEEK.
Abscissa shows how many different food items entered into the dishes composing menus for one week. Ordinate shows number of messes in which any given number of food items occurred.
FREQUENCY
NUMBER OF DIFFERENT ARTICLES ON INVENTORY FOR ONE WEEK
A
B
C
D
EXPLANATION OF CROSS-HATCHING
- Data from one-week surveys of 390 messes in barracks. The arithmetical mean of this group is 52.8 (shown at "A").
- Data from 14 messes in organizations on a 3-day hike. The arithmetical mean of this group is 15.9 (shown at "B").
- Data from 11 officers messes. Arithmetical mean of this group is 12.8 (shown at "C").
At "D" is shown the number of articles entering into a week's menus in the average family. This number is 39.

Chart 11

VARIETY

One of the most important factors in the diet for the maintenance of morale is variety. In studying this question one is met at the outset by the difficulty of assigning a value to such a factor in the mess. The most obvious way in which to measure variety is to count the number of different articles of food on the inventory for a given period and consider this an approximate measure of this particular dietary factor. Chart 11 represents graphically the results of a study of this sort made on 390 army messes. In making the estimate of articles on the inventory everything purchased by the mess sergeant for preparation of food for the week of the survey was counted. This included spices, tea, coffee, etc. The average number of articles used per mess per week was about 55. For organizations on the march, where difficulties of preparation and transportation of food necessarily demanded a simpler menu, the number of articles used per mess for a three-day period was 15.9. For an average family[4] 39 different articles enter into the weekly food inventory. At first glance, therefore, one would say that there was less monotony in army diet than in the diet of the average household. However, it is not entirely certain that the count of household articles was made on exactly the same basis as the army count. All that may safely be said is that army feeding, so far at least as variety is concerned, compares very favorably with household feeding.

[4] From dietary studies made by the Bureau of Markets of the United States Department of Agriculture in the autumn of 1917.

VARIATIONS IN STRENGTH AND IN THE CONSUMPTION OF FOOD BY RECRUITS AND SEASONED TROOPS

PAUL E. HOWE, C. C. MASON AND SANFORD C. DINSMORE

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, United States Army

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Nutritional Survey Party no. 2[1] of the Division of Food and Nutrition was asked to take up the question of the use of the Martin (1) strength test as a practical means of classifying and assigning men for particular duties in the army and as a means of studying problems relating to food consumption. Preliminary tests were made at Camp Fremont, California with the aid of Dr. E. G. Martin. At our next station, Camp Lewis, Washington, the tests and surveys detailed in this paper were carried out to determine whether or not there is a relation between the food consumption and variation in strength of recruits. Due to certain difficulties, the original object of the work was not attained. The results are, however, of considerable value with regard to *a*, the variation in strength and weight of recruits during the first weeks in camp, and *b*, the variation in food consumption of the same group of men from week to week in the course of three and four weeks and of different groups of men at the same time and under almost identical conditions. Such data are of use in connection with the evaluation of studies of groups of people.[2]

Determinations were made of the variations in strength and of weight of approximately forty men from each of eight companies of recruits; Company 6 of the Depot Brigade was quarantined during part of the time and the results are not included in the strength tests. The studies extended for three consecutive weeks; a smaller number of men were continued for the fourth week. Weights and strength were determined

[1] Capt. Paul E. Howe, Lieut. C. C. Mason, Lieut. S. C. Dinsmore and Lieut. B. H. Harrison.

[2] The question of the number or length of studies of this nature which must be made to obtain a correct estimate of the *average* food consumption has been treated by Lt. Col. J. R. Murlin (2).

at seven-day intervals. The food consumption of the messes from which the men were selected for strength tests was determined in weekly periods. The men were inoculated and vaccinated during the first two weeks of study.

The recruits arrived at the camp between April 25 and April 28, 1918. They passed through the usual routine of physical examination, and were inoculated with vaccine against typhoid and small pox. The men lived in barracks and received their preliminary training during the time of observation. During the first two weeks they were in quarantine. The character of the work varied from day to day but was nearly uniform for all companies on the same day. No work was done on the day following inoculation. The mean temperature was practically constant throughout the work. Two of the most influential factors affecting the quantity and quality of food consumed in an army mess, the menu prepared by the mess sergeant and the character of the cooking, were at least in part minimized through the use of the same menu for each of two groups of four companies and by the presence of trained cooks belonging to the School for Bakers and Cooks, who were permanent members of the Depot Brigade. The cooks were under the constant supervision of a sergeant cook-instructor detailed to us to supervise the cooking. We feel therefore that the factors affecting food consumption aside from the inclination of the men to eat what was put before them were eliminated as much as can be expected.

The men of Companies I, K, L and M of the 364th Infantry represented seasoned troops; they had been in the organization since October 1917 and this work was conducted in April and May, 1918. These men had received the maximum of training given in this country. All the companies were in the same battalion and did practically the same work. No attempt was made to control the menus in these organizations since all were well established.

Strength tests. Data with regard to the strength tests are given in tables 1, 2 and 3, pages, 559, 560 and 561. Ten tests were made on each man (right and left pectorals, right and left wrist flexors, right and left forearm flexors, right and left thigh abductors, and right and left thigh adductors). The results according to Martin represented 17.7 per cent of a man's strength. The choice of the men was made directly from the rolls without knowledge of individual sizes or weights. Men with venereal diseases were excluded.

The following facts will be found from an examination of the data:

TABLE 1

Average variations in strength of individual companies, expressed as pounds pulled per man, and in weight, 166th Depot Brigade

PERIOD	NUMBER OF MEN	VACCINATION DAYS BEFORE TEST	WEIGHT	STRENGTH				TOTAL
				Pectoral	Wrist flexors	Forearm flexors	Thigh abductors adductors	
				Company 5				
			pounds					
1	35	3	156	135	61	97	67	844
2	35	3	156	125	42	91	59	750
3	35	3	158	125	57	95	59	797
				Company 7				
1	34	6	158	118	36	90	52	694
2	34	6	151	115	42	95	55	724
3	34	6	157	133	47	96	54	790
				Company 8				
1	33	5	158	126	49	95	65	824
2	33	5	157	111	42	86	56	723
3	33	5	157	114	50	92	61	773
				Company 9				
1	34	6	150	114	38	85	55	695
2	34	6	149	110	42	86	53	689
3	34	6	151	118	45	91	57	734
				Company 10				
1	35	5	151	118	42	88	57	726
2	35	5	152	115	42	87	54	702
3	35	5	153	125	46	93	62	775
				Company 11				
1	35	4	151	121	52	94	56	798
2	35	4	151	114	42	85	53	690
3	35	4	151	109	42	86	49	668

TABLE 1—*Continued*

PERIOD	NUMBER OF MEN	VACCINATION DAYS BEFORE TEST	WEIGHT	STRENGTH				TOTAL
				Pectoral	Wrist flexors	Forearm flexors	Thigh abductors adductors	
Company 12								
			pounds					
1	26	2	154	120	62	92	54	761
2	26	2	154	116	42	83	55	698
3	26	2	156	110	55	85	51	700
Company K 364th Infantry								
1	24	—	152	132	64	94	52	789
2	24	—	152	123	57	85	49	724
3	24	—	153	121	57	92	47	726

Note: Average values for muscle groups represent pull for a single group of muscles, e.g., right pectoral, left thigh abduetor.

TABLE 2

Average values for total strength as pounds pulled per man for men tested on four successive periods

ORGANIZATION	NUMBER OF MEN	STRENGTH				WEIGHT			
		1	2	3	4	1	2	3	4
Company 5	32	846	762	802	800	156	156	156	157
Company 7	14	711	730	793	738	157	154	156	157
Company 8	24	810	706	753	804	152	152	151	152
Company 9	17	684	670	720	705	147	147	149	155
Company 10	13	701	678	752	732	145	145	146	147
Company 11	9	709	635	632	701	151	150	152	152
Company 12	24	770	704	708	760	154	154	156	157
Company K, 364th Infantry	24	789	724	726		152	152	153	

Values for strength represent the sum of the actual weights pulled or 17.7 per cent of total strength according to Martin.

a. Every company, except Company 10, showed an initial loss in weight averaging 1 pound. By the end of the third week there was an average gain of one pound over the original weight.[3]

[3] Similar losses and gains, but of greater magnitude, were observed by Lieut. W. A. Perlzweig in his studies of recruits.

TABLE 3

Average results obtained from all strength tests on the 166th Depot Brigade, expressed as pounds pulled per man

PERIOD	NUMBER OF MEN	WEIGHT	STRENGTH				TOTAL
			Pectoral	Wrist flexors	Forearm flexors	Thigh abductors adductors	
A. All men							
1	232	153	122	48	92	57	760
2	232	152	115	42	88	55	708
3	232	154	119	48	91	57	746
B. Men tested four times							
1	133	152	123	51	92	59	768
2	133	152	116	41	88	55	710
3	133	153	118	51	91	57	748
4	133	155	125	47	92	58	761

b. Data with regard to strength show an initial loss with recovery practically completed by the third week and then strength held during the fourth week.

The men were at low ebb physically at about the end of the second week. The second injection had been given, they were not fully accustomed to the new life, they were all tired from the unusual drill and most of them were more or less homesick. We noted in making the tests that the second one aroused little interest.

By the time of the third test most of the men had returned nearly to normal and their mental attitude had improved. The results are reflected in the strength tests. The data obtained from a smaller number of men in the fourth week bear out the fact that recovery was practically complete by the third week.

An analysis of the percentage losses and gains in strength of the various muscle groups tested with relation to the total weight pulled on the first test shows the following average results:

PERIOD	PECTORALS	WRIST FLEXORS	FOREARM FLEXORS	THIGH ABDUCTORS ADDUCTORS
2	−0.6	−0.8	−0.5	−0.4
3	+0.4	+0.8	+0.5	+0.3

TABLE 4

Average food consumption per man per day during three successive weeks, 166th Depot Brigade

	PROTEINS	FAT	CARBOHYDRATES	CALORIES
Company 5, 4/30–5/ 6/18	126	124	546	3912
5/ 7–5/13/18	110	121	457	3448
5/14–5/20/18	126	121	543	3863
Average	121	122	515	3741
Company 6, 4/30–5/ 6/18	104	117	421	3241
5/ 7–5/13/18	110	127	466	3538
5/14–5/20/18	102	113	407	3145
Average	105	119	431	3308
Company 7, 4/30–5/ 6/18	89	111	375	2931
5/ 7–5/13/18	100	130	349	3046
5/14–5/20/18	127	152	423	3669
Average	105	131	382	3215
Company 8, 4/30–5/ 6/18	155	172	495	4264
5/ 7–5/13/18	82	91	335	2557
5/14–5/20/18	134	133	598	4244
Average	124	132	476	3688
Company 9, 4/30–5/ 6/18	90	82	420	2852
5/ 7–5/13/18	110	116	482	3505
5/14–5/20/18	147	141	533	4096
Average	116	113	478	3484
Company 10, 4/30–5/ 6/18	85	75	517	3162
5/ 7–5/13/18	119	121	395	3231
5/14–5/20/18	115	112	525	3661
Average	106	103	479	3351
Company 11, 4/30–5/ 6/18	108	72	465	3022
5/ 7–5/13/18	115	93	538	3542
5/14–5/20/18	97	92	397	2879
Average	107	86	467	3148
Company 12, 4/30–5/ 6/18	129	108	627	4105
5/ 7–5/13/18	106	103	421	3119
5/14–5/20/18	118	106	518	3591
Average	118	106	522	3605
Average of all	112	114	469	3483

The percentage losses in the second week are practically made up in the third week. The wrists show the greatest loss; the men had just received their rifles at this time and this may be a factor.

There does not appear to be a direct relation between loss in strength and the proximity to the time of inoculation.

TABLE 5

Average food consumption per man per day during four successive weeks, 364th Infantry, seasoned troops

	PROTEINS	FAT	CARBOHYDRATES	CALORIES
Company I, 4/23–4/29/18	133	150	555	4212
4/30–5/6/18	139	153	522	4127
5/7–5/13/18	125	117	604	4074
5/14–5/20/18	113	98	526	3527
Average	128	130	552	3985
Company K, 4/23–4/29/18	100	102	535	3548
4/30–5/6/18	142	151	485	3972
5/7–5/13/18	112	119	603	4034
5/14–5/20/18	132	106	543	3752
Average	123	120	542	3827
Company L, 4/23–4/29/18	125	142	475	3778
4/30–5/6/18	156	136	560	4200
5/7–5/13/18	127	120	483	3616
5/14–5/20/18	116	117	429	3322
Average	131	124	487	3729
Company M, 4/23–4/29/18	149	133	510	3936
4/30–5/6/18	113	109	427	3230
5/7–5/13/18	123	124	448	3497
5/14–5/20/18	116	110	459	3385
Average	125	119	461	3512
Average of all	126	124	510	3764

The results on Company K, 364th Infantry, are not of value for comparison with the Depot Brigade for the training schedule prevented our getting the men in approximately the same condition for the different tests. In the second and third tests the men were fatigued as the result of active and heavy work. The values obtained indicate the effect of fatigue.

TABLE 6

Average food consumption of different companies using the same menu at the same time. 166th Depot Brigade

DATE		PROTEINS	FAT	CARBO-HYDRATES	CALORIES
4/30–5/7/18	Company 5	126.0	124.0	546.0	3912
	Company 6	104.0	117.0	421.0	3241
	Company 7	89.0	111.0	375.0	2931
	Company 8	155.0	172.0	495.0	4264
	Average	118.5	131.0	457.5	3587
	Company 9	90.0	82.0	420.0	2852
	Company 10	85.0	75.0	517.0	3162
	Company 11	108.0	72.0	465.0	3022
	Company 12	129.0	108.0	627.0	4105
	Average	103.0	84.0	507.0	3285
5/7–5/13/18	Company 5	110.0	121.0	457.0	3448
	Company 6	110.0	127.0	466.0	3538
	Company 7	100.0	130.0	349.0	3046
	Company 8	82.0	91.0	335.0	2557
	Average	100.5	117.0	402.0	3147
	Company 9	110.0	116.0	482.0	3505
	Company 10	119.0	121.0	395.0	3231
	Company 11	115.0	93.0	538.0	3542
	Company 12	106.0	103.0	421.0	3119
	Average	110.0	108.0	459.0	3349
5/14–5/20/18	Company 5	126.0	121.0	543.0	3863
	Company 6	102.0	113.0	407.0	3145
	Company 7	127.0	152.0	423.0	3669
	Company 8	134.0	133.0	598.0	4244
	Average	122.0	129.7	492.7	3730
	Company 9	147.0	141.0	533.0	4096
	Company 10	115.0	112.0	525.0	3661
	Company 11	97.0	92.0	397.0	2879
	Company 12	118.0	106.0	518.0	3591
	Average	119.0	112.7	493.0	3557
	Average of all studies	112.2	114.0	469.0	3483

Food consumption. The data relating to the food consumption are included in tables 4, 5, 6 and 7, pages 562, 563, 564 and 565. Tables 4 and 5 give the data from the same companies in each of successive periods. Tables 6 gives the results obtained for the same groups of companies when using the same menu. Table 7 gives the average values for each company during the periods studied.

TABLE 7

Company average food consumption per man per day for 3 one-week periods, 166th Depot Brigade, and 4 one-week periods, 364th Infantry

	PROTEINS	FAT	CARBO-HYDRATES	CALORIES
166th Depot Brigade. 3 one-week periods, 24 studies				
Company 5	121	122	515	3741
Company 6	105	119	431	3308
Company 7	105	131	382	3215
Company 8	124	132	476	3688
Company 9	116	113	478	3484
Company 10	106	103	479	3351
Company 11	107	86	467	3148
Company 12	118	106	522	3605
Average Depot Brigade	112	114	469	3483
364th Infantry. 4 one-week periods, 16 studies				
Company I	128	130	552	3985
Company K	123	120	542	3827
Company L	131	124	487	3729
Company M	125	119	461	3512
Average, Infantry Companies	126	124	510	3764
Grand average, 40 studies	118	118	485	3573

In considering the data it is to be remembered that each weekly average represents the average of the food consumed by approximately 220 men or equivalent to 1540 men for one day. The results are particularly interesting in that they give some indication of the variable food consumption which may exist under the very similar conditions. Even when using essentially the same kinds of food in the form of similar dishes there is a variation in the food consumption of different groups of men.

That the results are characteristic of recruits is not borne out by the results obtained in the 364th Infantry, for here we find just as great variations as with the recruits. The average food consumption is somewhat higher, a fact which is substantiated by other data obtained by the Division of Food and Nutrition.

BIBLIOGRAPHY

(1) Martin: Proc. Amer. Physiol. Soc., This Journal, 1918, xlv, 538.
Martin and Lovett: Journ. Amer. Med. Assoc., 1915, lxv, 1512.
(2) Murlin: This Journal, 1919, xlix, 531.

NOTE ON THE ACID-BASE BALANCE OF ARMY RATIONS

N. R. BLATHERWICK

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, United States Army

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Interest in the acid-base balance of dietaries has increased greatly in recent years. Sherman and his collaborators gave us the basis for work along this line when they made more accurate determinations than had hitherto been available of the ash constituents of the common foods. It will be recalled that meats and cereals show a predominance of acid-forming elements, while on the other hand fruits and vegetables have an excess of base-forming elements. Working under the direction of Professor Mendel, (1) it was my privilege to demonstrate the specific influence of the ash of foods upon the composition of the urine. This study showed that acid-forming foods lead to the formation of more acid urines and base-forming foods cause the excretion of less acid or alkaline urines. Certain exceptions were found, namely, plums, prunes and cranberries, which although yielding a basic ash, nevertheless increase the acid excretion due to the presence of benzoic acid, excreted as hippuric acid.

Although the question, whether or not an acid-forming diet eaten for some period of time is productive of undesirable results is debatable, probably the consensus of opinion is in favor of diets in which the acid-forming and base-forming elements are approximately balanced. Accepting this, it then becomes a matter of importance to determine whether soldiers' rations are well balanced in this sense.

Our soldiers in the training camps are subsisted on what is known as the garrison ration, the chief components of which are fresh beef, flour and potatoes. This ration, when completely eaten, is abundant in protein and energy content and shows an excess of acid-forming elements corresponding to about 44 cc. N/1 solution per day. Owing to the ration savings privilege, the mess sergeant is able to decrease the amounts of the various components consumed and receives credit for the same from the supply officer. With the money thus obtained,

TABLE 1

Acid-base balance of army rations

CAMP	ORGANIZATION	MILLIGRAMS PER RATION			REACTION		PROTEIN CALORIES
		Ca	P	Fe	Excess Acid	Excess Base	
					cc. N/1	*cc. N/1*	*per cent*
Bowie	132 F. A.	572	2077		11		18
Chanute	267 A. S.	1045	2600			6	13
Cody	125 F. A.	666	2615	37.4	19		16
Devens	301 F. S. Bn.	834	2138	25.7		9	12
Devens	303 F. A.	1060	2279	28.7	12		17
Dix	303 Eng.	691	1983			14	16
Dix	307 F. A.	556	2085	26.7		1	15
Funston	C. & B. School	780	2758	29.6	9		13
Greene	39 Inf.	653	2428	49.4	11		14
Greenleaf	Recr. No. 1	513	1645		28		10
Jackson	Off. Mess B. H.	782	1874			13	13
Johnston	Mess Co. No. 1	759	2229			24	14
Kearny	134 F. A.	685	1808			24	14
Lee	320 Inf.	643	2181	29.6	18		14
McClellan	112 H. F. A.	833	2065	30.2	4		15
Rockwell	63 Inf.	583	2140	29.1	13		16
Sevier	105 Eng.	649	2482	29.7	15		12
Selfridge	40 Squad.	749	1915	24.0		15	11
Gordon	2 Inf.	374	1516	22.8	0	0	15
Travis	260 Inf.	481	1975	23.3	39		14
Kelly	324 Squad.	627	2103	26.9	24		13
Fremont	12 Inf.	856	2050	30.3	1		12
Taylor	336 Inf.	956	2845	43.3	6		15
Scott	262 Squad.	695	2092	27.7	12		15
Upton	152 D. B.	573	2170	26.2		7	19
Wadsworth	107 Inf.	508	1992	24.3		1	15
Chanute	268 A. S.	868	2340	27.5	2		16
Gerstner	143 Squad.	659	1732	20.3			15
Lewis	166 D. B.	996	2632	34.4		20	15
Hazelhurst	357 A. S.	704	2215	29.4		13	13
Douglas	War prisoners	717	2130	25.0		25	13
Vancouver	404 A. Squad.	687	2402	26.7	19		14
Average		711	2171	29.1			14
Sherman's Standard		680	1440	15.0			

he purchases foods in the open market which give variety and, incidentally, serve to introduce larger amounts of the base-forming elements.[1] Table 1 shows in cubic centimeters of N/1 solution the excess of acid or base in the rations of various organizations from many camps. These values were obtained by determining the average amounts of all foods consumed per man per day for a period of seven days. Acid and base values were calculated from Sherman's tables. It is seen that some of the diets are strongly acid-forming, some about neutral and others contain an excess of base. There is, undoubtedly, a tendency for the rations, as actually consumed, to be acid-forming in character. The rations were found to contain more calcium, phosphorus and iron than the standards proposed by Sherman require.

TABLE 2

Acid-base balance of rations, Camp Cody

DATE	ORGANIZATION	Ca	P	Fe	EXCESS ACID	EXCESS BASE
		mgm.	*mgm.*	*mgm.*	*cc. N/1*	*cc. N/1*
August 3–9, 1918.....	Co. H, 135th Inf.	539	1790	22.1	30.2	
August 3–9, 1918.....	Co. I, 135th Inf.	416	1671	22.0	21.2	
August 3–9, 1918.....	Co. L., 135th Inf.	473	1662	21.0	21.5	
August 3–9, 1918.....	Co. M., 135th Inf.	542	1801	21.3	19.1	
August 11–17, 1918.....	Liquid Diet, Base Hosp.	1279	1240	67.0		21.0
August 11–17, 1918.....	Light Diet, Base Hosp.	872	2087	28.0		25.0
August 11–17, 1918.....	Patients' Mess, Base Hosp.	680	1585	23.0		2.0

Examination of base hospital dietaries reveals the interesting fact that base-forming elements are practically always in excess. Table 2 shows the acid-base balance of the rations of four infantry companies and of the base hospital diets at Camp Cody (data supplied by Capt. Paul E. Howe). This condition, perhaps, is more strikingly exhibited in a study made at the Base Hospital, Camp Custer, by Lieut. C. N. Frey, who undertook this work aided by the great interest of Lieut. Colonel Irons, the Commanding Officer. Table 3 indicates that the convalescent patients had only one of seven days in which the acid-forming elements of the food were in excess of the base formers. It seems significant that these hospital dietaries show such a marked contrast with many rations consumed by the well soldier. Is this alkaline reaction of the dietaries of the sick merely a coincidence, or

[1] Since April 1, 1919, all supplies have been obtained from the Quartermaster.

has a process of selection hit upon the seeming fact that neutral or base-forming diets are best suited to the needs of convalescents? The greater amounts of bases in hospital diets are secured by a more extensive use of milk, fruits and vegetables.

The possibility that the continued use of acid-forming diets may lead to a greater susceptibility to diseases of the less infectious type has seemed worthy of investigation. The statement of Hindhede (2) regarding his children who are vegetarians may be recalled, "I may add that with the exception of whooping cough and measles, which attacked them very lightly, and a slight tonsillitis in one of them, I do not recall that any of my children have ever been really ill."

TABLE 3

Summary of ration study, convalescent patients' mess, Base Hospital, Camp Custer

DAY	CALORIES (TOTAL)	ANIMAL PROTEIN	VEGETABLE PROTEIN	PROTEIN	EXCESS ACID	EXCESS BASE
		per cent	*per cent*	*grams*	*cc. N/1*	*cc. N/1*
Monday	3175	74.4	25.6	119		1.8
Tuesday	4258	65.1	34.9	117		15.6
Wednesday	3673	67.7	32.3	122	0.7	
Thursday	4418	59.0	41.0	146		113.0*
Friday	2855	61.3	38.7	109		4.1
Saturday	3690	59.2	40.8	109		25.0
Sunday	3420	76.0	24.0	132		24.4
Average	3638	66.1	33.9	122		30.6

* Foods contributing base: potatoes, spinach, milk, beans, figs, apples.

Chittenden has also reported a better state of health when living on a low protein diet.

At Camp Wheeler, conditions seemed favorable for undertaking an investigation of the possible relationship of diet to disease. During the winter of 1917–18, this camp had numerous cases of measles, mumps, influenza and pneumonia. When Nutritional Survey Party no. 8, under the leadership of Major H. A. Mattill, arrived there last spring, we were impressed with the peculiar dietary habits of the soldiers. A preliminary examination of the bills of fare showed that many organizations were consuming about one pound of meat per man per day and only about one-fourth pound of potatoes, instead of the usual pound of this food. Potatoes, with their alkaline ash, were substituted by rice and hominy, with their excess of acid-forming

elements. Chart 1 shows the relationship found between amounts of meat supplied and the duration of disease for fifteen companies of the 124th Infantry during February and March, 1918. A rough parallelism is indicated.

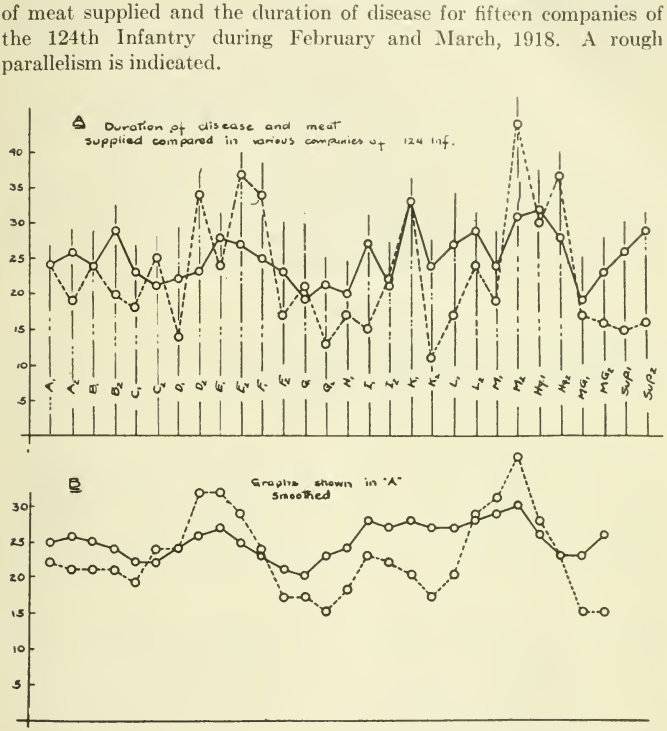

Fig. 1. A, Duration of disease and meat supplied compared in various companies of 124th Infantry; B, graphs shown in "A" smoothed. Solid line represents amount of meat supplied the messes, one scale division representing 4 pounds. Dotted line represents duration of disease.

The author studied the food consumption of certain organizations at Camp Devens during the epidemic of influenza-pneumonia. The three companies of the 36th Infantry with the lowest influenza rates and the three companies having the highest influenza rates failed to show any relationship whatsoever to the amount of excess acid in the rations (excess acid varied from 6.8 to 31.8 cc. N/1 solution per man

per day). Neither was any relationship between influenza rate and calorific intake or the partition of the calories indicated. It is extremely difficult, one might say impossible, to demonstrate any such influence in the case of influenza. The virulence of the organism or organisms is so great as to sweep away all ordinary powers of resistance.

The results obtained at Camp Wheeler are suggestive of a possibility that an acid-forming diet consumed for long periods of time may lead to a greater susceptibility to disease of the less infectious type. Those who have control of the feeding of the large numbers of persons in our public institutions might well undertake an investigation of this problem. In such institutions the necessary control of the diet and of the other factors should be more easily obtainable than in the army.

BIBLIOGRAPHY

(1) Blatherwick: Arch. Int. Med., 1914, xiv, 409.
(2) Hindhede: Protein and nutrition, London, 1913, 8.

DRIED VEGETABLES FOR ARMY USE

SAMUEL C. PRESCOTT

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, United States Army

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A famous wit once remarked that there is nothing new except what has been forgotten. The period since 1914 has proved the aptness if not the truth of this remark, for the conservation of food by drying has been strongly urged as a "new" method, whereas in reality it is perhaps the oldest method of preserving foods extant. Even in their relations to army subsistence we cannot claim that the dried or, as we now are more likely to speak of them, "dehydrated," foods can claim the quality of novelty for they were used to some extent in our Civil War, and to a considerable extent by the British Forces in the Boer War, and had been largely used by the Allies before the United States became an active participant in the struggle of the past few years.

It is true, however, that the European war has forced on our attention the possibilities of drying as a commercial process of food preservation in much the same way that the Civil War emphasized the great applicability of canning to the general problem of subsistence.

By "dehydration" is understood the rapid removal of water from vegetables, fruits or other foods through evaporation by the use of air currents or vacuum combined with moderate heat, to such an extent that the resulting product generally feels dry to the touch, and is hard, brittle or condensed in character. Dehydrated vegetables do not contain in general more than 10 per cent of water. The cellular structure should be unchanged, the membranes remaining intact, and the cell contents losing only water. When cooked they will return to approximately the bulk, appearance and character of the material before drying. If prepared properly the color, flavor and texture of the products when cooked will be essentially as in cooked fresh material.

The vegetables here considered include those ordinarily purchasable only in fresh, green or canned condition, and do not include mature dried beans, peas, lentils, corn or other vegetables of similar character.

During the Civil War desiccated vegetables in compressed form were used somewhat sparingly with the intent of improving the army ration from a hygienic standpoint, and especially as a means of preventing outbreaks of scurvy among the troops fed almost continuously on salt pork, salt beef, hard bread and the few vegetables which could be secured in the surrounding country. Some of the desiccated foods were said to be good while others were said to be very unpalatable. Among the anti-scorbutics issued to the Army of the Potomac in three months in 1864 were 600 pounds desiccated potatoes, 5320 pounds mixed vegetables and 551,812 pounds dried apples.

The following extract from the report of operations of the Medical Department of the Army of the Potomac from its organization in July, 1861, until the change of Base to the James River in July, 1862, by Surgeon C. S. Tripler, U. S. A. Medical Director, shows the faith which was placed in the use of these products as a means of preventing scurvy:

> While still at the White House, I received a telegram from the front that scurvy had appeared in two brigades of the Army one of which were the regular troops. I could scarcely credit the accuracy of the information. I knew that the brigade had obeyed the orders issued in relation to the use of vegetables, and the manner of cooking their rations; still I did not think it prudent to disregard the report, and accordingly I telegraphed to Washington for lemons and potash. . . . .
> . . . . I investigated the report with regard to scurvy and found it to be erroneous. I, however, requested the Adjutant General to compel the men to use desiccated vegetables and to make soup daily. . . . .
> . . . . General Dana says he cannot comprehend why the men should have scurvy with their present ration; but I am informed that the desiccated vegetables are so disagreeable to the taste that the men cannot eat them. . . .
> Antiscorbutic ordered. . . . .

During the Boer War dried soup vegetables which had been stored in paraffined barrels for over fifteen years, were used by the troops, thus indicating the keeping quality of these dehydrated products.

For about a year and a half I have had opportunity to study products prepared by a large number of methods, as manufacturers have sent them to Washington for possible sale to the Government for army use. There is a marked difference in products which have been studied. They have been examined as to:

a. Physical appearance.

b. Soaking back properties, whether good, bad, fairly rapid, or very slow.

c. Keeping quality. Danger of invasion by moulds, bacteria and insects.

d. Action of enzymes.

e. Absorption of moisture from air.

f. Cooking quality. Retention or loss of flavor.

g. Effect of type of container on the character and keeping quality of the foods.

As has been stated, marked differences have been noted. The causes for these differences may be referred to age and quality of raw materials, methods of pre-treatment, methods and time of drying and conditions of storage after drying. It may be stated that to secure good dehydrated vegetables the raw material should be fresh and of first-class quality. The pre-treatment by steam or hot water dipping should be brief and carefully managed to avoid excessive changes and the time and method of drying should be so regulated as to prevent intense surface evaporation at the beginning of the drying period with the consequent "case hardening" or sealing over of the surfaces so as to prevent rapid loss of the water from the inner tissues.

In a report to the Surgeon General in January, 1918, I pointed out certain advantages which might accrue as a result of the extensive use of dried vegetables and fruits in army subsistence. Most of these advantages were economic and a few were distinctly nutritional. The principal gains from such use were:

1. Lower cost of actual units.
2. Great saving of space in transportation.
3. Guaranteed keeping quality with no loss by freezing or spoilage.
4. Saving of storage space and labor in camp.
5. Wider range of vegetable foods.
6. Generally improved diet, through increase in roughage, alkaline salts and variety of combinations possible.

In the early part of the war some apprehension was felt regarding the use of these products, and while this fear was unnecessarily great it was based on certain sound observations. The ordinary dried vegetables before the war were neither attractive in appearance, appetizing in flavor nor good in food quality. Improvements have gradually taken place in the methods of preparation during the past four years, however, and while the bulk of material made is still in-

ferior, it is possible to secure some dehydrated products of excellent appearance and flavor closely approximating that of the cooked fresh vegetable, and of practically unimpaired food value. This improvement has been brought about by the recognition of the fact that a dehydrated food is not merely dried, but the drying must be done in such a way as to preserve cellular structure, prevent undue decomposition by heat, and forestall changes by microbic agencies or by oxidizing, proteolytic or sucroclastic enzymes. Attention must also be paid to the age, quality and soundness of the raw materials and to the methods of preparation, rate of drying and sanitation of the finished product.

During the past two years there have been shipped for our forces overseas approximately 40,000 tons of these products, principally potatoes, but also carrots, turnips, onions and large quantities of soup mixtures of six to eight or more vegetables. While no special reports on the use of these products are obtainable at this time, I am indebted to officers of the Quartermaster Corps for the statement that these dried foods have resulted in great saving in labor and have been found fairly acceptable to the men, satisfactory to the subsistence officers and cooks, and in general an excellent and easily handled form of vegetable food. This too in spite of the poor quality due to haste in manufacture to meet the emergency, and a letting down in specifications.

Extended studies conducted by the Section of Food and Nutrition of the Surgeon General's Office, and later by the Division of Dehydration of the Bureau of Chemistry have shown that from the standpoint of general use in large masses, chemical analysis, fuel value and maintenance of bodily strength, properly made products are practically equal to fresh materials. Reports from a number of army hospitals and camp messes have demonstrated their practicability.

The main question from the nutritive standpoint is in relation to antiscorbutic and growth-promoting properties. During the siege of Kut in the Mesopotamian Campaign it was reported that some of the British forces were afflicted with scurvy as a result of the constant and exclusive use of dried foods. Animal investigation with guinea pigs has shown that a diet composed entirely of dried vegetables of non-acid character is greatly weakened in antiscorbutics or has lost them altogether, while with dried fruits of acid character, such as tomatoes, oranges and lemons, scurvy does not result even after long continued feeding. With rats different results are obtained

and it seems to be practically impossible to produce symptoms of scurvy with any dried vegetables, if I am correctly informed. The bearing of these results on the use of these foods for the army, while of interest in a general way, is of secondary importance for it is only under most unusual conditions that a diet would be made up entirely of dried foods and then only for limited time periods. No experiments have been conducted on human subjects as would seem to be extremely desirable before we come to any final conclusion as to the deficiencies of these dried foods in antiscorbutic substances when applied to general use. The findings are of sufficient interest, however, to stimulate investigations for the purpose of discovering whether slight changes in the method of preparation or drying may not entirely overcome this academic objection and plans are now being formulated to take up these investigations on a large scale by the Division of Dehydration. Whether the mere presence of oxy-acids of the malic type is sufficient to prevent scurvy is problematical, but at least it seems probable that foods designed for use in localities where it is impossible to secure any fresh material can be so treated by changes in pre-treatment, by fermentation or otherwise as to secure the necessary antiscorbutic substance.

With a reduction in weight amounting to 90 per cent in most cases, a reduction in bulk amounting to more than 50 per cent and a guarantee of keeping qualities for long continued periods and the obviously greater ease of transportation, the military advantages in the use of these foods are so great that there can be no doubt but the army will constantly make use of them in increasing quantity for all expeditionary forces and in posts and camps during the winter and when an abundant and cheap supply of fresh materials cannot be obtained.

AMERICAN MILITARY HOSPITAL DIETARIES[1]

R. G. HOSKINS

From the Section of Food and Nutrition, Division of Sanitation, Medical Department, United States Army

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The problems of military hospital nutrition are, in general, much like those of any large institution, i.e., problems of supply, preparation and distribution. There are, however, certain features in the military hospitals not generally observed in civilian institutions. These arise mostly from the superposition of a military regime on that of an ordinary hospital. The proportion of patients who are practically well and merely waiting for discharge is notably higher in military than in civilian hospitals, these being essentially normal men, temporarily in confinement. The per capita food consumption, therefore, averages relatively high. The fact that the patients are subject to a high degree of arbitrary control is also reflected in totals of consumption and waste.

Although there are certain variations in details, the general plan of administration of the base and general hospitals in this country at present is much the same in all. Directly under the commanding officer is the mess officer, usually either an experienced hotel manager, or a former army mess sergeant commissioned in the Sanitary Corps. Associated with him, sometimes under his immediate direction, and sometimes immediately under the commanding officer, is the dietitian. She usually has one or more assistants. The dietitians are, for the most part, women who have had courses in domestic science, but who often have had little or no previous training in hospital work. The mess officer has the assistance of several sergeants who deal with such

[1] The quantitative data herein reported have been collected by several survey parties of the Section of Food and Nutrition, Office of the Surgeon General. Since thirty or more individuals have contributed, it is not feasible to assign credit, except for certain specific studies. The field notes added by the writer have been accumulated in the course of nine months' study of nutritional problems in some fifty military hospitals.

matters as supplies, accounts and kitchen and dining room management. The actual preparation of the food, and the care of the utensils and the premises are attended to by cooks, usually enlisted men, and kitchen police. Separate messes are typically maintained for sick officers, nurses, enlisted men, patients and hospital corps men. The officers' mess proper, and the nurses' mess are usually quasi-independent institutions, in which, however, the mess organization takes a benevolent and coöperating interest.

In theory, at any rate, dietetics is a branch of therapeutics, and each soldier in the hospital should receive the diet best adapted to his case, just as he should receive individual therapeusis in other respects. Practically, however, conditions have not permitted anything very closely approximating this ideal. It is true that occasionally special diets are encountered, which have been devised for individual patients, but for the most part, conventional "lights," "liquids" and "regulars" are provided for the men in large groups, with, in some cases "softs" interposed between the "liquids" and "lights," especially for the surgical convalescents. This is not an ideal state of affairs, but under existing conditions is probably as satisfactory as can be hoped for. The problem then resolves itself into one of best utilizing this system to secure for each patient the diet best suited to his individual needs.

Exclusive of officers and nurses, there are, from the point of view of dietetics, at least eight classes of individuals to be dealt with. These are:

1. Normal men at fairly active work, needing approximately 3600 calories daily.

2. Normal men at sedentary work, needing approximately 3200 calories each.

3. Convalescent patients, metabolically normal, walking about the wards and requiring approximately 2600 calories. Aside from a rather low caloric demand, there are no special indications to be met in the diet of this class.

4. Bed patients, metabolically normal, requiring approximately 2000 calories. This class is usually over-fed, receiving meals intended primarily for class 3, or even classes 1 and 2.

5. Sub-acute febrile patients, those with empyema especially, needing high caloric and high-protein feeding. Experience at Camp Lee has shown that empyema results in a constant drain on the body nitrogen, which should be compensated in the diet. The specific

dynamic action of protein is probably also of importance in this type of patients.

6. In this class may be included a considerable variety of patients who are not sufficiently ill to receive special diets, but whose convalescence could be hastened by careful attention to their dietaries. They include convalescents from pneumonia, and others in poor physical trim, who need a fairly generous diet, but who, from lack of appetite, eat but sparingly. Such patients should have meals planned especially for attractiveness and high caloric content. A common practice is to give these men the ordinary regular diet, supplemented by egg nogs and other special foods between meals. This is a makeshift device, which has obvious defects.

7. For that class of patients troubled with constipation when not actively exercising, a diet including especially laxative components should be provided. Rather than establishing a special diet for this type of patients, however, the situation could be met fairly well by deliberately making the meals of all regular patients laxative. The routine use of cathartics in the wards is a reflection upon the dietitians.

8. There is, of course, a special group of diseases, including nephritis, diabetes, cardio-vascular disorders, gastro-intestinal and acute surgical cases, which by general consent must be treated by special dietary therapeusis. The problem of meeting the needs of such cases does not ordinarily confront the mess administration, since the diets usually are, and should be prescribed by the surgeons in charge.

In table 1 are included the quantitative data available on food consumption in army hospitals and medical organizations. The number of rations upon which the study in each case is based, is stated in column 3. This, it will be seen, varies from 131 to 15,897. The ration, it might be stated, comprises the food of one man for one day. The studies have usually been carried out for a period of one week. In making such studies, the general plan has been to invoice the food materials in the kitchen and store rooms at the beginning of the study, to record all accessions during the period, and to make a second inventory at the end. Subtracting the amount at the second inventory from the sum of the first inventory and the accessions gave the food used during the period. From this were subtracted the various components of the garbage, giving the actual consumption during the period. In certain of the hospital studies, a more direct method of weighing the food actually used was more practicable.

TABLE 1

PATIENTS	TYPE OF HOSPITAL	RATIONS TOTAL FOR PERIOD STUDIED	NUMBER CALORIES CONSUMED	DISTRIBUTION			COST PER DAY	COST PER 1000 CALORIES	EDIBLE WASTE P.M.P.D.
				P.	F.	C.			
				per cent	*per cent*	*per cent*	*cents*		*pounds*
Liquid diets	Base	537	900	14	36	50	17.37	19.30	None
Light diets	Base	2,254	2,535	13	24	63	41.97	13.93	0.78
	Base	1,480	1,916	16	33	51	47.31	20.06	0.68
Regular diets	Base	4,109	3,788	13	33	54	55.34	14.61	0.22
	Base	5,341	2,743	13	29	58	46.06	13.40	0.98
	Base	614	3,458	12	35	53	48.23	12.12	0.72
	Base	709	3,242	11	36	53	39.83	10.84	0.91
	Base	236	3,906	14	34	52	57.90	13.74	0.48
	Base	728	3,043	13	35	52	45.64	12.44	0.96
	Base	4,516	3,270	14	37	49	55.13	14.85	0.68
	Base	10,125	3,165	12	34	54	45.48	12.18	0.73
	Aviation	131	2,986	15	38	47	54.14	17.19	0.18
	Base	3,338	2,759	12	34	54	52.20	14.53	1.16
Average		29,827	3,236	13	35	53	49.99	13.59	0.70
Officers' ward	Base	137	3,570	15	39	46	77.47	18.65	0.71
Patients and corps men combined	General	2,391	3,629	13	30	57	43.03	11.17	0.30
	Aviation	318	3,998	10	36	54	55.73	13.15	0.29
	Base	3,310	2,624	17	32	51	39.50	14.32	0.19
	Field	386	3,605	13	26	61	35.42	9.48	0.19
Average		6,405	3,464	13	31	56	43.42	12.03	0.24
Non-patients									
Med. Det.	Base	2,581	3,863	12	39	49	59.05	13.78	0.53
Nurses	Base	791	2,859	14	31	55	40.72	13.01	0.35
Hosp. Det.	Base	1,392	3,472	12	24	64	36.89	9.54	No data
Hosp. Det.	Base	1,863	3,682	14	29	57	51.26	13.68	0.20
Officers	Base	513	3,695	12	39	49	52.58	13.96	0.91
122–106 S. Tr.	Field	354	3,714	13	34	53	54.11	12.37	0.84
123 F. Hosp.	Field	248	4,047	14	36	50	57.08	12.67	0.64
F. Hosp.	Field	519	3,407	14	32	54	35.89	10.07	0.23
141 F. Hosp.	Field	556	3,387	12	35	53	40.15	11.60	0.09
Hosp. Corps	Aviation	187	4,522	13	31	56	58.50	12.58	0.12
U. S. A. A. S.		15,897	4,052	15	39	46	67.58	14.72	0.80
Sanitary Co.		1,148	3,638	13	31	56	47.80	11.73	0.71
Sanitary Co.		1,467	4,145	13	25	68	44.40	9.97	0.64
Amb. Co.		697	4,637	13	31	56	51.50	10.68	0.19
Amb. Co.		841	4,880	12	41	47	51.91	10.21	0.32
Amb. Co.		399	3,778	11	36	53	39.66	9.77	0.35
Amb. Co.		714	2,992	14	33	53	48.07	15.54	0.09
Amb. Co.		819	4,000	13	30	57	46.67	11.34	0.17
Amb. Co.		723	3,962	14	34	52	55.12	13.51	0.22
Average		31,709	3,828	13	33	54	49.94	12.14	0.39
Average of 427 army messes			3,633	14	31	55	47.26	13.01	0.38

One study of liquid diets only is available. In this the caloric content amounted to 900, with a fairly normal distribution of protein, fat and carbohydrate. Such diets are generally used for but a short time, and from a nutritional point of view often amount to little more than agreeable fluids.

Light diets in two hospitals, including 3734 rations, were studied. The caloric consumption in one was 2535, and in the other, 1916. The protein components amounted to 15 per cent as compared with an average of 14 per cent in 427 army messes elsewhere summarized. The fat components averaged 29 per cent, as compared with 31 per cent in the average army mess. Even this proportion seems somewhat high, however, for a "light" diet. The fact that such diets usually comprise considerable amounts of milk and eggs accounts for the cost of 45 cents per day, as compared with 47 cents for a full 3600 calorie ration. The edible waste in this group amounted to 0.73 pound per man per day. This is somewhat high as compared with average hospital waste, and is about twice that of the average army mess. This is to be ascribed partially to the capricious appetites characteristic of men ill enough to require light diets and partially to inefficiency in serving. This waste can be held at a much lower level.

Of the regular diets, ten studies are available, comprising 29,827 rations. The caloric consumption varied from 2743 to 3906, averaging 3236. The protein and carbohydrate components were slightly below the army average, while the fat component amounted to 35 per cent as compared with 31 per cent in the average army mess. This is to be ascribed partly to a higher consumption of butter and cream, but also, unfortunately, partly to unskilful dietaries in which greasy food played a part. The edible waste in this group was 0.7 pound per man per day. Experience at the Camp Custer Base Hospital has shown that the edible waste can be held below 0.3 ounce per man per day.

Only one study of a sick officers' ward is available. This comprised 137 rations. The caloric consumption amounted to 3570, which is probably somewhat below the average for this type of patients. The writer has been struck by the frequency with which officers in hospital systematically overeat. Heavy breakfasts, such as bacon and eggs, cereal with cream, fruit, potato and coffee with cream and sugar, are the usual type. The dinners and suppers are correspondingly generous. The fat consumption in the study reported, it will be noted, is 39 per cent, as against a general army average of 31 per cent. It is generally recognized by the dietitians and mess officers that such diets

are highly improper for sick men, but the demand is insistent, and officer patients are likely to get approximately what they want. The edible waste in this particular study was 0.7 pound per man per day. This is probably less than average. No specific figures are available, but the waste in the officers' messes is notoriously higher than that of others in the army. The cost per day in this case was 77 cents, whereas the allowance for this type of patient is $1.00 per day.

In a considerable number of hospitals, owing to ignorance and indolence in some cases, and to lack of equipment in others, the hospital attendants and the patients have been fed the same meals. Four studies of such hospitals are available, comprising 6405 rations. The caloric consumption was 3464, which is intermediate between that of patients proper and of medical personnel. The fat consumption was normal; the protein slightly low and the carbohydrate correspondingly high. The waste in this group averaged 0.24 pound per day. This favorable figure is not representative of this type of mess. The highest waste seen in any hospitals has been found in those in which the sick and well men are fed together. In one case it amounted to approximately 2 pounds per man per day.

Of non-patient groups, nineteen studies have been treated together. These include medical detachment, nurses, officers, sanitary train, field hospital, ambulance section and ambulance companies, giving a total of 31,709 rations. The caloric consumption varied from 2859 to 4880 averaging 3828, and the distribution of protein, fat and carbohydrate was approximately normal but with fat slightly high. The cost per day and the edible waste per capita were slightly above the army average.

In the nurses' mess it will be noted that the caloric consumption amounted to but 2859, distributed in exactly the ratio found as average in 427 army messes. It is believed that this mess is fairly typical of its kind. An officers' mess comprising 513 rations is also included; in this class the caloric consumption was 3695. Observations in more than 50 such messes have convinced the writer that this amount is lower than average. It has been a matter of some interest to note the dietary idiosyncracies of medical officers. Over-consumption and inadequate balance are so common as to constitute the rule rather than the exception. The high wastage in such messes has been previously commented on.

In table 2 are summarized the results of a detailed ration study, made by Lieut. C. N. Frey at the Camp Custer Base Hospital. This table is interesting as showing the variability of a mess from one day to another. The low figure of 2855 calories consumed on Friday is to be ascribed, probably, to fish for dinner and for supper. This commodity is usually unpopular. In this hospital a special study has been made for months of the technique of waste control. The patients are well fed and contented and wastage almost eliminated. The edible waste for the week studied amounted to 0.26 ounce per capita daily, a quantity which might well serve as model for any institution

TABLE 2

Summary of ration study

	CALORIES PER MAN	PER CENT CALORIES PROTEIN	PER CENT CALORIES FAT	PER CENT CALORIES CARBOHYDRATE	PER CENT ANIMAL PROTEIN	PER CENT VEGETABLE PROTEIN	POUNDS OF FOOD PER DAY	CALORIES PER POUND OF FOOD ISSUED	EDIBLE WASTE PER DAY AVERAGE	CALORIES OF FOOD WASTED, OUNCES	PER CENT OF CALORIES OF FOOD ISSUED WASTED IN GARBAGE	GRAMS PROTEIN PER MAN PER DAY
Monday	3175	15	39	45	74	26	4.76	667	0.37	16.0	0.50	118.95
Tuesday	4258	11	47	42	65	35	6.04	705	0.25	11.0	0.25	117.13
Wednesday	3673	14	37	49	68	32	5.65	650	0.20	8.0	0.22	121.67
Thursday	4418	14	43	44	59	41	6.16	733	0.25	11.0	0.25	145.73
Friday	2855	16	28	56	61	39	5.60	510	0.25	8.0	0.28	108.96
Saturday	3690	12	35	53	59	41	5.69	647	0.25	10.0	0.37	108.90
Sunday	3420	16	38	46	76	24	6.02	559	0.25	9.0	0.27	131.60
Average	3638	14	38	48	66	44	5.70	639	0.26	10.3	0.305	121.85

or any home kitchen. This amounts to approximately 20 pounds of edible waste per thousand men, or 10 calories per capita.

In table 3 is summarized the result of a special study made by Lieut. A. G. Hogan in an ambulance company. The records of the men showed their weights at the time of enlistment; second weighings were made in connection with this study. It was found that the men had gained during their six months' service from September to March, an average of nine pounds each. The average height of the company was 172.8 cm.; the average weight was 67.3 kgm; the surface calculated by the DuBois formula amounted to 1.81 sq.m., giving a basal metabolism of 1725 calories. The total metabolism by actual quantitative

determination of the food consumption amounted to 3778 calories, leaving a difference of 2053, representing the activity metabolism. During the week of the study the weather was moderate, the time and place being early spring in Kentucky. The men in this company were by previous occupation mostly farmers and laborers.

TABLE 3

Metabolism of ambulance company, March 4, 1918

Gain in weight during service,—September to March	9.0	pounds
Average height	172.8	cm.
Average weight	67.3	kgm.
Surface, Du Bois Formula	1.81	sq. m.
Basal metabolism	1725	calories
Total metabolism, by consumption	3778	calories
Difference	2053	calories

Sixty-two men studied one week. Work, moderate; temperature, moderate. Previous occupation, largely farmers and laborers.

TABLE 4*

Average daily expenditure for certain foods for six months. August, 1918, to January, 1919, at Base Hospital, Camp Kearny, California

	MEAT	EGGS	FRESH MILK‡	BUTTER	FRESH FRUIT	FRESH VEGE-TABLES	CANNED MILK	TOTAL FOR 7 ITEMS	TOTAL EXPEN-DITURES
Patients' mess	13.1¢	5.5¢	7.3¢	3.4¢	1.9¢	4.3¢	2.8¢	38.3¢	56.4¢
	23.3%	9.7%	12.8%	6.0%	3.4%	7.6%	5.1%	68.0%	100.0%
Detachment's mess	17.6¢	4.4¢	0.8¢	3.4¢	3.8¢	3.4¢	2.0¢	35.4¢	48.6¢
	36.1%	9.0%	1.6%§	6.9%	7.7%	7.0%	4.0%	72.0%	100.0%
Officers' mess	33.8¢	8.3¢	12.9¢	6.5¢	9.9¢	6.9¢	1.7¢	80.0¢	101.0¢
	33.4%	8.2%	12.7%	6.4%	9.7%	6.8%	1.6%	79.0%	100.0%
Nurses' mess	10.3¢	3.3¢	6.6¢	3.8¢	4.1¢	3.9¢		32.0¢	46.1¢
	22.3%	7.2%	14.3%	8.7%	8.9%	8.5%		70.0%	100.0%
Average 87 general messes	42.3%	4.4%	†	3.7%	†	6.0%	6.0%	62.4%	41.58%

* Compiled by Capt. Paul E. Howe, Sanitary Corps.

† Less than 1 per cent.

‡ Including cream and ice cream.

§ Ice cream, only.

Although it is intended to supply at army hospitals everything needed for adequate nutrition, the actual ration allowance for the individual patient is, of course, arbitrarily fixed. One of the major problems confronting the mess officer, therefore, is how to spend a limited sum to the best advantage.

In figure 1 is shown the relative expenditure for each of the 35 components most commonly used in 87 army messes. In table 4 is summarized the average expenditure in the Camp Kearny Base Hospital

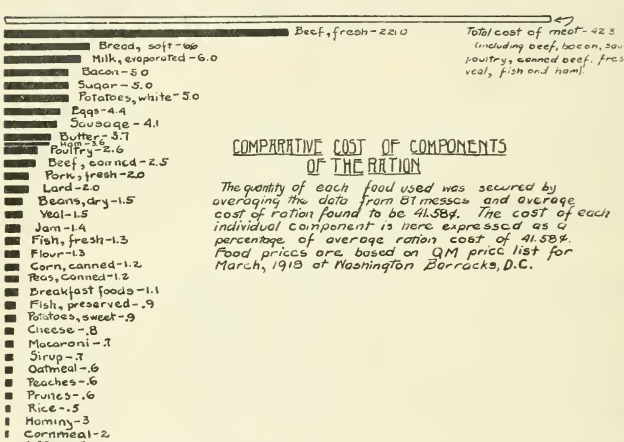

Fig. 1

for seven items comprising about 70 per cent of the total expenditure. These are based on daily determinations for a period of six months. This study was made by Capt. Paul E. Howe.

Figure 2 represents graphically the relative expenditure for each of the seven items in the patients', detachment's, officers', and nurses' messes respectively, as compared with the result for 87 general army messes. It will be noted that the hospital expenditure for meat and for condensed milk was less and that for eggs, fresh milk, butter and fresh fruits materially higher than that of the army at large. The expenditure for fresh vegetables was only slightly greater in the hospital.

On the whole, the table indicates a nearer approach to a proper balance in the hospital than in the army at large. In this it is representative of hospital consumption in general.

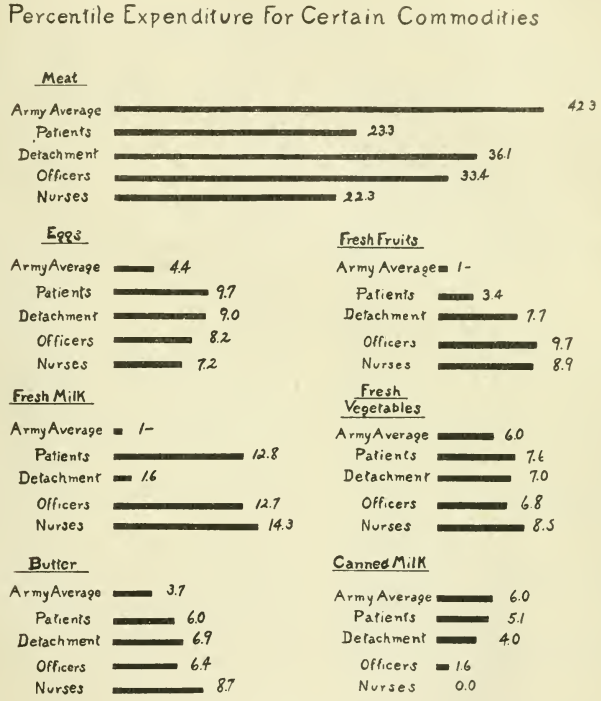

Fig. 2

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