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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

ON LOCALIZED CONTRACTION IN SKELETAL MUSCLE.

By Gertrude Frances Barbour and Percy G. Stiles.

We have attempted to determine whether the gastrocnemius of the frog contains any localized reserve of inactive tissue when it is moderately contracted, the stimulation being reflex or spinal. To this end we have secured simultaneous records of the movement of the tendon of Achilles and of various points on the surface of the muscle. It seems clear that in smaller contractions there is relatively less participation by the upper third of the muscle than is the case in maximal movements, or, in other words, that there is, near the femoral attachment, a reserve of fibres rather difficult to excite through the spinal centres.

THE SOURCE OF THE IMMUNE BODIES IN THE LYMPHS.

By F. C. Becht and A. B. Luckhardt.

After having established the facts as regards the concentration of antibodies in normal and in actively and passively immunized animals, the problem of the passage of the antibodies from the blood to the lymphs and other body fluids was begun.

The method employed was to immunize a dog by intravenous injections with a foreign blood, until a high state of immunity was established. Samples of cervical and thoracic lymph were collected, then the animal was cross-circulated for from seven to fifteen minutes

with a normal animal, from which samples of both the lymphs and blood had already been drawn. A sample of blood was drawn from each immediately after cross-circulation and then in most cases the actively immunized animal was killed. Samples of lymph and blood were then collected from the passively immunized animal under light ether anæsthesia at regular intervals for several hours. The blood was centrifugated after being defibrinated by whipping, and the lymphs were defibrinated immediately after collection. Cerebrospinal fluid and aqueous humor were collected at the end of the experiment only. The hemolytic, agglutinating, and opsonic power of the fluids was then tested.

We find that the antibodies — hemolysins, agglutinins, and opsonins — pass at about the same rate from the blood into the lymphs, but they make their appearance in a shorter time in the thoracic than in the cervical lymph. They are nearly always in higher concentration in the former than in the latter, although the reverse may be true occasionally after the experiment has been in progress for several hours. The antibodies hardly pass into the cerebrospinal fluid at all. The same is true of the aqueous humor. The concentration of the antibodies in the various body-fluids in the animal rendered passively immune by this method soon reaches an equilibrium, which is the same as that in the actively immunized animal of the same degree of immunity. From our experiments we have concluded that the source of the antibodies of the lymphs is the blood, and that the antibodies obey the laws of lymph formation as do the other constituents of the lymphs.

SOME OBSERVATIONS ON THE NATURE OF GASTRIC PERISTALSIS.

By W. B. Cannon.

Gastric peristalsis is similar to antiperistalsis in the colon in not being stopped by nicotine, in not being accompanied by a forerunning inhibition, in being rhythmically repeated, and in starting at a pulsating ring. Pulsations start at once if the organ, while in a state of tonic contraction, is distended, — they seem therefore to result from tension. Intragastric pressure measures the degree of tension. If

the pressure is increased, the pulsating ring is moved towards the pylorus; if diminished, towards the cardia. At first, therefore, the waves do not originate in any special region; later they start at a pulsating tonus ring, as in the colon.

Interruption of the myenteric plexus by several incisions, through both muscular coats, passing entirely around the stomach, does not stop the passage of the waves.

The necessary tonus is probably given initially by the vagi, and later maintained intrinsically by the stomach; for cutting the vagi before digestion begins greatly delays the appearance of peristalsis, whereas cutting the nerves after digestion has begun does not affect peristalsis. The recovery of motor activity after vagus section is attended by the development of independent tonus.

THE RECEPTIVE RELAXATION OF THE STOMACH.

By W. B. Cannon and C. W. Lieb.

From two to four and a half seconds after a cat swallows, the intragastric pressure which prevails during gastric digestion begins to fall. The lowest point of pressure, less than 1 cm. of water, is reached between six and ten seconds after the fall begins. In a few seconds more pressure begins to rise again and is soon at the former height. With a continued pressure in the gastric content between 3 and 4 cm. of water, the increase of gastric volume may be as much as 8 or 10 c.c. during the period of relaxation. Repeated swallowings keep the pressure at the lowest point. The phenomenon disappears if both vagi are cut.

The relaxation is at its maximum when the esophagus would naturally propel a bolus into the stomach.

ATTEMPTS TO PRODUCE EXPERIMENTAL HYPERTHYROIDISM.

By A. J. Carlson, J. F. Rooks, and J. F. McKie.

The work is primarily a search for tests for the thyroid secretions in the body fluids in order to determine whether in hyperthyroidism

(experimental and clinical) there is excess of these secretions in the body fluids or in the tissues.

Desiccated sheep and dog thyroids were fed to groups of pigeons, chickens, ducks, mice, rats, guinea pigs, rabbits, cats, foxes, dogs, and one monkey.

1. The different genera exhibit great variations in their resistance to thyroid feeding. Dogs, cats, foxes, and ducks seem the most resistant, doses of 20 gm. per day to dogs using from 3 k. to 7 k. producing no symptom, save increased appetite.

2. When thyroid is fed in sufficient quantity, the most constant symptoms produced are *emaciation* and *diarrhœa*. Both of these symptoms may, however, be absent even in fatal cases. Death in convulsions or in depression.

3. The most constant post mortem findings are hyperemia of the intestines with hemorragic infiltration of the intestinal mucosa.

4. Exophthalmus and nervousness cannot be detected. Many of the animals continue to eat until a few hours before death, so that the post mortem reveals a full stomach, or crop. In dogs and cats the largest doses so far tried have not produced tachycardia.

5. Control experiments with feeding of desiccated testes, pancreas, liver, and muscle seems to indicate that the above symptoms are mainly due to the thyroid feeding.

6. It would seem, then, that in the case of normal healthy animals there is a great variation in the resistance to thyroid substance in the different genera, and that when the thyroid substance reaches the toxic concentration the nervous and cardiac phases of the symptom complex in the birds and the lower mammals differ from those in man.

A SHADOW PUPILLOMETER FOR THE ACCURATE STUDY OF PUPILLARY REACTIONS.

By G. W. Fitz.

The principle underlying the apparatus is that an opaque object held close to the eye and in its line of vision will not cast a shadow (*umbra*) upon the retina unless it is at least as large as the (apparent) pupil.

It follows that an opaque object, as a slender cone or thin wedge (pencil point), which can be made to vary in size by exactly determinable amounts, can be used to measure the diameter of the pupil. In the shadow pupillometer this is accomplished by inserting a section of thin steel ribbon 20 mm. by 6.5 mm., into the end of a rotatable rod, which carries on its other end a protractor so adjusted that its zero is at its index when the plane of the steel ribbon is in the line of vision. For convenient application to the eye, the rod is suitably supported (as by the head piece of a stereoscope).

The steel ribbon is rotated until a shadow line is projected across the field of vision, and the angle of rotation is read. The diameter of the pupil is then calculated by the formula, diameter = sine $a \times$ 6.5 mm., and the area of the pupil by the formula, area = $\left(\frac{d}{2}\right)^2 \times \pi$. A graphic table which gives both of these values upon inspection, is readily constructed. If preferred, a quadrant graduated to diameters and areas may be substituted for the protractor. The apparatus is adapted to the testing of either one or both eyes, alternately or simultaneously. It is easily accurate to $\frac{1}{100}$ mm. in diameter of pupil, and discloses quick variations of diameter of even less amount, such as the arterial pulsations in the iris.

THE ALLANTOIN-PURINE EXCRETION OF THE MONKEY.

By Andrew Hunter and Maurice H. Givens.

Our findings confirm the observation of Wiechowski,[1] and support the general conclusion of Wells.[2] From 75 c.c. of the urine of one monkey we have isolated by Wiechowski's method 8.5 mgm. of allantoin in typical crystals. From 500 c.c. of the mixed urine of two monkeys we obtained 172 mgm. of crystalline, though not entirely pure, allantoin. In another 500 c.c. sample of mixed urine from the same two animals we could detect no uric acid, and only 4.5 mgm. of nitrogen in the form of purin bases. These samples cannot, un-

[1] Wiechowski: Hofmeister's Beiträge, 1908, xi, p. 101.

[2] Wells: Journal of biological chemistry, 1910, vii, p. 171.

fortunately, be directly compared, but the analyses demonstrate at least the great relative importance of allantoin as end-product of purin metabolism in our animals.

THE PART PLAYED BY THE SPLEEN IN THE FORMATION OF IMMUNE BODIES.

By Arno B. Luckhardt and Frank C. Becht.

As a working hypothesis we assumed that, other things being equal, an animal possessed of a spleen would develop antibodies more rapidly and in greater ultimate concentration than a splenectomized animal. Splenectomy, therefore, was one of the procedures adopted to test the validity of our assumption, active immunization of the animals being effected by a single intravenous injection of antigen (goat's or rat's corpuscles) administered at various intervals previous to or following splenectomy. For each splenectomized animal we provided on the same day a control animal having same age, weight, and size, and in order to make conditions as comparable as possible, performed a laparotomy at which occasion the spleen was temporarily removed from the abdominal cavity and replaced (anesthesia, mechanical irritation, shock). After immunization the animals were bled daily for the first nine days and thereafter at regular intervals for about three weeks. The sera were kept surrounded by a freezing mixture and were tested under the same conditions and on the same suspension of corpuscles at the end of that period.

Our report on this phase of the work is based on the results obtained from seven series of dogs comprising 21 animals which were immunized: twenty-four hours previous to splenectomy and laparotomy (3 dogs); immediately after splenectomy and laparotomy (4 dogs); three-fourths of a year (2 dogs), twenty-one days (4 dogs), sixteen days (4 dogs), eleven days (2 dogs), and five days (2 dogs) after splenectomy and laparotomy. Briefly the results are as follows:

(1) The animals possessed of a spleen produced the specific antibodies (hemolysins, hemagglutinins, and hemopsonins) more rapidly.

(2) The ultimate concentration of these antibodies in the serum was usually much higher than in the splenectomized animal; never was the concentration lower.

(3) In this relation of immunity there seems to be no compensation for the spleen, at least within a period of eight months.

2. Intraperitoneal introduction of spleen emulsion from dogs immunized three to twenty-four hours previously by an intravenous injection of antigen (goat or rat blood) resulted in the appearance of the specific antibodies in the serum of the recipients. No increase in antibodies was noted in the sera of those animals into whose peritoneal cavity normal spleen emulsion was introduced. The introduction of "immune" heart muscle, liver, bone marrow, and lymph glands did not give positive results.

3. The method of transplantation of the spleen *in toto* has so far not proved feasible in our hands.

THE OSMOTIC PROPERTIES OF SMOOTH MUSCLE.

By E. B. Meigs.

Striated muscle maintains its weight, as a general rule, only in salt and sugar solutions isotonic with the blood of the animal from which it was taken. Smooth muscle, when placed in isotonic or even hypertonic sugar solution, gains in weight almost as fast as it does in distilled water. In Ringer's solution having the formula NaCl, 0.65 gm.; KCl, 0.02 gm.; $CaCl_2$, 0.025 gm.; $NaHCO_3$, 0.02 gm.; H_2O, 100 c.c. the tissue usually gains slowly until at the end of twenty-four hours it may be 20 per cent or 30 per cent heavier than originally, though still quite irritable. These facts indicate that the fluid interchange between smooth muscle and its surroundings is controlled by factors widely different from those which operate in the case of striated muscle and other cells.

THE EFFECTS OF EXTRACTS OF THE DIFFERENT PARTS OF THE HYPOPHYSIS.

By J. L. Miller, D. D. Lewis, and S. A. Matthews.

Extracts of the pars intermedia, freed of the depressor substance, when injected intravenously gave a distinct pressor effect. Extracts

of the pars nervosa, freed of the pars intermedia and its own depressor substance, also give a pressor effect when injected intravenously. The pressor substance is apparently secreted by the pars intermedia, but passes into the pars nervosa. It does not need to be activated by the pars nervosa before it can exert the pressor effect.

Extracts of the stalk of the ox hypophysis never gave any pressor effect when injected intravenously. There is, therefore, a distinct interruption in the path of secretion of the pressor substance from the pars nervosa to the ventricle.

The portion of the anterior lobe immediately adjacent to the cleft contains groups of cells belonging to the pars intermedia. Extracts of this part of the gland freed of the depressor substance give the same pressor effect as that obtained from extracts of the pars intermedia in the posterior lobe. This tissue probably accounts for the secondary rise noted by Hamburger after injections of the anterior lobe extracts and for the Ehrmann reaction occasionally obtained by Franchini when using anterior lobe extracts.

We have obtained a decided pressor effect from the contents of a cyst of the pars intermedia. The substance which slows the heart, noted first by Howell, is confined mostly or at least in its most active form to the pars nervosa. Extracts of the pars intermedia in their purest form do not give this reaction.

THE HEART ACTION IN RELATION TO THE RESPIRATORY METABOLISM.

By J. R. Murlin and J. R. Greer.

Experiments on dogs were devised in which the absorption of oxygen and the output of carbon dioxide were determined by means of a small Benedict respiration apparatus attached directly to the dog's trachea. Simultaneously the blood pressure was recorded. The effects of anæsthesia were controlled.

Similar experiments on several different men in widely different nutritive condition and in varying degrees of muscular activity (lying

on a bed, standing, standing and lifting weights, shivering, etc.) were also done by means of the same respiration apparatus and the Erlanger sphygmomanometer. The results show a fairly close correlation in the same individual between the heart output expressed as the product of the pulse pressure and the heart rate on the one hand, and the absorption of oxygen and the elimination of carbon dioxide on the other. The relationship between carbon dioxide elimination and heart action is on the whole a little more constant than that between the oxygen absorption and heart action. Data will also be given on the volume output of the heart determined both directly and indirectly.

THE OLFACTORY SENSE OF FISHES.

By G. H. Parker.

That the olfactory surfaces of fishes are bathed with water has led many physiologists to conclude that these surfaces are organs of taste rather than organs of smell as they are in the air-inhabiting vertebrates. The common catfish, Amiurus nebulosus, will attack wads of cotton cloth containing hidden bits of earthworm, but is unstimulated by similar wads containing no such fragments. After cutting the olfactory tracts in this fish, it treats both kinds of wads with indifference. The killifish, Fundulus heteroclitus, will frequently seize wads of cloth containing hidden dogfish meat, but it will seldom touch similar wads containing no meat. When the anterior nasal apertures of this fish are closed by being stitched up, the fish reacts to wads containing meat as it does to those without meat. On reopening the nasal apertures by loosening the stitches, the ability to distinguish between the two kinds of wads is again shown by the fish. As the power to discover distant hidden food is lost by both kinds of fishes when their olfactory organs are prevented from acting, even though their organs of taste are in normal condition, it is concluded that their olfactory organs are as truly organs of smell as are those of the higher vertebrates, and that their olfactory organs are, therefore, properly classed as distance receptors.

MEASUREMENT OF THE BLOOD FLOW IN MAN. (DEMONSTRATION.)

By G. N. Stewart.

Method. — The blood flow in the hand is calculated from the formula

$$O = \frac{H}{T - T'} \cdot \frac{1}{S},$$

where O is the quantity of blood passing through the hand in the time of observation, H the quantity of heat given off by the hand to a calorimeter, S the specific heat of blood, T the temperature of the

Grams of blood per 100 c.c. of hand per minute.		Mean temperature of calorimeter.		Remarks.
Right hand.	Left hand.	Right hand.	Left hand.	
10.8	10.3	23.2	27.2	M. C., normal man, standing.
12.6[1]	12.3[1]	26.1	26.1	M. C. sitting.
17.3	5.2	24.3	23.6	M. C. standing. Right hand made to execute movements in the calorimeter.
14.5	6.5	26.8	26.5	N. M. standing. Boy with left hand paralyzed (infantile paralysis).
9.6	9.0	28.0	27.96	M. H. Man with left hand affected by spastic paralysis (birth palsy). Standing.

[1] Mean results of double observations with interchange of hands between the calorimeters.

arterial blood entering the hand, and T' the temperature of the venous blood leaving the hand. The heat produced in the hand is negligible in comparison with the heat exchange associated with the blood flow. T is taken as approximately rectal temperature; T' for a part like the hand, where nearly the whole blood flow takes place through quite superficial vessels, is, for a certain range of temperature, approximately the temperature of the calorimeter when the hand has been immersed for a sufficient time in a large bath at the temperature of

the water in the calorimeter. Outside of this range of temperature, O, when calculated on the assumption that T' is the mean temperature of the calorimeter, gives us a minimum below which the blood flow cannot lie.

PULSE-PRESSURE VARIATIONS IN THE PULMONARY CIRCUIT.

By Carl J. Wiggers.

Method. — A simple or T-cannula was inserted into the central end of a pulmonary artery, the chest rendered air-tight, a negative pressure equal to that previously existing in that animal was created, and the animal allowed to breathe naturally. The cannulas communicated by a tube passing through the chest wall either with a membrane manometer or with two valved manometers recording maximal and minimal pressures.

In normally breathing dogs with arterial pressures ranging from 100 to 112 mm., the maximal pressure in the pulmonary artery averaged 36 mm., while the minimal averaged 3 mm. Both systolic and diastolic pressures were lower in inspiration than in expiration. Increasing the negative pressure within the chest affected only the systolic pressure during expiration, which was increased. Decreasing the heart rate by weak vagus stimulation caused scarcely any change of pressure during expiration, but acted to increase the systolic and decrease the diastolic during inspiration.

While the mean pressure fell during inspiration, the pulmonary pulse pressure increased, due to the fact that the diastolic pressure was much lowered. The pulse-pressure quotient (ratio of pulse pressure to systolic pressure) was increased, tending to show that changes in the output of the right ventricle are not the only influences causing a decrease in pressure.

The vagus nerve was stimulated and the heart stopped, causing the pressure in the pulmonary arteries to fall to 2 or 4 mm. With each inspiration a decrease in pulmonary arterial pressure occurred, and with each expiration an increase. Further eliminative experiments showed that these results could be explained only by an effect of intra-thoracic pressure on the lung vessels.

THE FUNCTIONS OF THE CORPUS LUTEUM.

By Leo Loeb.

The corpus luteum has at least two functions:

1. To make possible the formation of the maternal placenta by supplying a sensitizing substance to the uterine mucosa.

2. To prolong the sexual cycle of the female organism in the pregnant as well as in the non-pregnant animal.

It can be shown experimentally that the former function is independent of nervous connections between the uterus and the ovaries.

THE MECHANISM OF THE ASPHYXIAL RISE OF BLOOD PRESSURE IN THE SPINAL ANIMAL.

By F. H. Pike.

Experiments on animals (cats) after decerebration and transection of the spinal cord or after paralysis of the brain, including the medulla oblongata, by depriving it of its blood supply, show that the first asphyxial use of pressure is due to the skeletal muscles.

A METHOD OF REMOVING GLYCOGEN FROM THE HUMAN SUBJECT.

By Graham Lusk.

In order to compare the results obtained upon phlorhizized dogs through the influence of cold, experiments have been made upon men with a view to causing the removal of glycogen from their bodies. The procedure was as follows: The evening meal contained only protein and fat; the breakfast was a cup of coffee. During the morning the metabolism of a resting period was obtained by use of a small Benedict respiratory apparatus. Then followed a cold bath in a bathtub filled with ice blocks, the temperature of the bath

being 10°, and its duration from six to twelve minutes. The metabolism was then determined for a second time during the period of shivering. This procedure was repeated.

The results in one case showed a fall in the respiratory quotient from 99 during the first period of rest to 75 during the period of shivering, and quotient 75 was also found during the resting period which followed. This corresponds to the quotient found by Benedict after prolonged fasting.

In a second case a thin man showed a respiratory quotient of 67 during a resting period following a period of shivering in which the quotient was 85. This indicated the exhaustion of glycogen from the body, and the low quotient is only to be interpreted by assuming a production of glycogen from protein.

In a third case the individual experimented on was a muscular athlete, and prolonged cold was not able to reduce his quotient at any time below 80. The shivering was sufficient in these cases to increase the metabolism from 100 to 200 per cent above the normal.

EFFECT OF INTRAVENOUS INJECTION OF EXTRACTS OF THE PINEAL BODY.

By J. A. E. Eyster (with H. E. Jordan).

An aqueous extract of the pineal body of the sheep causes, on intravenous injection, a fall of mean arterial blood pressure in the dog, sheep, cat, and usually in the rabbit, greater than the fall obtained from a similar extract made from other portions of the brain. The fall of blood pressure is associated with a vascular dilatation in the intestines, and since there is no important change in pulse rate and no important effect on the excised mammalian heart, the dilatation would seem to be the sole cause of the decrease in blood pressure. Acidulated aqueous extracts of the pineal body cause on intravenous injection in the rabbit a moderate and transitory diuresis. There is no very definite effect on respiration in the anæsthetized animal. The results, as a whole, would seem to indicate a relatively low degree of physiological activity of extracts of the pineal body so far as

the organs studied are concerned. One of us (Jordan) is making a detailed study of the microscopic anatomy of the pineal body in various animals. It is our intention to extend our physiological work to a study of the effects of extirpation in the sheep and other animals.

ACUTE ANAPHYLACTIC DEATH IN RABBITS.

By J. Auer.

Rabbits highly sensitized by repeated injection of horse serum respond in a characteristic fashion to the toxic injection when given intravenously. The train of symptoms is ushered in by a marked slowing of the respiration; without further premonitory symptoms the animal suddenly falls over with clonic and tonic convulsions, gives a few weak cries, and respiration ceases permanently. The heart is usually no longer palpable a few minutes after cessation of the convulsions. Autopsy shows the gut pale, without any hemorrhages; the splanchnic vessels are full. The lungs collapse well, but show usually traces of pulmonary œdema. The heart is distended with blood, especially the right ventricles. The ventricles usually do not beat, nor do they as a rule respond with a contraction to mechanical or electrical stimulation. The auricles usually beat regularly.

Blood pressure tracings show a fairly abrupt fall to the 10–20 millimetre level, the fall usually being preceded by a slight rise.

In order to rule out the central nervous system and the splanchnic region from the production of this fall of blood pressure, a rabbit was pithed including the medulla and the basal portions of the brain and the aorta and vena cava inferior were clamped. Artificial respiration was, of course, given. Injection of the toxic dose under these conditions showed the same characteristic fall of blood pressure and the same reduction or loss of irritability of the heart muscle.

It is therefore justifiable to conclude that the vital cause for acute anaphylactic death in rabbits lies in the heart itself, the toxic injection producing a reduction or abolition of contractility of the ventricles.

ON INTESTINAL PUTREFACTION DURING COPIOUS AND MODERATE WATER DRINKING WITH MEALS.

By W. M. Hattrem and P. B. Hawk.

1. The drinking of copious (1000 c.c.) or moderate (500 c.c.) volumes of water with meals decreased intestinal putrefaction as measured by the urinary indican output.

2. Copious water drinking caused a more pronounced lessening of the putrefactive processes than did the moderate water drinking.

3. In copious water drinking the total ethereal sulphate output was *increased* coincidently with the *decrease* in the indican output. This observation furnishes strong evidence in favor of the view that indican has an origin different from that of the other ethereal sulphates, and that they cannot correctly be considered as indices of the same metabolic process.

4. When Ellinger's method is employed, the determination of indican should be made on fresh urine before any preservative has been introduced. Especially is this true when thymol is used as the preservative.

5. The decreased intestinal putrefaction brought about through the ingestion of moderate or copious quantities of water at mealtime is probably due to an inhibition of the activity of indole-forming bacteria following the accelerated absorption of the products of protein digestion.

BIOLOGICAL ANALOGIES IN SOIL OXIDATION.

By Oswald Schreiner and M. X. Sullivan.

The soil is the seat of many biochemical activities which directly or indirectly affect soil fertility. Many of the processes in the soil are analogous to those occurring in plants and animals. Soils may show fatigue under a one-crop system and likewise under unsanitary conditions contain material which is retardative of plant growth. Many other compounds, some of which are known to be products of proteo-

lytic digestion, occur in soils. The soil *per se* has oxidizing and catalyzing powers which in cropped soils are due partly to activities of plant roots, but in air-dried soils are due mainly to non-enzymotic soil constituents, inorganic and organic, working separately, conjointly, or in reinforcing and activating combinations. The recently discovered activating action of salts of organic hydroxyacids and the discovery that alfalfa laccase is a mixture of salts of organic hydroxyacids have a close counterpart in soil oxidation studies.

THE ACTIVITY OF THE PANCREATIC FUNCTION UNDER THE INFLUENCE OF COPIOUS WATER DRINKING WITH MEALS.

By P. B. Hawk.

The activity of the pancreatic function as measured by the fecal amylase was found to be greatly facilitated when additional volumes of water ranging from 1500 to 4000 c.c. were daily ingested at meal by normal men maintained upon a uniform diet. Wohlgemuth's method was employed in determining amylase. The reaction of the feces may have been a disturbing factor in the determination. This point will be further investigated.

FEEDING EXPERIMENTS WITH MIXTURES OF ISOLATED FOOD SUBSTANCES.

By Thomas B. Osborne and Lafayette B. Mendel.

The experiments reported form part of an extensive study planned primarily to throw light upon the significance of the individual proteins in nutrition. The present series has been conducted with white rats; the methods and devices in use were demonstrated. Observations on food intake, nitrogen balance, digestibility, and body weight were made over long periods, and attention was devoted to such

questions as the palatability of the ration, monotony of diet, and inorganic salts, in addition to the nutrient rôle of the proteins used. The investigation has taken account of the food requirement during growth as well as the maintenance ration. The authors have already succeeded in maintaining rats on diets containing a single, isolated protein over a longer period than in any records heretofore published. The experiments are being continued.

ARE THE PARATHYROIDS CAPABLE OF REPLACING THE THYROIDS FUNCTIONALLY?

By Sutherland Simpson.

In the course of some experimental work on the sheep the thyroids were removed completely from nineteen lambs and twelve adults. Most of them were allowed to live from five to six months after the operation and were then slaughtered. Since during this period they showed practically no symptoms of thyroid insufficiency, it was thought that an examination of the parathyroids which had been left behind might throw some light on the question of their compensatory function. Accordingly all the parathyroid tissue discoverable at the time of death was removed, fixed in various fluids, sectioned in paraffin, stained and examined microscopically. For comparison the parathyroids from a few normal sheep and lambs were fixed and stained in the same way.

In the sheep the parathyroids are usually four in number — two internal, included in the substance of the thyroid, and two external, one on either side, imbedded in or closely related to the head of the thymus. The latter are situated so far away from the thyroid that they are not disturbed in any way by the operation of thyroidectomy.

The gland in the normal sheep is surrounded by a thin connective tissue capsule from which delicate septa pass inwards subdividing the organ into compartments that are occupied by the secreting cells. These are usually arranged in solid columns, but in some parts of the field small lumina can be made out in these columns and here the gland is of the tubular or acinar type. In the human subject several

types of cells have been described, but in the sheep there appears to be only a single type. The cytoplasm is granular, the nucleus relatively large, rounded or oval and rich in chromatin. The glands are extremely vascular

Microscopical examination of the two external parathyroids removed from the thyroidectomized sheep and lambs when they were slaughtered from five to six months after the operation, failed to show that they differed in any respect from the normal gland. Vesicular formation of the cells and the presence of colloid substance in the acini were particularly looked for, but neither was found, and the structure did not in any way appear to approximate to that of the thyroid. In the sheep, therefore, there does not seem to be any histological evidence in favor of the view that the parathyroid can functionally replace the thyroid.

THE CONSTANTS OF PUPILLARY REACTION. (A PRELIMINARY REPORT OF EXPERIMENTATION WITH THE SHADOW PUPILLOMETER.)

By G. W. Fitz.

The shadow pupillometer through its ability accurately to measure instantaneous changes upon the experimenter's own eyes, opens up a new field in the study of pupillary reactions. Experiments with it show that prevalent conceptions of these reactions require extensive modification.

Pupillary movements. — The iris shows marked pulsations of somewhat irregular character, which are apparently associated with the slight twitchings of the lids, although the latter are not always perceptible. The resulting changes in the pupil may be as great as 25 per cent of the total area, and occupy a quarter of a second. They are superposed upon much more regular and frequent variations of a fairly uniform character, which are too irregular for pulse beats, although they blend with them and assume the character of exaggerated beats. The pulse beats themselves can be distinguished best when the shadow of the pupillometer is reduced to its permanent

minimum for the illumination used. They amount to about 0.05 mm. in diameter. The changes in the pupil due to ordinary respiration are negligible. Enforced respiration, however, produces some change in size.

Pupillary adjustments. — In studying pupillary reaction, the area of the apparent pupil (*i. e.* of the pupil as seen from without, magnified by the lens effect of the cornea) was adopted as the best basis of comparison, since it gives more nearly the light-gathering power of the eye, and therefore the intensity of the illumination upon the retina, than does the area of the actual pupil. Much of the adjustment of the eye to strong light (above 10 metre-candle power) appears to be due to a diminishing sensitiveness of the retina rather than to an extreme narrowing of the pupil, which remains of nearly fixed size (slightly under 1 mm.). The pupil dilates markedly in lights below 4 metre-candle power. The adjustments of the pupil to sudden changes of light, as, for example, when the eye is opened after a closure of several seconds, are completed in approximately one half second. Light which does not strike the fovea affects the size of the pupil inversely as its distance from the visual pole. Accommodation makes a difference in the pupil's area of from 10 to 30 per cent, depending upon the strength of the illumination; hence all tests of pupillary reactions must have as a condition an equal convergence of the eyes.

THE MIGRATION OF SOLUTIONS IN ANIMAL BODIES DEPRIVED OF THEIR CARDIAC CIRCULATION.

By S. J. Meltzer.

Six years ago the author showed that an injection of adrenalin into one of the lymph sacs of a normal frog causes a maximal dilatation of the pupil. In this case the adrenalin is carried to the iris through the circulation and the dilatation appears a few minutes after the injection. Since the pupil of the frog reacts to the adrenalin for many hours after the death of the animal, I used it as a definite reaction for the study of the question whether there can be a migration of fluid in an animal body deprived of its cardiac circulation. In one method

the heart of frogs was exposed, firmly ligated and removed. In another method a curved needle was passed through the intact wall around the large vessels which were firmly ligated around the sternum, and then a second ligature was applied in the same manner around the middle of the heart. The results were positive. One cubic centimetre of adrenalin injected into the dorsal lymph sac invariably brings on a maximal dilatation of the pupils which sometimes appears as early as half an hour after the injection. When injected into the lateral lymph sacs, it may take two hours before the dilatation appears. Positive results can be obtained also from an injection into the thighs or legs. Positive results were also obtained in many instances, even when the animal was suspended by the head. The adrenalin migrated even against gravity.

The experiments with strychnine gave still more striking results. One cubic centimetre of a 1 per cent solution of strychnine brings out a definite tetanus when injected into the lymph sacs of the trunk, or of the extremities, or into the abdominal cavity. *A strong dose of strychnine causes paralysis.*

Since the firm ligation and removal of the heart excludes the participation of the heart, blood vessels, heart lymphs and lymph vessels, *the migration can take place only through the lymph spaces.* The experiments demonstrated that there is in the body a mechanism which is capable of carrying on in a slow but fairly efficient manner the migration of solutions in the body without the aid of the cardiac circulation. It is not improbable that this mechanism is active also in the presence of the cardiac circulation.

AN AUTOMATIC SHELLACKING DEVICE. (DEMONSTRATION.)

By D. E. Jackson.

A transverse supporting axle *D* permits the combination to revolve back and forth through an angle of forty-five degrees. The can *A* is six inches long by five inches in diameter. *C* is half-inch tubing. At *H* a shelf one and one half inches wide placed across the back end of

B forms a sort of reservoir in which the varnish can accumulate when *B* is suddenly elevated. This prevents the varnish from spilling out on the floor before it has had time to run back into *A*. *B* is eight and one half inches long, three inches deep, and five inches wide. At *I* a hole is made in *A* for equalization of the air pressure. *G* is a small lead weight used to pull *A* down when the treadle is raised. (A simple spring may be used for this if desired.) The uprights *M*, *M*, are attached to a wooden base (*N*) twelve inches long by ten inches wide. The ends of the axle *D* turn in holes in the upper ends of *M*, *M*. *N* is fastened to a table or shelf. At *O* a transverse bar of wood is nailed across between the uprights, and serves as a stop for the pan *B* when it has been pulled down to a horizontal position. The treadle *E* may be hinged at an angle to *B* when *B* is lowered to prevent dropping of varnish on the operator.

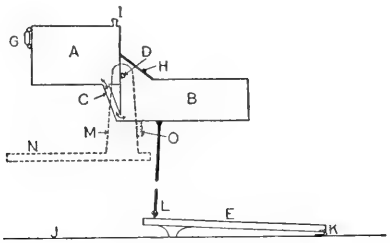

Diagram showing a lateral view of the apparatus.

INHIBITION OF THE DUODENUM COINCIDENT WITH THE MOVEMENTS OF THE PYLORIC PART OF THE STOMACH.

By D. R. Joseph and S. J. Meltzer.

In our present investigation we studied the normal contractions of the pyloric part of the stomach and of the duodenum simultaneously, by methods which we shall not describe here. We wish only to report here briefly the well-established fact that during each contraction of the pyloric part of the stomach the duodenum stops its rhythmic

activity and loses its tone, only to resume both again as soon as the contraction of the stomach passes off. We wish to add that we look upon this fact as a manifestation of the Law of Contrary Innervation. The contraction of the duodenum is antagonistic to that of the pyloric part of the stomach, and it is therefore a part of the normal function that the movements of the duodenum should be inhibited while the pyloric part of the stomach contracts. The phenomenon is identical in design with the relaxation of the œsophagus and cardia during deglutition as observed by Kronecker and Meltzer and with the observation by Cannon, reported at this meeting, of the relaxation of the stomach during the contraction of the œsophagus.

THE STIMULATION OF THE GASTRIC SECRETION UNDER THE INFLUENCE OF WATER DRINKING WITH MEALS.

By F. Wills and P. B. Hawk.

Under the influence of water drinking at mealtime by normal men the ammonia content of the urine is increased. This increase is considered an indirect index of the activity of the gastric function. This increased output of ammonia is directly proportional to the increase in the water ingestion. Within limits the quantity of ammonia excreted per 100 c.c. of ingested water is also practically the same whether the water ingestion is large or small. The above facts indicate that the animal organism attempts to maintain a constant acid concentration in the stomach contents under the influence of water drinking with meals.

FURTHER EXPERIMENTS ON THE ANTAGONISTIC ACTION OF SALTS.

By Jacques Loeb.

The author showed, first, that a pure solution of KCl of the same concentration as that in which this salt is contained in the sea water

kills half-grown fundulus in two days or less, while in a sodium chloride solution of the same concentration the fish live indefinitely; second, that potassium chloride solutions which are toxic can be rendered harmless through the addition of a definite quantity of sodium chloride; third, that the ratio between the toxic concentration of potassium chloride and the concentration of sodium chloride required to annihilate the toxic effect of the potassium chloride is a constant one; and fourth, that the antagonism exists in this case between the two kations, Na and K and not between K and Cl. From these facts the author draws the conclusion that the antagonistic action is ultimately due to a partition of the same presumably colloidal anion contained at the surface of the cells, espe cially of the gills of the fish, between the two metals, K and Na.

The author points out that as long as the ratio $\frac{C_{Na}}{C_K}$ remains below the critical value found in these experiments, namely, $\frac{i}{20}$ to $\frac{i}{25}$, not enough K ions get into the blood of the animal to produce a toxic action.

It was further found that there exists an upper limit for the concentration of KCl beyond which its toxic action can no longer be inhibited by NaCl.

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THE INFLUENCE OF ALCOHOL UPON NITROGENOUS METABOLISM IN MEN AND ANIMALS.

By LAFAYETTE B. MENDEL and WARREN W. HILDITCH.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut.*]

THE action of alcohol in the body may be twofold, pharmacodynamic and nutritive. Respecting certain features of its behavior in metabolism there is considerable unanimity of opinion.[1] When alcohol is ingested in moderate amounts, its influence on the utilization of the ordinary foodstuffs in the alimentary tract is minimal. Moderate doses are oxidized in the body and may thus exercise a sparing action on the metabolism of other foodstuffs. There is, further, an influence admittedly exerted by alcohol and alcoholic fluids on the elimination of uric acid; but the significance of this phenomenon is by no means clearly understood. Other incidental features of the influence of alcohol on metabolic processes, such as, for example, the oxidative functions, have been recorded from time to time.

The appearance of intermediary metabolic products, such as glycuronates, in the urine in cases of acute alcoholic intoxication suggested the possibility of discovering some details of the specific action of alcohol by making a more refined analysis of the urine than is commonly done in such instances. Just as peculiarities of purine

[1] The literature of the subject has been compiled to 1904 by Abderhalden: Bibliographie der gesammten wissenschaftlichen Literatur über den Alkohol und den Alkoholismus. The most recent general discussion of alcohol in relation to metabolism is by Rosemann: Oppenheimer's Handbuch der Biochemie, 1909, iv, Part 1, p. 413.

metabolism have been revealed in this connection, it was believed that other aberrant features might come to light.[2] Attention has been directed towards obtaining evidence of changes in the relative participation of various processes in the nutritive functions, particularly the partition of nitrogen in the urine. Heretofore the total nitrogenous metabolism and the total energy transformations have for the most part been the subject of study.

We have made comparisons, in men and dogs, of the output of urea-N, ammonia-N, creatine-N, creatinine-N, purine-N, and other constituents of the urine under fixed dietary conditions with and without intake of alcohol. The optical rotation of the urine was also observed in order to detect perversions of metabolism. The data for dogs supplement those recently presented by Salant and Hinkel.[3] Earlier observations on man, by Jackson and Blackfan,[4] are likewise of interest in this connection.

Experiments on Man.

The human experiments were conducted on two young men not accustomed to the use of alcohol: W. W. H., weighing 54 kilos; and J. F. L., weighing 67 kilos.[5] The daily diet consisted of

Breakfast, 8.30 A. M.		Dinner, 1 P. M.		Supper, 6 P. M.	
Grape fruit, about	175 gm.	Potato	100 gm.	"Uneeda" biscuit	25 gm.
Cereal ("Force") .	20 "	Hamburg steak .	100 "	Cream cheese . .	20 "
Banana, about . .	80 "	Pickles, about . .	30 "	Pickles, about . .	30 "
				Orange, about . .	125 "
				One egg.	

Milk, 900 c.c., and sugar, 100 gm., divided between three meals.
Bread, 125 gm., and butter, 60 gm., divided between two meals.
This was calculated to contain 13.4 gm. N. and yield about 2600 calories.

[2] A brief report of these studies was presented to the American Physiological Society, December, 1909. *Cf.* Proceedings, This journal, 1910, xxv, p. xi. The experimental data are taken from the thesis presented by W. W. Hilditch for the degree of Doctor of Philosophy at Yale University, June, 1909.

[3] Salant and Hinkel: Journal of pharmacology, 1910, i, p. 493.

[4] Jackson and Blackfan: Albany medical annals, January, 1907.

[5] These subjects also served in the experiments on the metabolism of purines by Mendel and Lyman: Journal of biological chemistry, 1910, viii, p. 115, which may be consulted for comparisons.

During the alcohol periods it was decided to make the intake of alcohol equal to that selected by Atwater and Benedict [6] in their well-known experiments on the energy exchange. They administered 72 gm. of absolute alcohol in divided doses, without noticeable psychic effects. Our two subjects received 96 c.c. of 95 per cent alcohol, furnishing about 500 calories, daily in six doses of 16 c.c. each. Three were taken with meals, the others at 10.30 A. M., 3.30 P. M., and 10 P. M., in milk or milk and water. The daily intake of fluid, including the milk, was 1300 c.c. After each dose a feeling of warmth was experienced, even at mealtimes. On several days when the mid-morning or mid-afternoon dose was taken somewhat later than usual, a slight and transitory dizziness was experienced, possibly owing to the fact that the alcohol reached an almost empty stomach. No other untoward features were noted. The diet was introduced three days before any of the analyses were begun.

The results of the analyses are summarized in Tables I and II.[7]

Discussion of the Experiments on Man.

In considering the tabulated results it is important to bear in mind that, aside from differences in body weight and individuality, the conditions of experiment were duplicated in the two subjects. This fact lends unusual significance to the deductions which are permissible.

Food utilization. — The diet itself afforded little more than a maintenance ration, and it was well utilized. The evidence for this is found in the study of the composition of the fæces.

The very slight increases in air-dry solids, nitrogen, and purine content during the alcohol period scarcely exceed the limits of ana-

[6] Atwater and Benedict: National Academy of Sciences, 1902, viii, sixth memoir.

[7] The analytical methods employed in all the work reported in this paper were those of Folin, for urea-, ammonia-, creatine-, and creatinine-N; of Krüger and Schmid, for urinary purine-N and uric acid; of Krüger and Schittenhelm for purine-N in the fæces; titration with uranium nitrate solution for phosphorus. Nitrogen was estimated by the Kjeldahl-Gunning process. Tests for sugar were made with the delicate reagent of Benedict: Journal of biological chemistry, 1909, v. p. 485. Optical rotation is expressed in degrees on the Ventzke scale (V°).

EXPERIMENT I.

MAN. — COMPOSITION

Date, 1909.	Body weight.	Alcohol taken 95 per cent.	Day of period.	Liquid intake.	Urine volume.	Spec. grav.	Total N.	Ammonia N.	Urea N.
Mar.	K.	c.c.		c.c.	c.c.	1.0–	gm.	gm.	gm.
12	67.4	..	1	1050	710	−30	11.45	.55	9.55
13	...	..	2	1150	760	−30	12.96	.55	10.98
14	67.5	..	3	1150	1000	−21	12.91	.57	10.77
15	67.4	96	1	1300	740	−28	10.83	.58	8.87
16	...	96	2	1300	1540	−18	12.53	.56	10.24
17	...	96	3	1300	1110	−21	11.29	.58	9.05
18	67.3	96	4	1350	980	−22	11.29	.53	9.19
19	...	96	5	1300	980	−24	11.96	.52	9.82
20	...	96	6	1300	1040	−23	11.88	.48	9.97
21	67.5	96	7	1300	1120	−21	11.99	.54	9.81
22	...	96	8	1300	700	−29	11.61	.54	9.48
23	...	96	9	1300	800	−27	11.72	.54	9.74
24	68.1	96	10	1300	820	−27	11.52	.52	9.42
25	...	96	11	1300	720	−25	10.48	.50	8.47
26	67.4	96	12	1300	1200	−21	12.04	.48	10.08
27	...	..	1	1300	920	−25	10.96	.49	9.04
28	68 2	..	2	1300	1070	−17	12.23	.49	10.00
29	...	..	3	1300	750	−28	12.27	.59	10.10
30	67.9	..	4	1300	1140	−21	13.20	.52	11.04

lytic error. If one were inclined, in view of their uniform occurrence, to assign a cause to them, it might be sought in the secretory effect of alcohol along the alimentary tract.[8] Such an application scarcely seems justified on the basis of the data at hand. Our utili-

[8] *Cf.* CHITTENDEN, MENDEL, and JACKSON: This journal, 1898, i, p. 164.

EXPERIMENT I.

OF EXCRETA. (J. F. L.)

Uric acid N.	Purine base N.	Creatinine N.	Creatine N.	Undetermined N.	Fæces N (average).	Total N output.	N balance.	Phosphorus.	Rotation.
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	V°
.156	.015	.57	...	.61	.71	12.16	+1.28	0.895	−.2
.156	.014	.59	...	.67	.71	13.67	−0.23	0.972	−.1
.156	.014	.59	...	.81	.71	13.62	−0.18	1.044	−.2
.210	.020	.59	...	.56	.79	11.62	+1.82	0.988	−.3
.230	.027	.59	...	.88	.79	13.32	+0.12	1.051	−.1
.205	.025	.59	...	.84	.79	12.08	+1.36	0.988	.0
.193	.018	.59	.020	.75	.79	12.08	+1.36	0.972	−.2
.185	.019	.59	.013	.81	.79	12.75	+.069	0.950	−.1
.182	.020	.58	...	.65	.79	12.67	+0.77	0.986	−.1
.186	.021	.61	...	.82	.79	12.78	+0.66	0.904	−.2
.198	.018	.58	...	.80	.79	12.40	+1.04	0.994	−2.
.208	.022	.59	...	.62	.79	12.51	+0.93	0.994	−.1
.193	.020	.61	...	.76	.79	12.31	+1.13	0.957	−.3
.191	.021	.62	...	.68	.79	11.27	+2.17	0.957	−.2
.190	.019	.60	...	.67	.79	12.83	+0.61	0.965	−.2
.162	.015	.62	...	.63	.69	11.65	+0.79	0.961	−.2
.176	.015	.59	...	.96	.69	12.92	+0.52	0.947	−.2
.169	.015	.58	...	.82	.69	12.96	+0.48	1.004	−.3
.174	.015	.58	...	.87	.69	13.89	−0.45	1.015	−.3

zation figures for nitrogen compare closely with those of Atwater and Benedict.

Protein-sparing action. — The quantities of alcohol consumed by our subjects could furnish about 500 calories per day. This is an equivalent of non-nitrogenous food sufficient to exert a distinct protein-sparing effect. As a rule, this favorable nutritive action

EXPERIMENT II.

MAN. — COMPOSITION OF

Date, 1909.	Body weight.	Alcohol taken 95 per cent.	Day of period.	Liquid intake.	Urine volume.	Spec. grav.	Total N.	Ammonia N.	Urea N.
Mar.	K.	c.c.		c.c.	c.c.	1.0–	gm.	gm.	gm.
12	54.4	..	1	1070	1100	−26	11.93	.32	10.05
13	...	..	2	1240	880	−25	11.77	.41	10.34
14	54.5	..	3	1240	980	−24	12.15	.38	10.26
15	54.5	96	1	1300	1100	−22	10.96	.37	9.21
16	...	96	2	1300	1310	−19	10.98	.43	9.09
17	...	96	3	1300	1140	−21	10.85	.41	9.55
18	54.7	96	4	1350	1030	−23	10.80	.41	8.91
19	...	96	5	1300	1400	−19	10.93	.37	9.14
20	...	96	6	1300	1080	−21	10.96	.40	9.27
21	55.2	96	7	1300	1210	−19	10.31	.40	8.41
22	...	96	8	1300	1200	−21	10.66	.40	8.96
23	...	96	9	1300	1100	−20	10.64	.37	8.73
24	55.2	96	10	1300	1120	−20	10.75	.32	8.99
25	...	96	11	1550	1040	−20	10.69	.35	8.91
26	55.2	96	12	1300	1440	−17	11.29	.34	9.14
27	...	..	1	1300	960	−26	11.23	.34	9.62
28	56.0	..	2	1300	1520	−18	11.69	.36	9.87
29	...	..	3	1300	1120	−21	12.10	.39	10.25
30	55.8	..	4	1300	1080	−21	11.66	.41	9.71

has been noted by previous investigators who have administered similar doses in a comparable way. Rosemann points out that although alcohol may replace fat or carbohydrate or produce an equivalent sparing effect in the later periods of its use, at first this may not happen. Some observers have recorded higher outputs of urinary nitrogen in the early period of alcohol administration. This has

EXPERIMENT II.

EXCRETA. (W. W. H.)

Uric acid N.	Purine base N.	Creatinine N.	Creatine N.	Undetermined N.	Faeces N (average).	Total N output.	N balance.	Phosphorus.	Rotation.
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	V₀
.150	.015	.51	...	0.88	0.90	12.83	+0.61	1.039	−.2
.150	.014	.50	...	0.36	0.90	12.67	+0.77	1.018	−.2
.159	.015	.51	...	0.83	0.90	13.05	+0.39	1.029	−.2
.173	.013	.51	...	0.68	1.06	12.02	+1.42	1.026	−.3
.187	.023	.48	...	0.77	1.06	12.04	+1.40	1.029	.0
.200	.020	.50	...	0.17	1.06	11.91	+1.53	1.004	.0
.189	.015	.52	.009	0.75	1.06	11.86	+1.58	0.950	−.2
.184	.021	.52	.009	0.68	1.06	11.99	+1.45	1.004	−.2
.181	.019	.49	...	0.60	1.06	12.02	+1.42	0.986	−.2
.191	.024	.51	...	0.77	1.06	11.37	+2.07	0.983	−.3
.204	.021	.49	...	0.58	1.06	11.72	+1.72	0.976	−.2
.192	.020	.51	...	0.82	1.06	11.70	+1.74	0.979	−.2
.206	.024	.52	...	0.69	1.06	11.81	+1.63	0.965	−.3
.194	.019	.53	...	0.69	1.06	11.75	+1.69	0.993	−.2
.195	.021	.51	...	1.08	1.06	12.35	+1.09	0.983	−.2
.160	.013	.50	...	0.60	0.97	12.20	+1.24	0.986	−.2
.170	.014	.50	...	0.78	0.97	12.66	+0.78	1.012	−.1
.172	.015	.52	...	0.75	0.97	13.07	+0.37	1.029	−.3
.156	.012	.51	...	0.86	0.97	12.63	+0.81	1.012	−.3

been ascribed to an increased elimination of nitrogenous katabolites as the result of an induced diuresis. Rosemann rather inclines to the view that the toxic (pharmacodynamic) action of alcohol overbalances its possible nutritive value in the early stages of its use, leading to cell katabolism. Tolerance may thus noticeably alter the influence of alcohol on metabolism.

COMPOSITION OF THE FÆCES — MAN — (AVERAGES, PER DAY).

	Wt. fresh.	Wt. air-dry.	Total nitrogen.		Purine nitrogen.		Ether extract.	
	gm.	gm.	gm.	p. c.	gm.	p. c.	gm.	p. c.
J. F. L.								
Fore period	64.1	16.8	0.71	4.25	.035	.21	2.55	15.2
Alcohol period	76.0	17.8	0.79	4.44	.041	.23	2.41	13.5
After period	67.8	15.6	0.68	4.36	.037	.24	2.40	15.4
W. W. H.								
Fore period	58.8	17.2	0.90	5.26	.050	.29	2.63	15.3
Alcohol period	73.3	19.1	1.06	5.55	.067	.35	2.60	13.6
After period	74.6	18.9	0.97	5.15	.057	.30	2.61	13.8

In our experiments there was no significant diuretic effect; and the protein-sparing influence of the alcohol made itself apparent from the first day. It may be emphasized that the intake of alcohol was favorably distributed and never induced obvious toxic or untoward results. The effect is expressed in the more favorable nitrogen balance during the alcohol period in both subjects.

NITROGEN BALANCE: MAN — SUMMARY OF DAILY AVERAGES.
(THE DAILY INTAKE IS ESTIMATED AS 13.4 GM.)

			Fore period.	Alcohol period.	After period.
			gm.	gm.	gm.
J. F. L.	Daily output	in urine	12.4	11.6	12.2
		in fæces	0.7	0.8	0.7
	Total		13.1	12.4	12.9
	Nitrogen balance		0.3	1.0	0.5
W. W. H.	Daily output	in urine	11.9	10.8	11.7
		in fæces	0.9	1.1	1.0
	Total		12.8	11.9	12.7
	Nitrogen balance		0.6	1.5	0.7

Partition of nitrogen in the urine: urea, ammonia, creatinine. —No alteration in the elimination of these constituents was noted beyond a reduction in the output of urea associated with the smaller

nitrogen elimination during the protein-sparing alcohol period. Jackson and Blackfan likewise found the creatinine output in man to remain unchanged in experiments with alcohol and a nitrogen-free diet of starch and cream. Since, as will be shown, the purine metabolism is altered, we may further conclude with Jackson that the creatinine and purines probably do not have a common origin; although such evidence as is presented does not eliminate the possibility that the influence of alcohol is exerted on the elimination or destructive factors rather than the origin of these compounds. Creatine was not a significant constituent of the urines examined by us.

Purine metabolism. — Our experiments leave no doubt that *even in the "moderate" doses used* alcohol increases the output of uric acid in man. This is in direct confirmation of the earlier experiments in this laboratory by Beebe.[9] The minimal changes in the output of purine bases under the influence of alcohol scarcely deserve note further than that they too were in the direction of an increase. Fig. 1 presents the results in graphic form.

Obviously the alcohol may affect either the endogenous or exogenous factor in purine metabolism, or both. The previously quoted studies of Jackson on nitrogen-free diet and newer experiments by Landau[10] on patients with purine-free diets indicate that alcohol may increase the endogenous output of uric acid in man. We have obtained direct positive evidence in the case of W. W. H. in two separate experiments.

In these experiments the diet, etc., were the same as in our previous trials, with the substitution of two eggs for 100 gm. Hamburg steak (at noon) and the omission of one egg at supper. An introductory period of three days on this purine-free dietary preceded the actual analytical period. The analyses were made in duplicate on one third of the daily urine.

The average daily augmentation of uric acid-N elimination in these trials on a purine-free diet was 13.6 mgm. in Experiment III and 15 mgm. in Experiment IV. The question arises: Is the increase in uric acid elimination under the influence of alcohol and with an

[9] Beebe: This journal, 1904, xii, p. 13.

[10] Landau: Deutsches Archiv für klinische Medizin, 1909, xcv, p. 280.

INFLUENCE OF ALCOHOL ON ENDOGENOUS PURINE METABOLISM — MAN — COMPOSITION OF THE URINE.[1]

Date.	Alcohol, 95 per cent.	Volume.	Specific gravity.	Uric acid nitrogen.	Purine base nitrogen.
	EXPERIMENT III.				
May	c.c.	c.c.		gm.	gm.
28	..	1300	1.022	.112	.012
29	..	960	1.026	.118	.014
30	96	920	1.027	.135	.016
31	96	970	1.027	.119	.017
June 1	96	860	1.027	.132	.015
	EXPERIMENT IV.				
Dec. 5	..	1065	1.019	.128	.013
6	..	1165	1.020	.126	.013
7	..	1085	1.022	.128	.014
8	96	1530	1.018	.151	.013
9	96	1215	1.020	.136	.012
10	96	1170	1.020	.141	.017
11	96	1500	1.019	.154	.015
12	96	1260	1.020	.148	.016
13	96	930	1.022	.131	.015
14	96	1050	1.021	.131	.014
15	..	960	1.022	.120	.013
16	..	960	1.023	.123	.013
17	..	1235	1.020	.132	.013

[1] The urine throughout these experiments was acid to litmus.

ordinary diet entirely accounted for by this increase in the endogenous component? Probably not, as a simple calculation shows:

AVERAGE DAILY OUTPUT OF URIC ACID-N. (W. W. H.)

	Endogenous output. Experiment IV.	Exogenous + Endogenous output. Experiment II.	Exogenous output (cal. by difference).
	mgm.	mgm.	mgm.
With alcohol	142	191	49
Without alcohol	126	153	27
Increased by alcohol	16	38	22

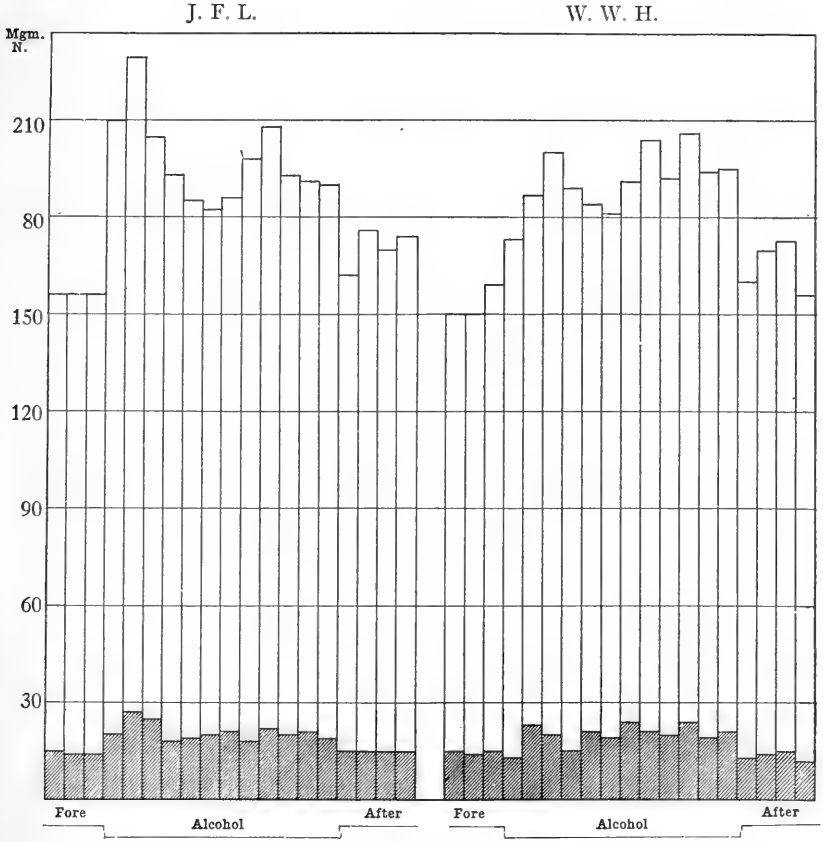

FIGURE 1. — Each vertical (ordinate) unit represents 30 mgm. of nitrogen. The horizontal (abscissa) units mark periods of twenty-four hours each. The boxed spaces represent the daily outputs of uric acid; and the shaded portions represent purine bases.

Landau's figures for the increase in endogenous uric acid output in five out of seven cases are of the same order, though somewhat larger than ours. His normal figures are, however, so widely variable from day to day that the exact value of his averages must be questioned. In regard to the excretion of exogenous purines he has

concluded that alcohol decreases the output as the result of diminished permeability of the kidneys towards uric acid. The considerations presented by him are by no means convincing and need not be reviewed here. It is difficult to understand why the kidney factors should not be similar, whatever the origin of the uric acid presented for elimination.

At the present stage of investigation a satisfactory hypothesis for the specific action of alcohol on purine metabolism is not forthcoming. Diuresis will not account for the augmented elimination in our experiments. There is no valid reason for assuming any unique synthesis of uric acid other than through the well-known sequence of enzymatic processes from purine precursors. If the increased output were confined to endogenous uric acid alone, one might be ready to accept Landau's explanation of a heightened (toxic) disintegration of cell nucleoproteins resulting in a hyperproduction of purines with consequent increase in uric acid formation and excretion. "One must conjecture," Landau writes, "that nucleoproteins are far more sensitive than the other proteins towards the administration of alcohol, and that the increased nuclear disintegration does not cease when the protoplasm has already become adapted to alcohol" (p. 308). On this view alcohol acts specifically to injure cells and damage the nucleus. Schittenhelm's[11] observations on dogs have also shown an increased endogenous output of purines during periods of alcohol administration.

The hyperproduction theory here reviewed is interesting in its relation to possible structural alterations in cells under the influence of alcohol. Histologists are in no way agreed as to what takes place. But if the suggestion presented by our calculations and indicating that *exogenous* uric acid or purine output is likewise increased by alcohol is substantiated by further evidence, the "cell destruction" theory will be inadequate to explain the facts. Some further indications of the disintegration of cellular constituents might be expected; for example, an increased output of phosphorus. On this point the experimental evidence is at present distinctly conflicting.

Another suggestion — that of increased hypoxanthine liberation in muscular tissues under the influence of alcohol — we are inclined to dismiss because of the constancy of our findings in respect to the

[11] SCHITTENHELM: Zeitschrift für physiologische Chemie, 1909, lxii, p. 93.

elimination of creatinine. Here again it must be admitted that this may not be an index of the chemical conditions in the muscular tissues. Plimmer, Dick, and Leib [12] have lately associated instances of heightened purine output with the special metabolism of the leucocytes. We have excluded this explanation for reasons presented elsewhere.[13]

There remains the more vague hypothesis of some disturbance in the enzymatic transformations of the purines under the toxic influence of alcohol — an explanation applicable to both the endogenous and exogenous components. Further discussion at the present moment appears unprofitable.

Other urinary constituents. — The remaining data presented in the tables call for little comment, since the conclusions to be drawn from them are obvious on inspection. The constancy of the so-called *undetermined nitrogen* derived by calculation is merely another expression here for the absence of marked alterations in the partition of the nitrogen. There is a very slight, perhaps insignificant, tendency towards a diminished urinary output of phosphorus (as estimated by uranium solution) during the alcohol period.[14] In view of the marked lævorotation which we have observed in the urine of chronic alcoholism this factor was considered in our subjects. No abnormal features were detected.

Experiments on Dogs.

In the preceding experiments on man the studies of the influence of alcohol on metabolism were confined to doses which produced no apparent untoward effects. For the obviously toxic conditions recourse was had to trials with dogs.

The animals, full-grown bitches, were catherized daily. Their daily diet consisted of 200 gm. hashed meat, 30 gm. lard, 30 gm. cracker meal, 200 gm. water; 20 gm. bone ash were added to improve the consistency of the fæces (Gies's method). The nitrogen intake was 7.3 gm., in about 600 calories (estimated). The alcohol ad-

[12] Plimmer, Dick, and Leib: Journal of physiology, 1909, xxxix, p. 98.

[13] Mendel and Lyman: Journal of biological chemistry, 1910, viii, p. 115.

[14] *Cf.* Salant and Hinkel: *Loc. cit.*, who found a diminished output in dogs and discuss the questions involved.

ministered was of 95 per cent volume strength and was added to the food in doses ranging from 1 to 7 c.c. per kilo body weight, as indicated.

EXPERIMENT V.[1]

DOG — COMPOSITION OF URINE. ALCOHOL GIVEN = 1–2 C.C. PER KGM. BODY WEIGHT; ONE MEAL PER DAY.

Date, 1908.	Body weight.	Alcohol taken 95 per cent.	Volume.	Spec. grav.	Total N.	Ammonia N.	Urea N.	Creatinine N.	Creatine N.	Rotation.
Dec.	kgm.	c.c.	c.c.	1.0 —	gm.	gm.	gm.	gm.	gm.	V°.
7	9.6	...	275	−20	6.21	.18	5.28	.09	.14	−.5
8	9.6	...	260	−19	6.09	.20	5.19	.09	.17	−.5
9	9.5	...	275	−19	6.23	.22	5.40	.09	.15	−.5
10	9.4	...	285	−18	6.75	.26	5.76	.09	.20	−.4
11	9.4	...	270	−18	6.20	.24	5.31	.09	.20	−.5
12[2]	9.3	18.5[2]	110	−34	4.20	.13	3.60	.09	.09	−.7
13	9.0	...	210	−26	6.99	.26	6.00	.09	.24	−.5
14	9.1	...	270	−18	6.51	.22	5.63	.09	.19	−.5
15	9.0	9.0	260	−19	6.09	.26	4.74	.09	.18	−.5
16	9.1	9.1	280	−19	5.55	.21	...	.09	.16	−.6
17	9.2	9.2	280	−19	5.76	.22	4.75	.09	.17	−.5
18	9.2	13.8	300	−18	5.78	.23	...	.08	.16	−.6
19	9.2	18.4	305	−19	5.79	.24	4.71	.08	.16	−.8
20	9.2	18.4	315	−19	5.79	.24	4.74	.08	.16	−.7
21	9.2	18.4	280	−19	5.58	.24	4.71	.08	.16	−.6
22	9.2	18.4	290	−20	5.88	.25	5.06	.08	.16	−.6

[1] Daily N intake = 7.3 gm.; 600 calories. The urine throughout the experiment was acid to litmus.

[2] On this day 18.5 c.c. alcohol = 2 c.c. per kgm. (diluted three volumes with water) were administered with a stomach sound just before the meal. Within an hour the dog vomited and refused to eat the vomitus again. To restore equilibrium two normal days were allowed to elapse before the alcohol feeding (with the meals) was resumed.

Experiment VI. — Dog — Composition of Excreta.

The same animal as in Experiment V was used. The dose of alcohol was 4 c.c. per kgm. per day. During the fore period and the first eight days of the alcohol period, the food and alcohol were divided into two meals given at 10 A. M. and 4 P. M. During the last eight days of the alcohol period 4 c.c. of alcohol per kgm. were given with one meal, instead of two separate portions of 2 c.c. per kgm., as before. The only visible effect of the alcohol was a slight unsteadiness of the hind limbs.

Experiment VII. — Dog — Composition of Excreta.

Alcohol given, 2–7 c.c. per kgm. Prolonged administration of alcohol.

A bitch weighing 12.3 kgm. was brought into nitrogen equilibrium on the same diet as in the previous experiments with a daily nitrogen intake of 7.3 gm. and 600 calories. The food was divided into two meals to which the alcohol was added in equal amounts beginning with 1 c.c. per kgm. at each meal. When the alcohol intake was less than 3 c.c. per kgm. twice a day, the food was eaten with more avidity. The alcohol intake for March 6 and 7 was somewhat less than is stated in the accompanying table, owing to evaporation, as the bitch refused to eat all of each meal at once. The morning meal contained 3 c.c. per kgm., while the evening portion contained 2 c.c. per kgm. For the next few days the morning meal was fed with a spoon, while the food in the afternoon was readily eaten, although the animal was scarcely able to stand. During the morning and early afternoon she seemed to be more amiable, while in the evening she was quiet, with slow respirations, and could be aroused only with difficulty.

Even the daily doses of 4 c.c. per kgm. caused distinct staggering, so that the distaste for alcohol which developed was not unexpected. Only 4 c.c. per kgm. were given on March 11, owing to the depressed condition of the animal. The daily analyses were now stopped, but the fixed diet was continued with 4 c.c. per kgm. of alcohol daily except when note is made of a higher intake in the analytical table.

EXPERIMENT VI.[1]

Date 1909.	Body weight.	Alcohol taken 95 per cent.	Day of period.	Urine volume.	Spec. grav.	Total N.
Jan.	Kgm.	c.c.		c.c.	1.0–	gm.
10	9.4	...	1	300	−21	7.22
11	9.4	...	2	310	−20	7.03
12	9.4	...	3	330	−20	6.68
13	9.4	...	4	320	−20	6.65
14	9.4	37.6	1	415	−19	6.32
15	9.4	37.6	2	300	−25	6.92
16	9.3	37.2	3	270	−24	6.23
17	9.3	37.2	4	350	−20	6.65
18	9.3	37.2	5	330	−22	6.79
19	9.3	37.2	6	350	−19	6.90
20	9.3	37.2	7	275	−23	6.92
21	9.3	37.2	8	350	−20	6.76
22	9.3	37.2	9	305	−23	6.55
23	9.3	37.2	10	315	−20	6.76
24	9.3	37.2	11	305	−20	6.63
25	9.3	37.2	12	320	−20	6.35
26	9.3	37.2	13	285	−22	6.81
27	9.4	37.6	14	380	−20	6.77
28	9.4	37.6	15	305	−20	6.78
29	9.4	37.6	16	310	−21	6.72
30	9.4	...	1	335	−22	7.06
31	9.4	...	2	295	−22	6.47
Feb. 1	9.4	...	3	315	−21	6.47
2	9.4	...	4	305	−21	6.66

[1] Daily N. intake = 7.3 gm.; 600 calories. The urine

EXPERIMENT VI.

Ammonia N.	Urea N.	Creatinine N.	Creatine N.	Fæces N.	Total N output.	Phosphorus.	Rotation.
gm.	gm.	gm.	gm.	gm.	gm.	gm.	V°.
.24	6.41	.09	.17	...	...	.41	−.4
.22	6.21	.09	.17	...	...	.39	−.3
.25	5.60	.09	.16	...	...	.37	−.3
.24	5.77	.09	.17	...	...	.37	−.4
.24	5.25	.09	.20	...	...	.31	−.6
.31	5.80	.09	.20	...	...	.38	−.6
.29	5.14	.08	.20	...	...	.34	−.6
.28	5.68	.08	.20	...	...	.33	−.5
.27	5.68	.09	.20	...	...	.38	−.5
.27	5.68	.09	.19	...	...	.34	−.4
.26	5.83	.09	.20	...	...	.34	−.5
.24	5.79	.09	.19	...	...	.31	−.5
.22	5.86	.09	.20	.51	7.06	.34	−.6
.27	5.74	.09	.20	.51	7.27	.32	−.6
.28	5.61	.10	.16	.51	7.14	.33	−.5
.24	5.38	.09	.20	.51	6.86	.32	−.5
.31	5.84	.10	.21	.51	7.32	.35	−.5
.24	5.76	.09	.19	.51	7.28	.32	−.6
.30	5.81	.09	.20	.51	7.29	.33	−.5
.26	5.65	.10	.19	.51	7.23	.35	−.5
.23	6.13	.09	.16	.54	7.60	.40	−.3
.24	5.49	.09	.15	.54	7.01	.38	−.3
.25	5.70	.09	.14	.54	7.01	.34	−.3
.25	6.11	.09	.16	.54	7.20	.37	−.3

throughout the experiment was acid to litmus.

EXPERIMENT VII.[1]

DOG — COMPOSITION OF EXCRETA.

Date, 1909.	Body weight.	Alcohol taken 95 per cent.	Day of period.	Urine volume.	Spec. grav.	Total N.	Ammonia N.	Urea N.	Creatinine N.	Creatine N.	Purine N.	Fæces N.	Total N output.	Phosphorus.	Rotation.
Feb.	K.	c.c.		c.c.	1.0-	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	Vo.
23	12.3	...	1	290	−22	7.05	.26	6.52	.13	.19	.002	.66	7.71	.43	−0.4
24	12.3	...	2	310	−24	7.27	.28	6.14	.13	.20	.003	.66	7.93	.52	−0.5
25	12.3	...	3	295	−20	7.22	.25	6.17	.14	.19	.003	.66	7.88	.52	−0.5
26	12.3	24.6	1	375	−20	7.31	.24	6.32	.14	.20	.021	.53	7.84	.49	−0.7
27	12.2	24.4	2	280	−20	7.20	.26	6.40	.13	.22	.018	.53	7.73	.49	−0.6
28[2]	12.2	36.6	3	345	−23	6.19[2]	.20	5.30	.12	.17	.022	.53	...	.37	−0.4
Mar. 1	12.1	36.3	4	265	−27	7.04	.24	5.97	.14	.20	.020	.53	7.57	.64	−0.9
2	12.2	48.8	5	340	−21	7.33	.21	6.30	.14	.18	.023	.53	7.86	.42	−1.0
3	12.2	48.8	6	315	−24	7.05	.23	5.98	.13	.22	.027	.53	7.58	.37	−1.1
4	12.2	61.0	7	350	−22	7.13	.21	5.99	.13	.20	.023	.53	7.66	.40	−1.3
5	12.2	61.0	8	465	−20	7.88	.22	6.56	.12	.20	.031	.53	8.41	.45	−1.9
6	12.1	60.5	9	265	−31	8.10	.42	6.43	.13	.28	.023	.53	8.63	.46	−1.9
7	12.1	60.5	10	250	−32	7.88	.41	6.50	.13	.26	.022	.53	8.41	.51	−1.6

8	12.2	61.0	11	295	−26	7.60	.40	5.98	.13	.20	.022	.53	8.13	.45	−1.8
9	12.2	73.2	12	335	−27	8.19	.36	6.50	.13	.20	.030	.53	8.72	.47	−1.8
10	12.2	85.4	13	495	−20	8.17	.31	6.55	.12	.28	.031	.53	8.70	.41	−2.6
11	12.1	48.4	14	290	−30	8.30	.45	6.61	.13	.26	.035	.53	8.83	.48	−2.7
17	12.2	85.4	20	330	−28	8.09	.43	6.40	.12	.28	.034	...	...	.40	−3.4
25	12.2	48.8	28	295	−20	7.62	.49	5.63	.11	.09	.014	...	...	.32	−1.2
29	12.4	49.6	32	295	−21	6.92	.24	6.00	.11	.12	.026	...	...	.36	−1.1
30	12.4	86.8	33	455	−20	6.63	.23	5.73	.11	.11	.015	...	...	.41	−1.8
Apr. 16	12.7	50.8	50	350	−22	7.11	.26	6.19	.11	.15	.017	...	...	.52	−1.1
26	12.7	50.8	60	320	−22	7.27	.28	6.22	.13	.19	.008	...	...	.41	−1.4
May 5	12.5	50.0	69	205	−26	7.57	.26	6.76	.13	.19	.022	...	...	.25	−1.2
6	12.6	50.3	70	335	−18	6.86	.29	5.93	.12	.20	.020	...	...	.42	−1.2
June 1	13.2	52.8	96	355	−19	7.00	.30	6.24	.15	.19	.021	...	...	.40	−0.9
2	13.2	52.8	97	445	−17	6.75	.30	5.77	.14	.18	.018	...	...	.40	−0.9
3	13.2	66.0	98	350	−20	6.57	.30	5.64	.14	.22	.022	...	...	.42	−1.1
4	13.2	79.2	99	435	−20	7.38	.30	6.12	.13	.22	.027	...	...	.51	−1.3
5	13.2	92.4	100[3]	...	...	...	...	...	...	...	...	...	...	...	...

[1] Daily N intake = 7.3 gm. 600 calories. The urine throughout the experiment was acid to litmus.

[2] Part of the urine was accidentally spilled.

[3] Urine contaminated by vomitus.

The food for each day was divided into two portions: one, containing the alcohol, was fed by spoon; the other was eaten voluntarily. Within four or five hours after the meal the dog was quiet and somewhat drowsy. She was easily aroused, and although willing, she was unable to respond when called, owing to apparent lack of control of the hind quarters. The drowsiness appeared to diminish after about the fiftieth day and the lack of control was rarely perceptible after the seventieth day, except at the end of the experiment, when the dose was larger than 4 c.c. per kgm.

During the alcohol period the urine was analyzed for the first fourteen days and then on the twentieth, twenty-eighth, thirty-second, thirty-third, fiftieth, sixtieth, sixty-ninth, seventieth, and finally during the ninety-sixth to one hundredth days. In the intervening days the dog was allowed more freedom in a larger pen, the diet (including 4 c.c. of alcohol per kgm. of body weight) remaining unchanged.

The adaptation of the body to the amount of alcohol given (4 c.c. per kgm.) is clearly shown by a study of the accompanying table of analyses. As early as the thirty-second and thirty-third days the distribution of the nitrogenous constituents of the urine is seen to be practically normal except for the purine nitrogen, which throughout the experiment has shown the first and most marked changes.

The approximate nitrogenous equilibrium of the thirty-second day was accompanied by a slight gain in body weight, with a gradual increase from 12.4 kgm. on that day to 13.2 kgm. at the end of the experiment. The total nitrogen output was increased somewhat when the alcohol intake was increased to 6 c.c. per kgm. The creatinine output remained practically constant. A trace of protein appeared in the urine beginning with the fiftieth day of the experiment.

The excretion of glycuronates as measured by the rotation of the urine gradually decreased from -3.4 V° on the twentieth day to $-.9$ V° on the ninety-seventh day with a slight increase for each of the next two days, when the alcohol intake was increased to 5 and 6 c.c. per kgm.

That the adaptation of the body to 4 c.c. per kgm. was disturbed when the daily intake of alcohol was increased was clearly shown by the reappearance of staggering and drowsiness together with the

changes in the excretion of total nitrogen, purine nitrogen, and glycuronates, with vomiting on the last day.

A post-mortem examination a few days after the close of the experiment failed to reveal any gross pathological changes in the organs.

Discussion of the Experiments on Dogs.

The three series represent the effects of varying doses of alcohol under comparable dietary conditions, the effects being pushed in the last prolonged experiment to the condition of chronic intoxication. In this case the dog even gained somewhat in weight. The utilization of protein was found satisfactory whenever it was determined. With the smaller doses (1–2 c.c. per kgm.) the protein-sparing action was apparent in the diminished urinary nitrogen output (see Experiment V). With the larger doses, however (Experiment VII), the toxic effect is visible in an increased output of nitrogen in the urine, so that the nitrogen balance became more unfavorable. That this condition did not become permanent, even after weeks of continued alcohol dosage, is shown by the figures recorded for the last week of Experiment VII. With a reduced intake of alcohol (4 c.c. per kgm.) the nitrogen output showed the characteristic tendency to return to its normal level.

The experiments show all stages of the effects of alcohol on protein metabolism, ranging from its sparing action with "moderate" doses to the toxic katabolism accompanying pronounced intoxication. The partition of nitrogen, if we except the purine metabolism, is scarcely disturbed in any case. Thus is furnished another illustration of the capacity of the organism to maintain its katabolic functions along certain normal channels, despite the interference of toxic agents. Herein doubtless exists an additional "factor of safety" for the body, further exemplified in poisoning with adrenalin,[15] hepatotoxic sera,[16] hydrazine,[17] cyanide,[18] and other instances.

A slight rise in ammonia-N output during the stages of severer intoxication may be associated with the production of organic acids

[15] *Cf.* Underhill and Closson: This journal, 1906, xvii, p. 42.

[16] *Cf.* Jackson and Pearce: Journal of experimental medicine, 1907, ix, p. 552.

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

[18] *Cf.* Richards and Wallace: *Ibid.*, p. 179.

which is suggested by the distinct increase in the lævorotation of the urine. There is some evidence that glycuronates are excreted under these circumstances.[19] We have repeatedly observed the same phenomenon in the urine of intoxicated animals and patients in the acute stages of delirium tremens.[20] The glycuronates disappear with the cessation of the intake of alcohol.

With regard to the influence of alcohol on the elimination of purine derivatives in the dog (Experiment VII) the same features as those recorded on man are brought to light. It must be borne in mind that the end product of purine metabolism in the dog is represented by allantoïn, the production of which we have not studied. The recent experiments by Schittenhelm[21] supplement our own in respect to allantoïn, the excretion of which he has found to be increased by alcohol in both endogenous and exogenous relations. In our experiments extending over weeks the intermediary purines (including uric acid) continued to be eliminated in quantities much larger than normal. What has been said from a theoretical standpoint in the earlier discussion on page 9 applies here also.

Is alcohol a food? — The argument commonly advanced in favor of the nutritive value of alcohol asserts that it can replace isodynamic quantities of carbohydrate and fat, and, like them, spare proteins. For "moderate" quantities (500 calories per day) this is doubtless true. But we know of no *nutrient* which will, in comparable amounts, increase the katabolic output of purines. Indeed, the tendency of foodstuffs is, if anything, in the reverse direction.[22] The contrast between what is doubtless a "toxic" (or pharmacodynamic) effect of alcohol and its real nutritive features always deserves to be kept in mind.

Summary.

A study of protein metabolism and utilization, and especially the partition of nitrogen in the urine, under the influence of alcohol has

[19] *Cf.* Neubauer: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 133.

[20] A review of these data will be published later.

[21] Schittenhelm: Zeitschrift für physiologische Chemie, 1909, lxii, p. 93.

[22] *Cf.* Kaufmann and Mohr: Deutsches Archiv für klinische Medizin, 1902, lxxiv, p. 141; Rockwood: This journal, 1904, xii, p. 38.

been carried out on man and dogs under fixed and comparable conditions of diet. In man the doses used were moderate, *i. e.*, 500 calories daily in the form of alcohol distributed in six portions. With the animals a range of dosage leading to distinct intoxication was employed.

The findings in general were as follows: There is no pronounced disturbance in the alimentary utilization of the foodstuffs. Moderate doses exert a protein-sparing action, which is succeeded by loss of nitrogen when larger quantities of alcohol are administered. The partition of urinary nitrogen remains remarkably unaltered with the exception of an increased elimination of ammonia-nitrogen (accompanying other evidences of perverted metabolism as indicated by the appearance of optically active (lævorotatory) compounds in the urine) following "toxic" doses, and a higher output of purines. The theoretical significance of the latter, which affects both the endogenous and exogenous fractions, is discussed at some length; and its bearing on the assumed nutrient properties of alcohol is indicated.

The most significant impression, perhaps, which the analytical data afford, is the absence of *pronounced* alterations indicative of markedly disturbed protein metabolism, even when comparatively large doses are continued for days and weeks. This has been interpreted as another evidence of the "factor of safety" in metabolism.

A STUDY OF THE ISOLATED KIDNEY. — THE INFLUENCE OF PULSE PRESSURE UPON RENAL FUNCTION.

By D. R, HOOKER.

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

IN 1904 Erlanger and Hooker[1] published a protracted study of the blood pressure in two men, one of whom had at the time orthostatic albuminuria. In the present connection two points are of especial interest: first, the amount of urine excreted varied *directly* as the magnitude of the pulse pressure; and, second, the amount of protein excreted varied *inversely* as the magnitude of the pulse pressure. Blood-pressure changes were induced by exercise, hot and cold baths, compression of the legs and abdomen, altering the position of the body with respect to the horizontal, etc. No constant relationship was observed between the functional activities of the kidneys and either the systolic or diastolic blood pressure. Finally, the tentative explanation of the facts observed was advanced that the nutritive state of the renal epithelium is dependent upon the pulsatile variations of the blood-pressure; that these variations act by affecting the amount of blood passing through the organ, and consequently the oxygen supply to the cells.

In both subjects the blood-pressure changes were of the same kind, but different in degree, as seen in Table I. The blood pressures were uniformly lower in the albuminuric, and showed a greater tendency to instability. This difference was further emphasized by greater fatigue upon standing still and liability to syncopal attacks.

A study of a second case of orthostatic albuminuria has resulted in similar findings.[2]

[1] Erlanger and Hooker: Johns Hopkins Hospital reports, 1904, xii, p. 145.

[2] Hooker, Hegeman, and Zartman: This journal, 1909, xxiii, p. xi.

These papers are the only ones known to the author[3] in which special emphasis is laid upon the pulse pressure as a factor influencing functional activity, although attention has frequently been called to the value of a pulsatile pressure, especially in maintaining a normal condition of tissues when studied under artificial perfusion.[4] Whether the beneficent effect is the result of an increased blood flow[5] or of

TABLE I.

	Normal individual.[1]			Albuminuric.[2]		
	Systolic.	Diastolic.	Pulse pressure.	Systolic.	Diastolic.	Pulse pressure.
Lying	126.8	87.2	39.6	115.8	85.5	30.3
Sitting	127.5	91.0	36.5	113.6	85.7	27.9
Standing	127.7	99.2	28.5	113.3	92.5	20.8

[1] Averages from five experiments under constant conditions.
[2] Averages from nine experiments under constant conditions.

the "shock" consequent to the pulse, remains an open question, although the latter has the stronger experimental support.[6]

The study of the relation of renal function to circulatory changes in man or in the intact animal is obviously complicated by many uncontrolled factors. Hence conclusive evidence of such a relationship must naturally depend upon the successful perfusion of the iso-

[3] For a résumé of the literature on orthostatic albuminuria see HOOKER: Archives of internal medicine, 1910, v, p. 491.

[4] JACOBI: Archiv für experimentelle Pathologie und Pharmakologie, 1892, xxix, p. 25; BRODIE: Journal of physiology, 1903, xxix, p. 266; HOFFMANN: Archiv für die gesammte Physiologie, 1903, c, p. 242; HAMEL: Zeitschrift für Biologie, 1889, xxv, p. 474.

[5] ERLANGER and HOOKER believed they were able to show that where the pulse pressure was the only factor changed, the velocity of blood flow varied *pari passu.*

[6] MELTZER: Johns Hopkins Hospital reports, 1900, ix, p. 135; MALL and WELCH, quoted by WELCH: Thrombosis and embolism, ALBUTT'S system of medicine, 1899, p. 254. See also FLEISCHL VON MARXOW: Eine neue Theorie der Respiration, Stuttgart, 1887.

FIGURE 1 *A*.

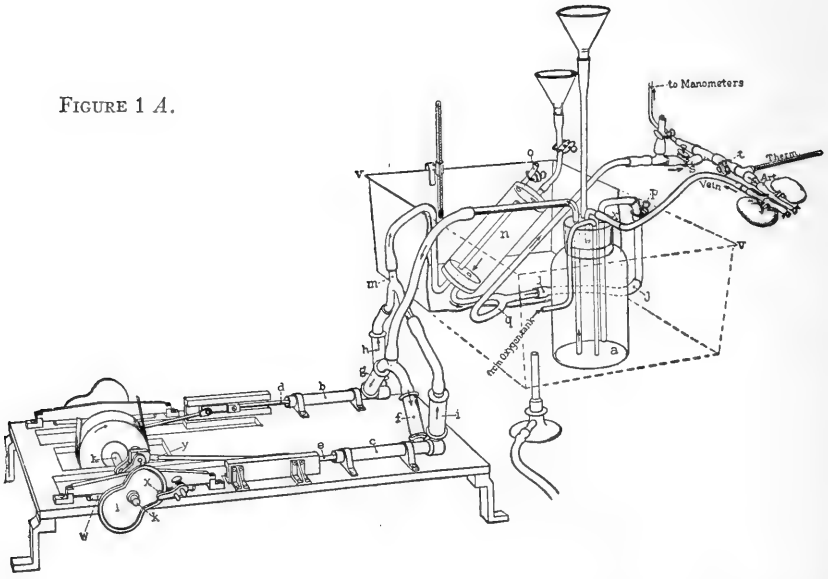

FIGURE 1.—Perfusion apparatus. The following letters are used: *a*, reservoir from which perfusion fluid is drawn to be injected into circulatory system and into which fluid returned from perfused organ is emptied. Oxygen is continuously bubbled through the fluid. *b*, *c*, cylinders from which fluid is alternately discharged into circulation. *d*, *e*, piston rods. *f*, *g*, *h*, *i*, valve boxes. Flow is permitted in the direction of the arrows. *j*, about 10 cm. rubber tubing in an otherwise rigid system. Serves to absorb secondary pulsatile waves caused by unavoidable mechanical imperfections in action of pump. *k*, crank axle to which power is applied and on which cam is fixed. The axle travels with sliding carriage (*w*). *l*, cam. Fixed on the axle (*k*) and bearing on point (*x*), it causes the sliding carriage (*w*) to move forward and back when the axle (*k*) revolves. This alters relative position of *k* with respect to cylinders (*b* and *c*), thus controlling the velocity of movement of pistons and so shape of pulse curve produced. *m*, point of union of tubes from cylinders. *n*, large air trap. *o*, vent through which air bubbles are allowed to escape. *p*, screw clamp. Serves to control peripheral resistance and so mean systemic pressure. *q*'

lated organ. It is the purpose of this paper to give some of the results obtained by the latter method of study.

THE PERFUSION APPARATUS EMPLOYED.

The essential parts are seen in the drawings shown in Fig. 1. Fluid is drawn out of the reservoir (*a*) and into the cylinder (*b* and *c*) by the back stroke of the piston (*d* and *e*), during which movement

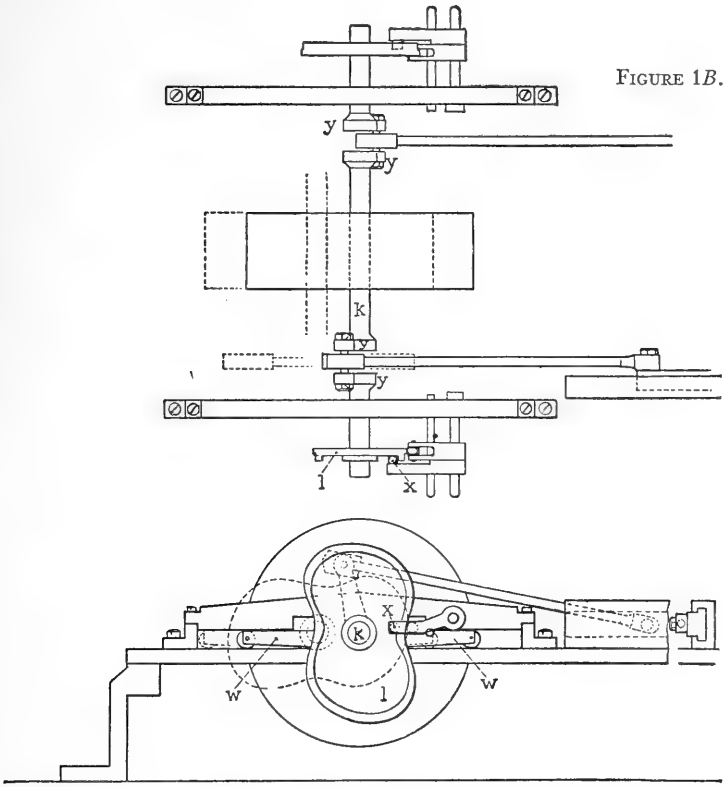

FIGURE 1*B*.

place where side tube leading to organ originates. *s*, screw clamp. Serves to control magnitude of pulse pressure in tube leading to organ. Beyond *s* tube divides, one arm going to organ and containing thermometer, the other arm going to maximum, minimum, and mean manometers. *t*, screw clamp. Substitutes for resistance of organ in preliminary adjustment of perfusion pressures. *v*, warm bath. *w*, sliding carriage, movements of which in response to changing cam radius alter piston velocity by altering relative position of *k* with respect to cylinders (*b* and *c*). *x*, bearing point of cam. *y*, crank axle.

the valve (*f* and *g*) is open. The forward stroke of the piston closes the valve (*f* and *g*) and opens the valve (*h* and *i*), giving a systolic discharge of the perfusion fluid into the circulatory system. During the forward stroke of one piston the other is returning, so that immediately one systolic discharge is completed another may be begun. The apparatus thus differs from the cardio-vascular system in that there is practically a continuous discharge into the circulatory tubes. The

latter are rigid,[7] so that each piston stroke must displace an amount of fluid equal to that forced into the tubular system. The waves of pressure within such an inelastic system must therefore have a shape which is the resultant of the forward velocity of the piston plus the resistance to the emptying of the system. Consequently the crest of the pulse wave so produced will occur at the time of greatest piston velocity, and all other points on such a wave will bear a direct relation to the piston velocity at corresponding times.

FIGURE 2. — To show the type of pulse curve produced by the apparatus when the crank axle (*k*) revolves about a fixed point.

The pulse curve. — The apparatus will yield a standard pulse curve when the crank axle (*k*) revolves about a fixed point. An example of this curve is given in Fig. 2. Obviously such a curve differs entirely from a sphygmogram. Since, however, it is essentially dependent upon the piston velocity, we can, by altering the latter, alter the type of pressure curve produced. This is accomplished by means of the cam (*l*) and sliding carriage (*w*). The sliding carriage (*w*) holds the axle (*k*) to which the power is applied and on which the cam (*l*) is fixed. A reduction in the radius of the cam at its bearing point (*x*) will cause the carriage and consequently the axle to move forward, thus adding velocity to the movement of the piston. Conversely an

FIGURE 3. — Pulse tracing obtained from the apparatus with the Hürthle manometer.

increase in the radius of the cam will cause the carriage and axle to move backward, thus reducing the velocity forward of the piston. Hence, with a properly constructed cam, we can control the relative velocity of the piston at any time of its stroke, and so control the shape of the wave of pressure produced by the discharge of fluid into the circulatory system. The modification of the standard curve as employed in the present research is reproduced in Fig. 3.

[7] Except for a short section (*j*). The slight elasticity thus provided serves to absorb secondary pulsatile waves caused by unavoidable mechanical imperfections in the action of the pump.

I wish here to express my indebtedness to my friend R. G. Van Name of Yale University, for the method of cam construction used, and for much valuable advice without which the development of the present method would have been impossible.[8]

The circulatory path. — This may be seen in the drawing. Both cylinders join a common tube (*m*), which empties into the upper part of the glass chamber (*n*). From the lower part of this chamber, which serves as an air trap and from which air bubbles may be permitted to escape through the vent (*o*), the tube continues to end beneath the surface of the fluid in the reservoir (*a*). At *p* a screw clamp is provided, by means of which the peripheral resistance and so the systemic pressure may be controlled. At the point *q* a branch tube leads to the organ to be perfused. This branch tube is provided with a thermometer and with a connection to the manometers for recording the perfusion pressures. The greater part of the system containing the perfusion fluid is submerged in the warm bath (*v*).

The venous flow from the organ is returned to the reservoir (*a*), after being measured by the outflow recorder described by Williams [9] and used in the United States Weather Bureau for recording the rainfall (Marvin's tipping bucket rain-gauge).[10] During an experiment oxygen or carbon dioxide, or a mixture of the two, is continuously bubbled through the fluid in the reservoir (*a*).

The control of the perfusion pressures. — The screw clamp (*p*) acts as peripheral resistance and so controls the mean systemic pressure. In the body alterations in the magnitude of the pulse pressure depend primarily upon alterations in the cardiac output.[11] In the apparatus this might be accomplished by a change in the length of the piston stroke (change in the length of the crank axle *y*). The necessary complication of construction makes such a method impracticable. The simple device employed serves the desired purpose without apparently any distortion of the pulse curve (see Fig. 4).

At the point *s* on the branch tube leading to the organ, another

[8] It seems unnecessary to occupy the space required to give the method of cam construction. I should be glad to furnish it upon request.

[9] Williams: Journal of pharmacology and experimental therapeutics, 1910, i, p. 457.

[10] Abbe: Report on the meteorology of Maryland, Maryland Weather Service, special publication, 1899, i, part III, p. 327.

[11] Erlanger and Hooker: *Loc. cit.*, p. 153.

screw clamp is placed. Constriction of this tube will alter the pressures distally by (1) lowering the mean pressure, (2) lowering the maximum pressure, and (3) raising the minimum pressure. If therefore it is desired to reduce the pulse pressure only, it is necessary to tighten both clamp *s* and clamp *p*. The proper adjustment of these

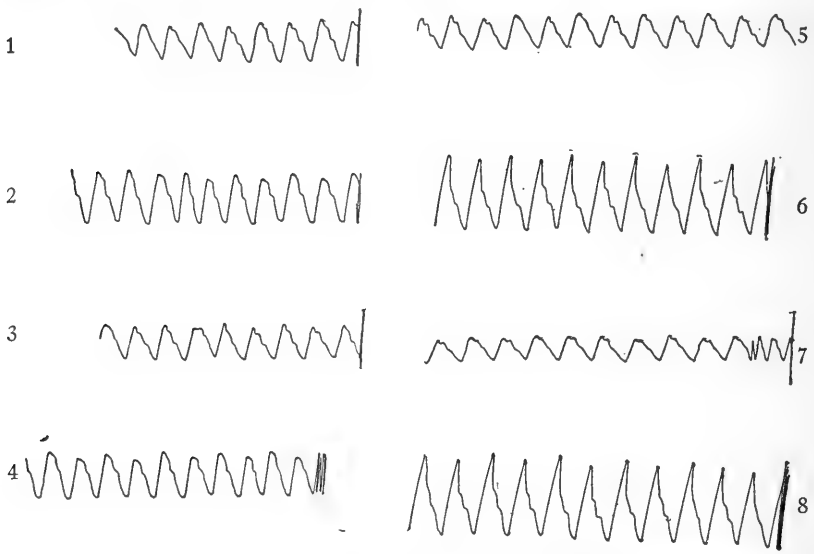

FIGURE 4. — Pulse tracings obtained with the Hürthle manometer during experiment of April 5, 1910. To show that the method employed to alter the magnitude of the pulse pressure does not seriously distort the pulse curve.

clamps is then obtained, when without alteration of the mean pressure the pulse pressure is reduced the desired amount. An increase of pulse pressure only is obtained by a similar manipulation, the clamps being opened instead of closed.

Preparation for an experiment. — The apparatus is set up as shown in the drawing. The temperature of the large water bath (*v*) is raised to about 40° C. The reservoir (*a*) and the glass chamber (*n*) are filled with the perfusion fluid. The arterial and venous tubes are temporarily connected and a screw clamp is applied at *t* with such a pressure that the amount of fluid passing this point is approximately equal to the normal outflow from the kidneys. The pump is now started and allowed to run until all air is removed from the system by collection and expulsion from the air traps. The pressures are then

approximately adjusted. The temperature is noted, and the gas to be used is bubbled through the reservoir (*a*).

The animal under morphia and ether is eviscerated, and the suprarenal glands are carefully tied off. The aorta and inferior vena cava are dissected out, and all branches except the renal are ligated for a distance of about 5 cm. below, and 2 cm. above the renal vessels. Cannulas are tied into the aorta and vena cava distally and looking towards the kidneys with the usual technique. Strong ligatures are laid, but not tied, about the great vessels above the kidneys. A cannula is tied into the urinary bladder, its distal opening being well below the body level, so that it acts as a siphon to keep the bladder empty. During this procedure the blood supply to the kidneys is at no time interrupted, and the organs have suffered very little handling.

Connection is now made between the arterial tube and the cannula in the aorta, all air being carefully excluded. The clamp which guarded the aortic cannula is removed, and for an instant the kidneys receive a mixture of perfusion fluid and normal blood. The clamp guarding the venous cannula is removed, and the ligatures previously placed on the aorta and vein proximally to the kidneys are quickly tied. The organs are thus isolated on the artificial perfusion without interruption of their circulation. After the perfusion fluid has had time to wash out all the blood previously contained by the kidneys, the venous cannula is connected with the tube leading back to the reservoir. During the time occupied in washing out the kidneys and while the perfusion fluid is thus wasting, opportunity is offered, if need be, to adjust the pressure conditions more accurately.

Discussion of Experiments.

The protocols of all the experiments here considered are collected at the end of the paper. The experiments reported were conducted to study the influence of the pulse pressure upon the amount of urinary filtrate formed and upon the presence of protein in the urinary filtrate. The technical difficulties of such a study are great, and many experiments failed completely. Successful experiments yielded, however, positive results in support of the hypothesis that the magnitude of the pulse pressure varies directly with the amount

of urinary filtrate and inversely with the amount of protein in the urinary filtrate.

The duration of the perfusions varied from forty minutes to two hours and a half. In general the longer experiments were those in which defibrinated blood in a dilution of one to two parts salt solution was used as perfusion fluid.

The perfusion fluid. — In the earlier experiments Locke's solution modified in various ways was employed. Œdema of the kidneys and of the surrounding tissues was very marked. In the other and later experiments defibrinated dog's blood diluted with either Locke's solution or with 0.9 per cent sodium chloride was used. Œdema, while not entirely absent, was rare and not so extensive. Both perfusion fluids yielded data. The defibrinated blood, however, naturally conserved normal conditions better. The addition of sodium nitrate to the Locke's solution was of no help in maintaining the blood flow.

Evidence of normal function. — The justification of conclusions drawn must depend largely upon the evidence that the organs approached the normal in function. The distinctly venous color of the outflow blood evidenced active oxidation. The urinary filtrate was neutral or very faintly acid to litmus, although the perfusion fluid was always alkaline. In the early stages of the experiments with defibrinated blood, the urinary filtrate contained very little protein. In one (April 21) the first urinary filtrate yielded a flocculent precipitate. The next seven samples contained much less protein (not enough to give a precipitate on boiling with dilute acetic acid). The remaining samples again yielded flocculent precipitates which progressively increased in amount. In these experiments also the urinary filtrate was free from blood coloration for a considerable time. Indeed in some of them it did not occur at all.

After varying lengths of time the formation of the urinary filtrate began to decrease. This usually occurred roughly coincident to a decrease in the venous outflow from the organs. It was possible to maintain the previous conditions by an increase of perfusion pressure. However, this change was taken as an indication that the organs were no longer normal, and served to delimit the experiments.

The progressive deterioration which invariably expressed itself by a gradual cessation of the blood flow might be due to œdema, the entrance of air bubbles producing embolism in the finer vessels, or to a

FIGURE 5. — About one third the original size. Part of the graphic record obtained in experiment of April 21, 1910. The two uppermost lines represent the maximum and minimum perfusion pressures in millimetres of mercury. The lowest line is the zero pressure. Each interruption of this line was produced by the dip of the bucket and represents a venous outflow of seven cubic centimetres. The second line from the bottom gives the time in periods of thirty seconds each. The line beneath the pressure tracings records the drops of urinary filtrate falling upon a tambour. The perfusion pressures were recorded by maximum and minimum valves leading to mercury manometers. The pressure values of the tracing must therefore be multiplied by two. The mean pressure was obtained by excluding the valves and interposing sufficient resistance to almost completely dampen the excursions of the mercury columns. The record shows the quick response of the urinary filtrate to changes of pulse pressure.

vaso-constriction due to the chemical nature of the perfusion fluid. Œdema almost invariably occurred in the experiments with Locke's solution. Frequently air bubbles could be seen to collect in the arterial cannula. This was especially true in the experiments with defibrinated blood. The viscosity of this fluid apparently retarded the escape of the minute bubbles into the air traps. The possibility of a chemical vaso-constriction need not be discussed in the absence of experimental evidence.

Amount of urinary filtrate. — The response of the urinary filtrate to changes in the magnitude of the pulse pressure is clearly shown in the accompanying graphic record (Fig. 5).

This is a part of the record obtained in the experiment of April 21, 1910. The pointer writing the minimum perfusion pressure is displaced about 6 mm. to the right of the vertical for the other writing

points. With each increase of pulse pressure there is an increase in the number of drops of urinary filtrate. The mean pressure is practically constant throughout (94–97 mm. Hg). The protocols of all the experiments show clearly that this small difference is insufficient to account for the urinary changes. Undoubtedly considerable changes in mean pressure do affect the amount of urinary filtrate,[12] but in this paper we are dealing with practically constant mean pressures.

The minimum pressure is lower during the periods of greater excretory activity. Probably the decrease of this factor as such plays no part in renal activity.

The maximum pressure is higher during the periods of greater excretory activity. Change in the value of this pressure is parallel with the pulse pressure change, and is a necessary concomitant of the latter. In the method of study employed the maximum pressure could not be changed independently. It is unlikely that such a change can occur in the normal circulation. Therefore it is probable that this factor alone could not influence renal function.

The conclusion that the amount of urinary filtrate formed is dependent upon the magnitude of the pulse pressure is based upon six experiments in which the value of the pulse pressure was changed over thirty times.

Blood flow through the organs. — The venous outflow responds to perfusion pressure changes, as does the urinary filtrate. In the graphic record reproduced in Fig. 5 each break in the lowermost line represents an outflow of 7 c.c. In this, as in all of the experiments, the rate of outflow shows a steady decrease. The influence of the pressure changes upon the rate of outflow, the figures of which are given in the protocols, is graphically shown in the accompanying plotted curves (Fig. 6). The curve of outflow tends to fall in a step-like descent. The steps are, however, not of the same depth. A considerable fall occurring in a period of low pulse pressure is much lessened, entirely stopped, or converted into an increase in the following period of high pulse pressure. This would indicate that the higher pulse pressure has a beneficial effect upon the blood flow. The influence of the magnitude of the pulse pressure upon the rate of blood flow through the kidneys may be reproduced upon an artificial capillary bed. Thus, when the organ was replaced in the perfusion system by

[12] Sollmann: This journal, 1905, xiii, p. 253.

a screw clamp on the arterial tube, the rate of flow through the constricted area was with a large pulse pressure 48 c.c., with a small pulse pressure 36 c.c., and with an intermediate pulse pressure 44 c.c. per minute.

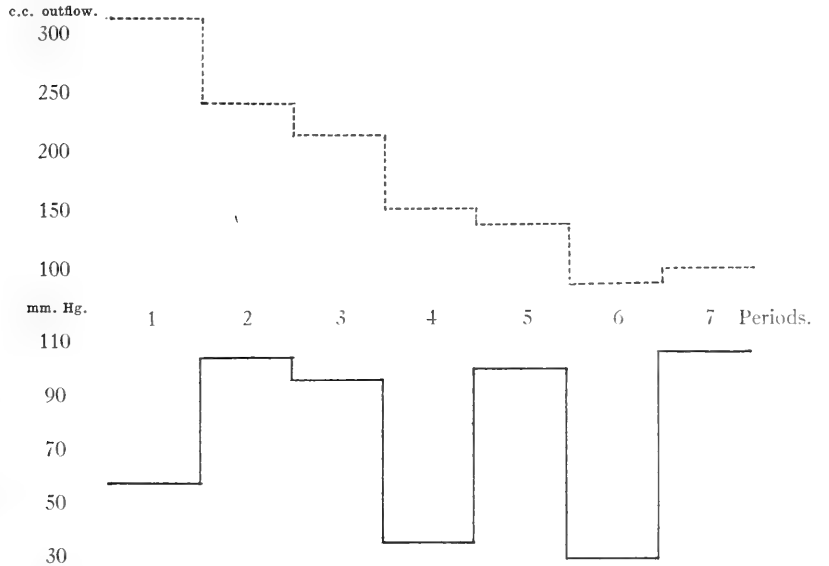

FIGURE 6. — The results obtained in experiment of April 5, 1910, plotted to show the relation of the venous outflow from the organs to the pulse pressure. The horizontal sections of the lower (solid) line represent the values for the pulse pressure in millimetres of mercury during the different periods. The corresponding horizontal sections of the upper (broken) line represent the rate of venous outflow in cubic centimetres; a continuous decrease in the rate of outflow, except in the last period, will be noted. This decrease is not, however, uniform, but is more marked in the periods of low pulse pressure. An exception is seen in the second period when an increased pulse pressure is accompanied by an unusually large decrease in outflow. This may perhaps be accounted for by the incomplete adaptation of the organs to the experimental conditions.

The beneficial effect of the pulse pressure upon the rate of blood flow through the organ is evident in each of the three experiments on this point.

The effect of the pulse pressure upon the protein content of the urinary filtrate. — Two experiments were performed in this connection. They both showed clearly that the amount of protein in the urinary filtrate bears an inverse relationship to the magnitude of the pulse pressure. In several of the experiments with defibrinated blood, primarily directed to a study of the amount of urinary filtrate,

the urine was examined for protein. The protocols state that there was no apparent difference in the protein content to correspond with the differences in the magnitude of the pulse pressure. This observation was undoubtedly due to the brevity of the periods in which the urine was collected. When these periods were increased in length, the differences in protein content came out strikingly enough to leave no doubt.

Protocols of Experiments.

The protocols of the experiments above discussed follow:

May 19, 1909. About 1200 c.c. blood obtained from a large dog. Defibrinated by whipping. Filtered through paper and diluted to approximately 5000 c.c. with 0.9 per cent NaCl.

Small bitch eviscerated and the kidneys prepared for perfusion in the usual manner. Peptone (0.3 gm. per kilo) injected into left femoral vein.

Pump started at 1 P. M. Dog connected and proximal ligatures tied. At 3 P. M. it was discovered that both ureters had been tied off. They were distended with urine, which began to flow as soon as the ligatures were removed.

The rate of blood flow from the organs was not recorded; it was distinctly venous in color. The blood was "arterialized" simply by exposure to the air. The urinary filtrate was slightly blood-tinged.

It was subsequently found that the proximal ligature on the vena cava occluded the left renal vein. Consequently the experiment represents the results from the perfusion of the right kidney only.

Perfusion pressures in mm. of Hg.				Urinary filtrate drops in 5 min.	Temperature.	Time.	Procedure.
Mean.	Max.	Min.	Pulse.				
115	150	85	65	10	39.5° C.	3.07–3.12	
115	135	95	40	7	39.	3.13 3.16–3.21	Pressure changed
115	150	85	65	10½	38.5	3.22 3.25–3.30	Pressure changed
118	135	95	40	6	37.5	3.31 3.34–3.39	Pressure changed
110	180	70	110	9	...	3.40 3.43–3.48	Pressure changed

The data obtained would indicate that the amount of the urinary filtrate formed is independent of (*a*) the temperature, (*b*) the mean perfusion pressure, (*c*) the maximum perfusion pressure, and (*d*) the minimum perfusion pressure. The only factor which varies consistently with the urinary filtrate is the magnitude of the pulse pressure.

Jan. 17, 1910. Perfusion with Locke's solution, made up as follows:

In 4 litres	36.0 gm.	NaCl
	1.22 gm.	$CaCl_2$
	1.68 gm.	KCl
	0.8 gm.	$NaHCO_3$
	4.0 gm.	Glucose

Fox terrier bitch eviscerated. Ligatures and cannulas as usual. No peptone used. Animal in excellent condition.

Pure carbon dioxide was fed to the perfusion fluid throughout the experiment.

Perfusion pressures in mm. of Hg.				Outflow from organs. No. of sec. required to fill given vessel.	Urinary filtrate. No. of sec. required for 10 drops to fall.	Temperature.	Period.
Mean.	Max.	Min.	Pulse.				Min.
85	120	66	54	7, 12, 8, 7½, 7½, 7½.	13 [1]	39.75° C.	30
84	100	76	24	27	16	39.75	10
84	120	66	55	24	11	39.	10
90	102	84	18	32	20	38.75	10
90	125	75	50	31	11	40.	6
90	96	84	12	54	27	39.	6

[1] Note that a decrease in the values of the figures indicates an increased rate of flow, and *vice versa*.

The experiment was interrupted at the end of one hour and thirteen minutes by the entrance of visible air bubbles into the arterial cannula. The duration of the periods of observation indicate the time which elapsed from the change of the perfusion pressures until the readings were made.

The figures show that change in the mean perfusion pressure has no conspicuous effect upon either the blood flow through the organs or the amount of urinary filtrate formed. Alterations in the magnitude of the pulse pressure cause both the amount of the urinary filtrate and the blood flow to vary in the same sense. There is quite a steady decrease in the rate of blood flow through the organs throughout the experiment, which is more prominent in the periods of low pulse pressure.

The urinary filtrate was neutral to litmus.

March 24, 1910. Perfusion with Locke's solution made up as follows:

In 1 litres		
	40.0 gm.	NaCl
	1.22 gm.	$CaCl_2$
	1.68 gm.	KCl
	0.8 gm.	$NaHCO_3$
	4.0 gm.	Glucose
	0.3 gm.	$NaNO_2$.

The sodium nitrate was added in this, and in the experiments of March 31, April 5, April 9, and April 11 with the hope that it would maintain vascular relaxation. The present data are insufficient for a positive answer as to its value.

Oxygen gas used. The temperature throughout was 35°–36° C. No peptone was injected. The accompanying figures were taken from a graphic record:

Perfusion pressures in mm. of Hg.				Drops of urine in three minutes.
Mean.	Max.	Min.	Pulse.	
115	140	102	38	17
115	152	90	62	24
115	136	116	20	23
115	150	98	52	25

The amount of urinary filtrate formed varies directly with the magnitude of the pulse pressure. The difference is not marked, due probably to the fact that the periods of observation were short

(three to five minutes), so that it was necessary to count the drops of urine almost from the instant the pressure conditions were changed. In those experiments in which time was allowed for the kidneys to become adjusted to the new conditions this difference is very much more striking. No record was obtained of the rate of venous outflow.

March 31, 1910. Perfusion with Locke's solution as used in experiment of March 24, except that 45 gm. instead of 40 gm. NaCl were used.

Oxygen gas was used. The temperature throughout was 38° C. Considerable œdema occurred. The figures were obtained from a graphic record.

Perfusion pressures in mm. of Hg.				Drops of urine in three minutes.
Mean.	Max.	Min.	Pulse.	
118	138	96	42	16
118	158	78	80	29
118	130	108	22	9
118	154	84	70	12
118	132	100	32	9

The urinary filtrate follows the pulse pressure. The amount formed obviously decreased as the experiment progressed. This decrease may have been due to the progressive œdema. No record was obtained of the rate of venous outflow.

April 5, 1910. Perfusion with Locke's solution made up as follows:

In 4 litres	NaCl	45.0 gm.
	KCl	1.68 gm.
	$CaCl_2$	1.22 gm.
	Glucose	4.0 gm.
	$NaHCO_3$	0.8 gm.
	$NaNo_2$	0.3 gm.

Oxygen gas was used. The temperature throughout was 38° C. No urinary filtrate, due probably to a severe hæmorrhage during the

operation. The experiment served for a study of the influence of the pulse pressure upon the rate of blood flow through the organs. The figures were obtained from a graphic record.

Perfusion pressures in mm. of Hg.				Venous outflow in four-minute periods.	Decrease of venous outflow.
Mean.	Max.	Min.	Pulse.		
				c.c.	c.c
90	130	70	60	315	...
90	150	48	102	245	70
90	146	50	96	224	21
90	122	82	40	161	63
90	152	54	98	147	14
90	118	83	35	98	49
90	152	48	104	105	+7

The venous outflow decreased throughout the experimental period here recorded, except at the end, when an increased outflow occurred. The figures show, however, that the amount which the outflow decreases is very distinctly less during the periods of large pulse pressure than during those of small pulse pressure. This fact is more obvious in the plotted curves shown in Fig. 6.

Apparently in this, as in all of the experiments here reported, the conditions are such that the rate of blood flow through the organs is abnormal, and this abnormality increases as the experiment proceeds. It is much more accentuated during the periods of low pulse pressure. In other words, the larger pulse pressure tends to maintain the normal condition of blood flow.

There are reproduced in Fig. 4 the sphygmograms obtained during the different periods of this experiment with the Hürthle manometer. The numbers on the tracings correspond serially to the blood pressures given in the table, except the last (tracing 8). It will be noted that the character of the pulse wave remains practically constant in the different periods in spite of the variation of the pressure of the writing lever against the drum. There is only the change in the vertical elongation of the curve which might be anticipated to occur with altered pulse pressure values.

April 14, 1910. Perfusion with defibrinated blood diluted with an equal part of Locke's solution (formula of March 31).

Oxygen gas was used and the temperature was maintained at 37° C. The figures were obtained from a graphic record. The rate of venous outflow was not satisfactorily recorded and is here omitted.

Perfusion pressures in mm. of Hg.				Urinary filtrate, drops in five minutes
Mean.	Max.	Min.	Pulse.	
92	114	66	48	26
98	102	94	8	22
94	116	64	52	23
90	98	86	12	20
92	126	62	64	25
94	96	90	6	14

The only factor conspicuously associated with the change in the amount of urinary filtrate is the pulse pressure, the amount of urinary filtrate varying directly with the magnitude of the pulse pressure.

The urinary filtrate was neutral to litmus.

April 21, 1910. Perfusion with defibrinated blood diluted with an equal part of 0.9 per cent NaCl.

Oxygen gas was used. The temperature was 33°–35° C. The figures were obtained from a graphic record, part of which is reproduced in Fig. 5.

This experiment is perhaps the most satisfactory of the series. It lasted for about two hours and a half. The urinary filtrate varies conspicuously with the magnitude of the pulse pressure, as does also the venous outflow from the organs. There is a tendency for the outflow to decrease in amount throughout the experiment. In the second and last periods, however, there is an actual increase coincident to the change to a larger pulse pressure, and in two of the other periods in which the large pulse pressure replaced the small, the decrease of outflow is interrupted.

Perfusion pressures in mm. of Hg.				Urinary filtrate, drops in five min.	Venous outflow in five min.	Decrease of venous outflow.
Mean.	Max.	Min.	Pulse.			
					c.c.	c.c.
94	115	82	33	10	95	...
94	148	64	84	25	111	+16
95	116	84	32	8	74	27
95	126	63	63	19	70	4
92	112	85	27	14	65	5
92	136	69	67	22	65	0
92	109	86	23	20	60	5
95	138	68	70	32	60	0
95	134	70	64	28	42	18
97	110	90	20	15	23	19
..	148	62	86	20	25	+ 2

The urinary filtrate collected during the first five periods was entirely free from blood coloration. Subsequently the "bloody" color steadily increased. All the samples when boiled and treated with dilute acetic acid were found to contain protein. The first sample yielded a flocculent precipitate. The following seven samples showed opacity, without evident precipitate. The remaining samples yielded flocculent precipitates which progressively increase in amount. It is perhaps fair to assume that the large amount of protein present in the first sample was due largely to the operation, and other manipulation of the kidneys, which by disturbing their circulation or otherwise affected the organs injuriously. With the return to more normal conditions there was a slight recovery, which lasted for some time.

There was no apparent association between the amount of protein in the urinary filtrate and the magnitude of the pulse pressure. This was doubtless due to the shortness of the observational periods (five minutes in the first eight), which did not permit of a complete adjustment to the new conditions before a change again occurred. This explanation is supported by the experiments especially directed

to this point, and by the graphic records which show that the formation of the urinary filtrate lags behind the change of perfusion pressure.

The urinary filtrate was neutral to litmus.

June 8, 1910. Perfusion with defibrinated blood diluted with two and one half parts of 0.9 per cent NaCl.

Oxygen gas was used. The temperature was maintained at 39° C.

Two periods only were observed in order to obtain enough urinary filtrate for accurate analysis and to insure that the organs were adapted to the pressure conditions under which the urinary filtrate was formed. The perfusion pressures were:

	Mean.	Max.	Min.	Pulse.
I	122	150	98	52
II	116	174	74	100

The urine taken from the bladder before the perfusion was begun contained a very slight trace of coagulable protein. The two specimens of urinary filtrate obtained during the perfusion contained sufficient protein to yield a flocculent precipitate on boiling with the addition of dilute acetic acid. The specimen obtained in the second period with the large pulse pressure contained very distinctly less protein than that obtained in the first period with the small pulse pressure. Assuming that the prolongation of the experiment increased the deviation of the functional activity from the normal, we might expect an increased protein content, such as occurred in the experiment of April 21, 1910, rather than the contrary. The results obtained, therefore, indicate that the magnitude of the pulse pressure bears an inverse relation to the protein content of the urinary filtrate.

Both specimens obtained reacted neutral to litmus, and were entirely without tinge of blood.

June 30, 1910. Perfusion with defibrinated blood diluted with an equal part of 0.9 per cent NaCl.

Oxygen gas was used. The temperature was maintained at 38° C.

The pressures were maintained until the urinary filtrate was sufficient in amount for satisfactory tests of protein content and to insure adaptation of the organ function to the new conditions.

The control sample of urine taken from the bladder was acid to litmus, and contained only a very faint trace of protein.

Period.	Perfusion pressures in mm. of Hg.				Protein in urinary sample.
	Mean.	Max.	Min.	Pulse.	
1	140	186	90	90	Faint trace; more than in control.
2	140	156	126	30	Flocculent precipitate; much more than in first period.
3	140	194	86	108	Faint trace; about the same amount as in first period.

There was no blood tinge to any of the samples thus collected. The last specimen was doubtfully alkaline to litmus.

This experiment shows clearly that the magnitude of the pulse pressure definitely influences the amount of protein in the urinary filtrate.

Summary.

1. A perfusion apparatus is described which yields a pulsatile wave of pressure similar to the normal pulse wave and which allows of alteration in the magnitude of the pulse pressure.

2. The use of the apparatus in a study of the isolated (dog's) kidneys yielded the following results:

a. With a constant mean perfusion pressure the amount of urinary filtrate formed varied *directly* as the magnitude of the pulse pressure.

b. With a constant mean perfusion pressure the amount of protein in the urinary filtrate varied *inversely* as the magnitude of the pulse pressure.

c. With a constant mean perfusion pressure, the rate of blood flow through the organs varied *directly* as the magnitude of the pulse pressure.

SENSORY CHANGES IN THE SKIN FOLLOWING THE APPLICATION OF LOCAL ANESTHETICS AND OTHER AGENTS. — I. ETHYL CHLORIDE.

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RECENT physiological studies of skin sensations and anatomical investigations of nerve endings in the skin have shown a much greater complexity of nerve structure and an association of the sensory functions of the nerves which had previously been overlooked. Following the division of nerves, Head and others have been able to find a dissociation of skin sensations different from that previously reported, and this work has led to a new hypothesis regarding the functions of the peripheral afferent nerves.[1] Examinations of individuals in whom the peripheral nerves have been cut or injured have shown a marked and hitherto undescribed variation in the sensibility to light touch, pressure, pain, and temperature stimuli.

The conclusions of Head regarding the combination of sensations following destruction of or injury to nerves are of special interest to us in the present work. Head finds that the sensory mechanism of the peripheral nerves consists of three systems:

"A. Deep sensibility, capable of answering to pressure and to movement of parts, and even capable of producing pain under the influence of excessive pressure or when the joint is injured. The fibres subserving this form of sensation run mainly with the motor nerves and are not destroyed by division of all the sensory nerves to the skin.

"B. Protopathic sensibility, capable of responding to painful cutaneous stimuli and to extremes of heat and cold. This is the great reflex system producing a rapid, widely diffused response, unaccompanied by any definite appreciation of the locality of the spot stimulated.

"C. Epicritic sensibility, by which we gain the power of cutaneous

[1] Head, Rivers, and Sherren: Brain, 1905, xxviii, p. 111.

localization, of the discrimination of two points and of the finer degrees of temperature, cold and warm."

The following is Head's account of the sensation changes coincident with lesions of nerves, although it must be understood that these conclusions give only the grosser results of his extensive work:

"Loss of epicritic sensibility abolishes: recognition of light touch over hairless parts or parts that have been shaved; cutaneous localization; discrimination of compass points; appreciation of difference in size, including the accurate discrimination of the head from the point of a pin apart from the pain of the prick (acuæsthesia); discrimination of intermediate degrees of temperature, from about 25° C. to about 40° C.

"Loss of protopathic sensibility abolishes: cutaneous pain, especially that produced by pricking, burning, freezing, together with that of stimulation with the painful interrupted current; over hair-clad parts plucking the hairs ceases to be painful; sensations of heat from temperatures over 45° C.; sensations of cold from temperature below 20° C.

"After destruction of all cutaneous afferent fibres the part is still endowed with deep sensibility; pressure can be recognized and its gradual increases appreciated; pain is produced by excessive pressure (measured by the algometer); movements of the muscles can be recognized; the point of application of pressure can be localized; the patient can recognize the extent and direction of movement passively produced in all joints within the affected area." [2]

These conclusions of Head have been accepted in a general way, but it is more than likely that they will have to be modified slightly in accordance with further studies and examinations of patients. For details of the analyses the reader is referred to the articles by Head and his collaborators and by others.[3]

The conclusions from these results so greatly alter the views of the functions of the sensory nerves that we considered it advisable to investigate the sensory changes following the application of local anesthetics to the skin. The present series of papers has a twofold func-

[2] Head and Thompson: Brain, 1906, xxix, p. 551.

[3] Head, Rivers, and Sherren: *Op. cit.*; Head and Thompson: *Op. cit.*; Head and Sherren: Brain, 1905, xxviii, p. 241; Rivers and Head: Brain, 1908, xxxi, p. 323; Trotter and Davies: Journal of physiology, 1909, xxxvii, 134; Franz: Journal of comparative neurology and psychology, 1909, xix, pp. 107, 215.

tion: to contribute to the analysis of the sensations from the skin, and at the same time to fill a gap in our pharmacological knowledge regarding the actions of the so-called local anesthetics and analgesics.

General Methods.

Throughout the present series of experiments certain areas on the hand and arm were selected for careful examination. These areas were carefully examined before any application of the agent which was to be investigated and the normal sensitiveness of the part was determined. For most of the work an area on the radial side of the forearm, about 4 cm. from the bend of the elbow, was selected. This area was 5 cm. square. It was accurately divided into twenty-five squares of one square centimetre each. The skin was marked with a 10 per cent solution of silver nitrate, so that at the time of the experiments the tests could be made at definite places, and so that the areas were accurately defined and kept constant throughout the series of experiments. After the solution of silver nitrate had been applied to and had dried on the skin, the lines were developed by the application of a photographic developer solution (hydroquinone). This produced a relatively permanent stain which made the areas distinct and which persisted for three or four weeks before it became too faint and had to be renewed. The application of the silver nitrate was always made at least a full day before experimental determinations on the part were made.

The area selected for the experiments was partly endowed with long hairs and partly free from them. In most of the experiments this part was carefully shaved to prevent disturbances of the light touch experiments, and the shaving was always done six hours, and sometimes a full day preceding the experiments, since there was the possibility (although we had no definite indication that this was the case) that the application of hot water, lathering, and shaving might produce changes in the sensitiveness of the skin. In certain experiments it was deemed advisable to leave the part unshaved, and this was always done when we wished to test the sensibility of the hairs.

In all the work the two authors acted as subjects. Both were in good health at the time of the experiments, and the tests were made at approximately the same time of the day. Each had considerable previous

experience in acting as a subject in similar experiments and may properly be considered a skilled subject. At the time of the experiments general introspective accounts were made by the subject in addition to the measurements, both of which were recorded by the experimenter.

In some preliminary experiments on the effects of different agents on touch sensations we tested the sensibility of the skin to light touch by means of a camel's hair brush. In these early tests we found certain deviations from the normal which could not be expressed in any but general terms, and it was therefore deemed advisable to use a more accurate instrument. For effects that pass away rapidly it is impossible to utilize the von Frey hairs for the determination of threshold touch values, for a number of touch hairs must be used and only a few points can be examined with any degree of thoroughness. The touch weights which have been utilized in this work have the same fault, for they require a considerable amount of time and are not suitable for the determination of conditions that are fugitive. The use of the single von Frey hair with the possibility of adjustment to different lengths saves some time, but it also requires more time than is given for these experiments. For these reasons, because it can be used with little expenditure of time and because it gives relatively accurate results, and, moreover, is at all times comparative in its reading, we selected the Block esthesiometer, which had previously been employed by one of us in other work on the skin sensations.[4] This instrument enabled us to determine the amount of pressure necessary to produce a sensation of light touch if the sensation could be produced by a pressure under 2000 mg. Since we were concerned with relatively increased or decreased sensibilities, as measured by the amount of stimulus necessary to produce sensations, we have given in the tables which follow the figures on the instrument rather than the estimations of unit-area-pressure such as have been deemed essential by von Frey.[5]

Pain sensations were tested with another instrument similar to the well-known Cattell algometer, but with a finely pointed needle as the

[4] Franz: *Op. cit.*; Franz: This journal, 1907, xix, p. 23.

[5] von Frey has worked out a formula for the threshold of pressure in relation to the cross section of the stimulating surface. This is of no special value in the present work because the area of stimulation was kept constant.

stimulating surface, and, consequently, with a much weaker spring. By the use of both instruments very small areas can be investigated, and the measurement of threshold values be made for the touch and pain points.

The ethyl chloride was applied to the skin from a tube which was held from 30 to 40 cm. from the part which was to be tested. We attempted to have the central part of the square on the arm affected by the spray and to have the surrounding area kept normal. At the time of applying the ethyl chloride the outer area of 16 sq. cm. was protected by pieces of a heavy rubber bandage and of gauze to prevent the action of the ethyl chloride upon this area, but in practice it was found that the spray affected not only the inner square but that some escaped and acted upon part of the outer area. The amount of area affected by the spray was always determinable, and in each experiment we noted the amount of area affected and compared the sensations from this area with those from the areas known to have been unaffected by the ethyl chloride. By the application of the ethyl chloride the skin was frozen, but little or no freezing of the underlying tissues took place. Not more than one application of the ethyl chloride was made to the same area on one day. In the application of other agents which were not to be made by means of spray the exact location of the application could be previously determined and kept constant. In each case careful notes of the location of the application were made, and, as in the case of the ethyl chloride, not only this area but the surrounding supposedly normal regions were carefully examined.

After the application of any agent, tests were begun immediately, *i. e.*, within ten seconds, or as soon as the ethyl chloride tube could be laid aside and the testing instruments taken up. For obvious reasons the different forms of sensibility were not tested at each sitting in the same order. At times the sensibility to light touch was first investigated; at times temperature sensations; at times the hairs were stimulated by a brush or by pulling; at times the pain thresholds were first investigated; and at other times the general feelings of the subject when his or the experimenter's finger were brushed lightly over the area were described and noted. At a sitting we were usually able to make tests of only two or three kinds of sensibility.

It is necessary to mention that it was impossible to have the application of the ethyl chloride and other agents exactly alike in all ex-

periments. It is well known that the amount of the freezing and the extent of the frozen area depend upon a number of factors: the condition of dryness or moistness of the skin, the amount of the ethyl chloride employed, the height of the tube from the skin and the consequent spreading of the spray, the temperature of the skin and of the room and the consequent rapidity of evaporation. A sufficient number of experiments were performed, however, so that we are satisfied the anesthetic or analgesic results, *i. e.*, from a sensation standpoint, are due to distinct temporary alterations in the irritability or conductivity of the end organs in the skin.

Results.

When the ethyl chloride is sprayed on the skin, there is at first a sensation like that from a light touch, probably produced by the rain-like dropping of the spray; with this there is a sensation of coolness. The sensation of touch from the application of the spray soon disappears, and the coolness sensation passes over into one of distinct cold, which in a very short time is felt only at the edges of the area affected by the spray. On the skin frost is formed, which disappears in a few seconds, leaving the skin blanched, and at this time it is relatively or absolutely anesthetic and analgesic. After the passing of the frozen stage the skin is reddened and continues to be so for a length of time varying with the amount of ethyl chloride used (or the amount of the freezing of the subcutaneous tissues). During the stage of anesthesia the sensibility of the skin to the different forms of specific stimuli is altered, the amount of alteration, *i. e.*, the degree of anesthesia, and the duration of the change varying for the different sensations.

Temperature sensations. — Immediately after the disappearance of the frost, temperature stimuli are not felt on the sprayed area except as pressures. Soon the area becomes sensitive to cold and cooled objects, but these have not the same sensation effect as they have on normal areas. At the time the sensations of coolness can be aroused the application of warm or even of hot objects produces no corresponding sensation and they are felt only as pressures. The correspondence in sensation between the normal and the sprayed areas does not occur for a relatively long period, the difference being noticeable for a longer time than the disturbance in touch sensation. Following are some records of the results of experiments in this field:

April 24, 1909. — Subject F. Anesthesia slight. Fifteen minutes after application of ethyl chloride, a cold glass rod was appreciated as cold in the normal area, but only as cool within the area acted upon by the ethyl chloride. Warm objects did not produce the sensation of warmth when applied to the anesthetic area.

June 10, 1909. — Subject R. Anesthesia marked. Two and a half minutes after the application: a warm metal rod (45° C.) produced the sensation of warmth, but it was much less distinct than when applied to the normal area, a cold stimulus (8° C.) was felt to be decidedly cold in the normal area but only cool in the anesthetic area. Forty-five minutes after application: cold (9° C.) was felt as cold in both normal and anesthetic areas, and warm (40° C.) as warm. In all the experiments the sensations appeared to be more distinct when the stimuli were applied to the normal area.

June 10, 1909. — Subject F. Anesthesia marked. Two minutes after application: hot (60° C.) was felt to be hot in the normal area, but only warm in the anesthetic area; cold (6° C.) cool on the sprayed area, cold on the normal area. Fifteen minutes after application: warm (40° C.) was felt as warm on the normal area, indifferent on the sprayed area; cold (8° C.), cold on the normal and only cool on the anesthetic area. Thirty minutes after application: warm (37° C.) and cold (8° C.) gave approximately similar sensations from both areas.

June 12, 1909. — Subject F. Anesthesia marked. One and a half minutes after application: 10° C. felt cold in normal, cool in anesthetic area; 40° C. warm in normal and indifferent in anesthetic area. Thirty minutes after application: 16° C., cold in both areas, but with a slight dulling in the area to which the ethyl chloride had been applied; 57° C. felt warm and apparently the same in both areas. Sixty minutes after application: the temperature sensations were alike in both areas.

June 13, 1909. — Subject R. Anesthesia marked. One minute after application: 10° C. felt cool in both areas, but colder in the normal zone; 50° C., warm in the normal but no temperature sensation in the anesthetic area. Thirty minutes after application: no difference in temperature sensations were discovered.

From these notes it will be seen that the loss of ability to properly sense warm and cold objects continued for only a short time after the application of the ethyl chloride and that partial recovery took place within about two minutes after the freezing of the part. One peculiarity was observed, *viz.*, that soon after the beginning of recovery the hot and cold stimuli did not produce corresponding sensations of hot-

ness and of coldness in the anesthetic part, but only the sensations of warmth and coolness. This difference in sensation was further brought out when, instead of applying the point of the stimulating areas to the skin, the length of the stimulant was applied so that it partly stimulated the normal and partly the anesthetic areas. The description of the resultant sensation given by the subject was that the stimulus felt hot at either end and only warm in the middle (the rod being laid across the anesthetic area so as to stimulate it and the normal areas on either side), or that it felt warm at the ends and indifferent (or only a pressure) in the middle. These latterly mentioned results are like those noted above, and are in line with results obtained by one of us in the examination of temperature sensibility after the lesion of nerves.[6]

Sensibility of the hairs. — When a hair on a normal part of the skin is lightly brushed, there results a sensation similar to that of light touch; when it is pulled, the sensation becomes clearer and more intensive, and when sufficient traction is exerted, there ensues a distinct feeling or sensation of pain. This pain appears to differ in character from that produced by extremes of pressure, as for example that produced by an algometer, for it is rather burning in character. When traction is further increased, the hair may be plucked out with its bulb, resulting in a stinging pain almost like that of a burn. Both the light and heavy tractions causing pain produce sensations which are accurately localizable, while the brushing of the hair gives a rather poorly localizable sensation. If the hairs be very long, the sensation resulting from light brushing may be localized on the average closer to the part of the skin over which the end of the hair lies, but the sensation from plucking is always localized, as has been said, accurately, *i. e.*, at the point of insertion of the hair in the skin.

In the experiments variations of both of these forms of sensibility were found. The following are protocols of a few experiments:

May 8, 1909. — Subject R. Slight amount of ethyl chloride, not sufficient to freeze the skin. Within twenty seconds after the application of the ethyl chloride there was found no variation in the sensibility of the hairs; the sensations like those of light touch (from brushing) and those of pain were apparently normal and were obtained from all the hairs stimulated.

[6] Franz: Journal of comparative neurology and psychology, 1909, xix. 19. See especially pp. 223–233.

May 8, 1909 (second series). — Subject R. Skin frozen to a medium degree. Immediately after freezing the skin, it was found that the sensations from traction were present, although at first there were no sensations from lightly brushing the hairs. The latter sensations returned rapidly, within three minutes, although for five minutes a small area of the arm, about 5 cm. in diameter, was still dull (to the stimulation of light touches on the skin, not on the hairs).

May 29, 1909. — Subject R. Marked freezing. Twenty-one seconds after the application: the sensations from lightly brushing the hairs were obtained, but they appeared to be dull; the sensations from traction were absent, and no pain resulted even when the hair was plucked out. In fifty-five seconds: a dull pain from traction was first felt, and this state continued for about a minute and a half. Two minutes after the application of the ethyl chloride, the sensations from brushing the hairs were normal, but in five minutes the pain resulting from the pulling of the hairs had not become normal in quality (or quantity).

May 29, 1909 (second series). — Subject R. Anesthesia of medium degree. Ten seconds after application: touches felt on the skin, but the movement of the hairs was not accompanied by a sensation. In twenty-three seconds: there was no sensation, no pain, on pulling the hairs, but the sensations similar to touch appeared to be normal. Sixty seconds after application: pain on pulling returned, but the sensation was not so keen as in normal parts. For two minutes the sensations accompanying traction remained different from the normal.

May 29, 1909 (third series). — Subject R. Medium degree of anesthesia. No change from the normal was noticed when the hairs were brushed or slightly moved, but on traction pain was first felt in one hundred seconds, and this kind of sensation continued to be duller than that on normal parts for five or six minutes.

May 29, 1909. — Subject F. Slight degree of anesthesia. The sensations from stroking the hairs was normal when first investigated, but no pain on traction appeared until fifteen seconds after the application of the anesthetic. The pain from pulling was acute at thirty seconds, and at sixty was diffuse and not localized as is the pain from normal parts.

June 10, 1909. — Subject R. Anesthesia marked. The brushing of the hairs was first felt in about thirty seconds, but at that time no sensations were obtained from the pulling of the hairs. The pain sensations from traction returned in two and one half minutes.

June 12, 1909. — Subject F. Anesthesia medium. Immediately after laying down the ethyl chloride tube the brushing of the hairs was begun and found to give a normal sensation. There was no pain from

traction until fifty seconds, and the sensations continued to be duller than normal for a comparatively long period.

June 12, 1909. — Subject R. Anesthesia medium. After one second brushing was felt; after ten seconds traction was felt, but it was not painful; the pain sensation from traction appeared at twenty-one seconds, but was much less acute than on normal parts; thirty-four seconds after application, the sensations from traction were quite acute, but apparently duller than normal.

From the accounts of the experiments it will be seen that after the anesthesia usually the sensations from lightly brushing or disturbing the position of the hairs returned sooner than the sensations from traction on the hairs. At times there appeared to be a marked dissociation of these two forms of sensation, and a result similar to that obtained on a case of nerve division was obtained.[7] It appears that these results indicate the normal dissociation of these two forms of sensibility. That the sensations from brushing returned sooner than the pain and pressure-like sensations from traction, and that the brushing sensations were sometimes present even from the first indicates that the traction sensations are not exaggerations of the brushing sensations, or, in other words, that the pain and pressure-like sensations resulting from traction are not due to the increased stimulus alone, but are separate kinds of sensation or are mediated by separate nerve endings.

Light touch. — The sensations of light touch differed in intensity or clearness in the different experiments, and appeared to be affected by the anesthesia a much longer time than the sensations of temperature and those from the stimulation of the hairs. Like the two forms of sensibility already described, the touch sensations were not much affected by slight degrees of anesthesia, but they were abolished if the anesthesia was marked.

The results of the experiments in this field are given in Table I. In this table are given the results of seven distinct experiments on the two subjects. The individual determinations have been averaged, given in the first lines of each test, and the average variations determined, given in the second lines of the test results.[8] The number of

[7] Franz: *Op. cit.*, pp. 215–223.

[8] The results of the experiments on Subject R, April 24, were grouped in sets of threes after the completion of the series, and the calculation of the average was

THRESHOLDS OF LIGHT TOUCH, NORMAL AND AFTER SPRAYING WITH ETHYL CHLORIDE.

Subject R.						Subject F.					
Dates.	Times.	Condition of anesthesia.	Average threshold values. Normal.	Average threshold values. Hypesthesic.	Hypesthesic / Normal.	Dates.	Times.	Condition of anesthesia.	Average threshold values. Normal.	Average threshold values. Hypesthesic.	Hypesthesic / Normal.
Apr. 24	2′	Slight	13.7 (13)	12.3 (12)	.90	Apr. 24	15′	Medium	10.7 7.3 (48)	20.7 11.2 (27)	1.93
May 8	Immed.	Medium	9.5 3.5 (50)	18.2 5.9 (25)	1.92		Immed.		1.5 .5 (10)	39.5+ (10)	26.33
	3.5′		....	9.3 2.4 (25)	.99	June 10	15′	Marked	2.6 1.0 (5)	18.3 11.2 (10)	7.04
	Immed.		12.7 1.6 (10)	40.0+ (10)	3.15+		30′		2.4 .7 (5)	6.2 3.8 (10)	2.58
June 10	15′	Marked	15.7 3.4 (10)	30.6 8.8 (10)	1.95		Immed.		8.8 3.8 (10)	40.0+ (10)	3.41
	30′		34.0 6.0 (10)	36.7 3.8 (10)	1.08	June 12	30′	Marked	7.6 3.6 (10)	11.2 5.5 (10)	1.47
	Immed.		17.8 3.8 (10)	40.0+ (10)	2.25		60′		3.4 1.9 (10)	4.1 2.5 (10)	1.21
June 12	30′	Marked	....	21.5 5.4 (10)	1.21	....	....	....	....	....	....
	60′		19.7 2.0 (10)	22.7 2.7 (10)	1.15	....	....	....	....	....	....

experiments we made in each case and from which the average was obtained is given in parentheses. The averages in the series of tests on the normal areas differed from day to day, partly owing to a difference in sensitiveness and partly to a difference in the estimation of the subject. At times the subject would wait for a very clear sensation before announcing its appearance, and at times a less distinct sensation was taken as the threshold. On any one day, however, the results are comparable for the normal and anesthetic areas. The average variation in all the experiments was quite large, and this was due to the actual sensory condition and partly to the inaccuracies in reading the instrument. Where there is a constantly increasing stimulus, as in the use of this kind of instrument, it is impossible for the experimenter to catch the exact point on the instrument at which the sensation appeared, and at the same time it must be remembered that the subject takes some time to announce the fact that he feels the stimulus. The error of reading the instrument may be considered in many of the experiments to be about one division on the scale, and the error of the subject, the reaction time we may say, was approximately two division, when the threshold value was above ten. In some of the experiments we were unable to determine the threshold value after the anesthesia on account of its amount. The instrument when pressed down to its greatest extent did not produce a sensation, and in these experiments, therefore, the threshold average is given as 40+. From some early experiments (April and May) it was found that the examination of a number of points showed a quite rapid return to the normal condition, and that the later determinations were on the average lower (nearer the normal) than the first determinations. For this reason the number of points examined in the later series was reduced to ten, which number could be made in a comparatively short time and have the sensational condition approximately constant during the whole time.

It will be seen that with slight degrees of anesthesia the touch threshold was not affected (Subject R, April 24). With the more marked degrees of anesthesia the threshold values were higher for a period of at least fifteen minutes. No variation of less than 10 per cent, perhaps it would be better to take 20, can be considered to be distinctive of a

made. The actual determinations have been mislaid (although those on pain sensations on the same day were preserved) and it was impossible to calculate the average variations.

sensory change. However, it should be noted that although the threshold measurements gave a normal or nearly normal average from the anesthetic area fifteen minutes after the application, the general feelings of the subjects were that the touch sensations were not at that time exactly like those from normal parts. The difference could not be described in any other terms than that the skin did not appear to give sensations as clear as those from the surrounding (normal) regions. The sensation difference just noted disappeared within an hour, and after that time there were neither subjective nor objective evidences of functional alterations.

Pain sensations. —The results of the measurements of the sensations of pain from pressure are given in Table II. The average, the average variation, and the number of experiments in each test are given in the same way as in Table I. It will be seen that in general the pain thresholds were higher in the areas sprayed by ethyl chloride, that the effect of the spraying persisted for about thirty minutes, although the period of complete analgesia was of only very short duration. In some of the experiments the algometer did not measure the amount needed to produce pain, and these results are indicated in the table by the plus sign. On June 10, subject R was so completely analgesic in the sprayed area that the limit of the instrument was reached in all of the first series and no pain occurred. On that day after fifteen, thirty, and forty-five minutes, there were some points that did not react to the stimulus by a pain sensation, although most of the area had recovered partly the sensibility to the extreme pressures. In the last-mentioned experiments the quotient of the division of the average threshold for the normal area into that of the anesthetic area is not high, and would have more nearly approached unity had it not been for the one or two abnormally insensitive points. The difference between the proportions for F on June 10, after fifteen and thirty minutes, is due to the fact that at the later time one of the ten determinations was abnormally large. This is also indicated by the average variation.

The effect of the ethyl chloride on the pain sensations may be summed up as follows: With a slight amount of freezing there was no pain threshold difference after eight minutes; with a medium amount of freezing there was a very slight or no effect in one subject after two minutes, and only a slight effect in the other subject after fifteen min-

TABLE II.

THRESHOLDS OF PAIN, NORMAL AND AFTER SPRAYING WITH ETHYL CHLORIDE.

Subject R.						Subject F.					
Dates.	Times.	Condition of anesthesia.	Average threshold values. Normal.	Average threshold values. Hypesthesic.	Hypesthesic / Normal.	Dates.	Times.	Condition of anesthesia.	Average threshold values. Normal.	Average threshold values. Hypesthesic.	Hypesthesic / Normal.
Apr. 24	8′	Slight	39.1 16.5 (13)	42.0 7.7 (12)	1.07	Apr. 24	15′	Medium	9.8 4.1 (16)	13.2 3.0 (9)	1.35
May 8	15″	Medium	37.0 4.7 (6)	75.8+ 5.8 (13)	2.05	June 10	15″	Marked	15.7 4.8 (10)	47.6 4.7 (10)	3.03
....	2′	....	39.2 7.5 (6)	45.7 7.2 (6)	1.17	....	15′	....	19.0 1.2 (5)	28.2 8.2 (10)	1.48
June 10	Immed.	Marked	47.1 11.6 (8)	80.0+ (10)	1.70	....	30′	....	17.2 4.9 (5)	33.0 15.6 (10)	1.92
....	15′	....	44.5 9.3 (10)	56.9 4.7 (10)	1.28	....	....	....	....	....	....
....	30′	....	45.2 4.6 (5)	55.7 6.0 (10)	1.23	....	....	....	....	....	....
....	45′	....	44.5 10.3 (6)	56.2 3.7 (10)	1.26	....	....	....	....	....	....

utes; with a marked degree of anesthesia the effect persisted for at least thirty minutes, and even though the general average threshold does not greatly differ, after forty-five minutes there was sufficient indication to show that some of the analgesia remained. To judge from the averages one could conclude that the hypalgesia remained for a long time at a fairly constant level after the return of the pain sensations, and it was noticed that the area did not appear to be normal even after the day's experiments were finished.

It is worthy of note that the pain on traction of the hairs returned more rapidly than that from pressure of the algometer. This would indicate that there were separate end organs for the initiation of the pain impulses from the two kinds of stimuli, and that the hair end organ regained its sensibility sooner than the end organ in the skin.

Summary and Conclusions.

Ethyl chloride is not only an analgesic, but also an anesthetic.

The anesthetic effect is of short duration, while that of analgesia is relatively persistent.

The sensibility of the hairs is affected in much the same way as the sensibility to touch and to pain. The sensations of lightly brushing them disappears for only a short time, while those for pain and for pressure on traction do not become normal for a much longer period.

The differences in the reappearance of the touch-like and the pressure-like and pain sensations from the stimulation of the hairs indicate that the hairs have two distinct sensory end organs for the appreciation of stimuli, and they contradict the assumption that the traction sensations are only exaggerations of the touch-like sensations obtained by lightly brushing the hairs.

The variation in temperature sensations is similar to that obtained on section of a nerve and its subsequent regeneration; *viz.*, on the part acted upon by the ethyl chloride the hot and cold stimuli, when they are first sensed, are appreciated as warm and cool rather than hot and cold. The results of the experiments do not indicate any difference in the nerve supply for these kinds of sensations.

CONCERNING THE SECRETION OF THE INFUNDIBULAR LOBE OF THE PITUITARY BODY AND ITS PRESENCE IN THE CEREBROSPINAL FLUID.[1]

By HARVEY CUSHING and EMIL GOETSCH.

[*From the Hunterian Laboratory of Experimental Medicine, the Johns Hopkins University.*]

THE suggestion was first advanced by P. T. Herring[2] in 1908 that the faintly staining, granular, hyaline, or colloid masses seen in the posterior lobe of the hypophysis cerebri represent products of secretory activity of the epithelial investment which find their way through the tissue interstices of the pars nervosa and ultimately discharge between the ependymal cells into the cavity of the third ventricle.[3] This conjecture, primarily based on Herring's studies of the cat's hypophysis, was strengthened by the appearance taken on by the gland after experimental thyroidectomy,[4] in which state he observed a marked increase of the hyaline bodies, which under normal conditions are seen in relatively scant numbers. As recorded heretofore,[5] in a report from this laboratory covering the main results of our earlier observations on the function of the canine

[1] Presented before the American Association of Pathologists and Bacteriologists, Washington, June, 1910.

[2] Herring: Quarterly journal of physiology, 1908, i, p. 151.

[3] It will be recalled that Haller (Morphologisches Jahrbuch, 1896, xxv, p. 101) believed that the secretion from the epithelial portions of the gland was collected in tubules which emptied directly into lymph spaces contained in the dural envelope of the gland — a view not supported by Edinger, Salzer, Sterzi, or Herring. Peremeschko, furthermore (Archiv für pathologische Anatomie, 1867, xxxviii, p. 329), described a direct communication between the cleft-like relic of Rathke's pouch and the ventricle, and in a single specimen from a kitten Herring has met with such a condition, though he believes that it is rare. In our long series of observations on nearly two hundred canine glands serial sections have never given evidence of any such communication.

[4] Herring: Quarterly journal of physiology, 1908, i, p. 281.

[5] Crowe, Cushing, and Homans: Johns Hopkins Hospital Bulletin, 1910, xxi p. 151.

gland as modified by various operative procedures, histological studies of the tissues so far corroborated Herring's description that his interpretation of the significance of the hyaline bodies was fully accepted.

Further confirmation of these views has come from the more detailed investigation of the tissues of a new series of animals operated upon during the present year (1909–1910). These findings, coupled with the ultimate demonstration of a substance in the cerebrospinal fluid of man which gives physiological reactions similar to those produced by extracts of the posterior lobe itself, seem to establish beyond peradventure not only that the hyaline bodies are the product of posterior lobe secretion, but also that their primary destination is the ventricular cavity, where they enter into solution in the cerebrospinal fluid.

In normal states of activity the pars nervosa of the posterior lobe is sharply demarcated from its narrow investment of epithelial cells (*Markschicht* of Peremeschko; *Epithelsaum* of Lothringer, *pars intermedia* of Herring) by a layer of capillary vessels which do not penetrate between the cells of the epithelial covering. Above the interlobular cleft, however, there is a massing of cells of the pars intermedia type. Here they fuse more or less intimately with the anterior lobe cells and send off a tonguelike process which closely envelops the anterior surface of the infundibular stalk. This portion of the pars intermedia, unlike the investment proper, is highly vascularized. Indeed it is the only subdivision of the entire gland which receives a collateral supply, being nourished partly by the vessels from the stalk destined for the anterior lobe, and partly by the more remote and distant posterior lobe vessels.[6] At this point, furthermore, there is no such clear demarcation between the anatomical subdivisions of the gland as exists elsewhere.

It is in this neighborhood that one sees the most striking examples of invasion by histologically unaltered pars intermedia cells into the very centre, at times, of the nervous lobe (Fig. 1). Instances of this are not at all uncommon in the supposedly "normal" glands of man,[7] and the appearance simulates the cellular invasion which one

[6] Dandy and Goetsch: American journal of anatomy, 1910 (to appear).

[7] For example, in a series of seventy-five pituitary bodies which we have had the opportunity of examining through the courtesy of Dr. Adolph Meyer, a con-

sees in certain of the infiltrating ectodermal epitheliomas. These pictures doubtless represent extreme deviations from the physiological normal. However, in glands which have been modified by experimentation or disease one may see conditions suggesting the

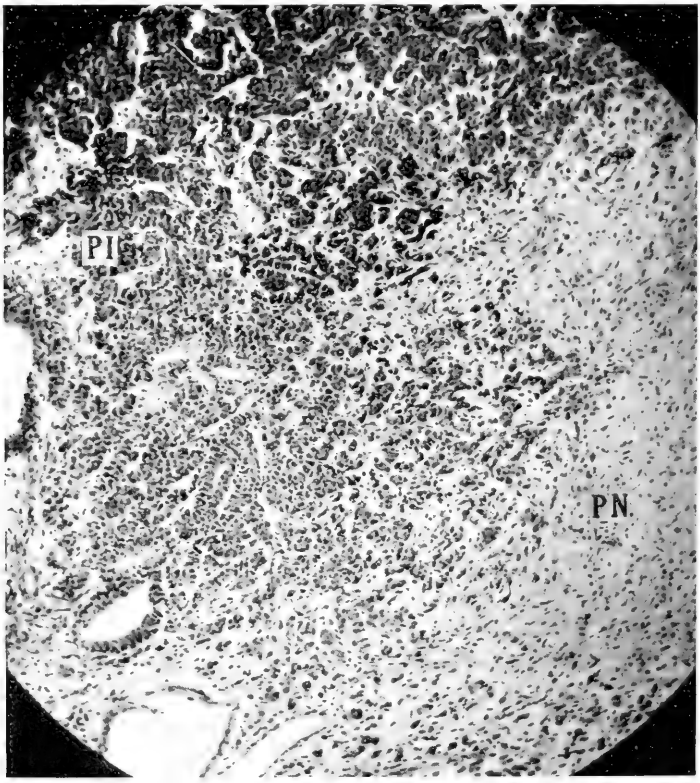

FIGURE 1. — Showing invasion of pars nervosa (*PN*) by unaltered cells of pars intermedia (*PI*). From a case of acromegaly, acute symptoms of glandular deficiency having occurred three days after an accidental surgical division of the stalk, resulting in extensive necrosis of anterior lobe.

same process, rare though it may be for completely unaltered pars intermedia cells to penetrate for any distance into the pars nervosa. Thus one may always discern at certain areas of the investment an

siderable number of the glands showed this condition in more or less marked degree. The material came from the routine autopsies of asylum patients and there were no histories of the individual cases.

apparent breaking through of cells into the pars nervosa proper. It is the rule for these cells, however, to undergo a prompt granular or hyaline transformation, and the resultant masses are found to distribute themselves in such a way through the posterior lobe as to give an appearance of streaming upward toward the infundibular cavity. Hence the suggestion that this is their destination was a natural one.

Herring's description of these hyaline bodies, observed chiefly in the cat, answers very well for their appearance in the canine gland. At the various points where cellular invasion occurs, the pars intermedia cells — at times forming islets or acini or tubules in the centre of which faintly staining hyaline material may be seen, at times seeming to wander in as individual cells — soon begin to lose their normal staining reactions and assume the characteristics of hyalin. Oftentimes when near their points of origin ghosts of nuclei may still be discernible in the faintly eosinophilic masses, but as they approach the infundibulum it is less common to find any traces of the original cell, the masses having become more or less irregular in shape and quite structureless. As stated, their general tendency seems to be in a direction toward the neck of the infundibulum, and the amount of hyalin in the tissue interspaces becomes obviously greater as the neighborhood of the ventricular cavity is reached. The substance seems to lie in distinct interneuroglial spaces which are radially disposed toward the infundibulum. Immediately underlying the ependyma, collections of the material may be seen (Figs. 3 and 6), and at times globular masses appear to be extruding themselves between the ependymal cells directly into the ventricular cavity, where not uncommonly an amorphous accumulation of the substance, not as yet entirely in solution, may be made out.

In the course of our experimental work we have met with far better histological examples of posterior lobe secretion than are furnished by the study of normal glands alone. Hyaline bodies in excess or showing an unusual distribution have been observed under a number of conditions, of which six types may be selected as illustrations: [8]

[8] Needless to say, it is requisite to the perfection of these histological studies that the tissues be removed not only with the greatest delicacy but within a few minutes after death, and that they be immediately fixed in a preservative which

1. **After extirpation of other ductless glands.** — In accord with Herring, the hyaline bodies seem to be more numerous subsequent to a thyroidectomy, but the most striking examples of their increase in our present series have been furnished by the glands of animals after nearly total extirpation of the pancreas. Though our studies on the functional interrelation of the pancreas and hypophysis will be reserved for another communication, the excess of hyalin in the

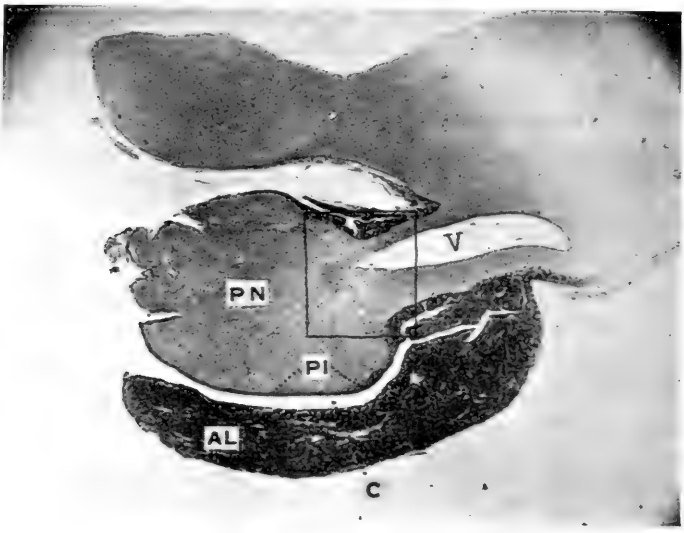

FIGURE 2. — Mesial sagittal section of the hypophysis of a dog sacrificed eight days after a total pancreatectomy. *V*, ventricle; *AL*, anterior lobe; *PN*, pars nervosa; *PI*, pars intermedia investing *PN*; *C*, cleft. (Squared area magnified in Fig. 3.)

posterior lobe after a total pancreatectomy is appropriate to our present theme, for there have been few better illustrations of abundant hyalin without associated tendency to colloid formation than

will cause the least distortion. Though fixation in ZENKER'S fluid gives satisfactory results, we have found that BENSLEY'S fluid is by far the best preservative. This fixative consists of equal parts of (1) a 2.5 per cent aqueous solution of bichromate of potash and (2) a saturated $HgCl_2$ solution in 95 per cent alcohol. The tissues have all been embedded in paraffin and the sections cut 4 or 5 microns thick. Unless great care is taken with these thin sections the hyalin will be dissolved out, leaving merely the wide-meshed spaces which contained it.

The sections here reproduced were stained in EHRLICH'S hæmatoxylin and alcoholic eosin.

are furnished by these glands. The normal-appearing gland (Fig. 2) under magnification shows a definite concentration of the widely distributed hyaline bodies near the tip of the infundibular cavity (Fig. 3), where they crowd the tissue spaces (Fig. 4) and break through the ependyma.

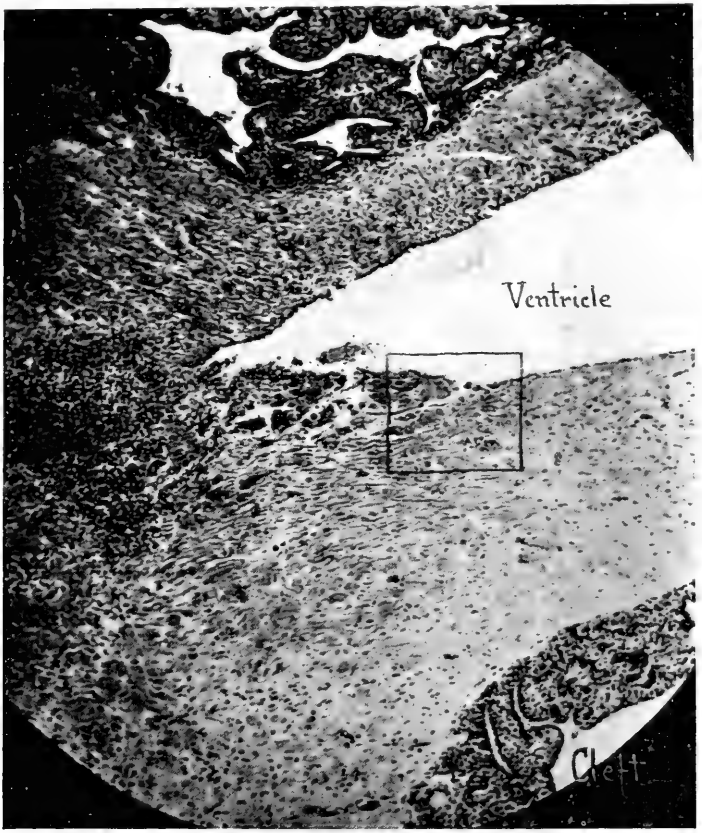

FIGURE 3. — Magnification of area squared in Fig. 2, showing tissue spaces filled with hyaline bodies, which are massed at the lower and anterior surface of the ventricle. (For squared area see Fig. 4.)

2. **After mechanical injuries.** — It is our impression that almost any direct mechanical injury will serve to increase the hyaline masses observed in the posterior lobe, and in view of the apparent close relation of the posterior lobe secretion to the storage of carbo-

hydrates, it is not improbable that the transient glycosurias which often follow traumatic injuries of the head are due to an increased discharge of hyalin in consequence of a posterior lobe lesion; but this, too, is another story. In a single case a particularly striking increase in hyalin was observed after an injection of India ink had been made

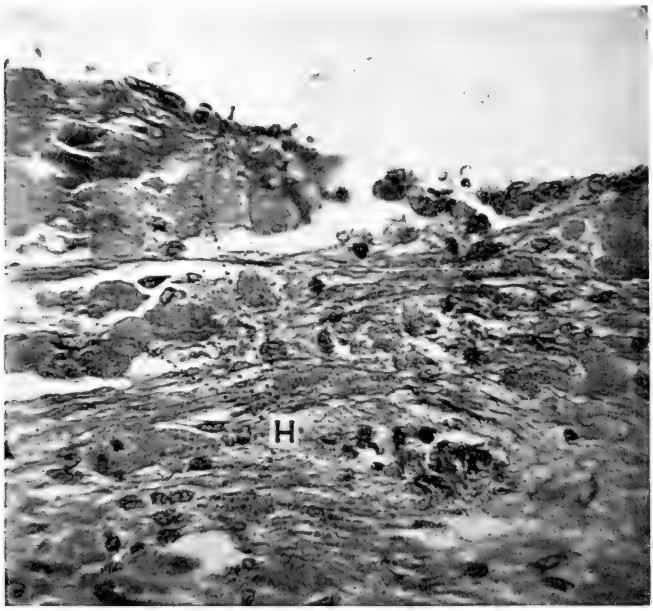

FIGURE 4. — Magnification of area squared in Fig. 3, showing interneuroglial spaces crowded with hyalin. Dotted lines indicate a few of the masses in the neighborhood of *H*.

in the substance of the posterior lobe and the animal sacrificed a few hours later. Here, in contradistinction to the condition shown in Fig. 2, one can see the accumulation of colloid [9] in a multitude of new-formed vesicles (Figs. 5, 6, and 7) at the junction of pars inter-

[9] Lest there be some confusion from an indiscriminate use of the terms *colloid* and *hyalin* it may be advisable to make clear at the outset that we believe that colloid accumulation in these new-formed vesicles described above is merely a precursor of free hyaline globules. These, however, may apparently originate also as a direct transformation of individual wandering cells; hence the nuclear remains occasionally seen in the hyalin bodies originating in this way rather than as a product of primary secretion into vesicles.

media and pars nervosa. Indeed, the injection mass itself and the resultant corpuscular extravasation appear to be tending, just as do the multitude of hyaline globules (Fig. 6), toward the infundibular cavity, where they may be seen (Fig. 6) massed under the ependymal lining of the ventricle.

3. **After partial hypophysectomies.** — Particularly interesting examples have been given by the hyalin secreted into the infundibular wall by tags of pars intermedia left after "total" hypophysectomies.

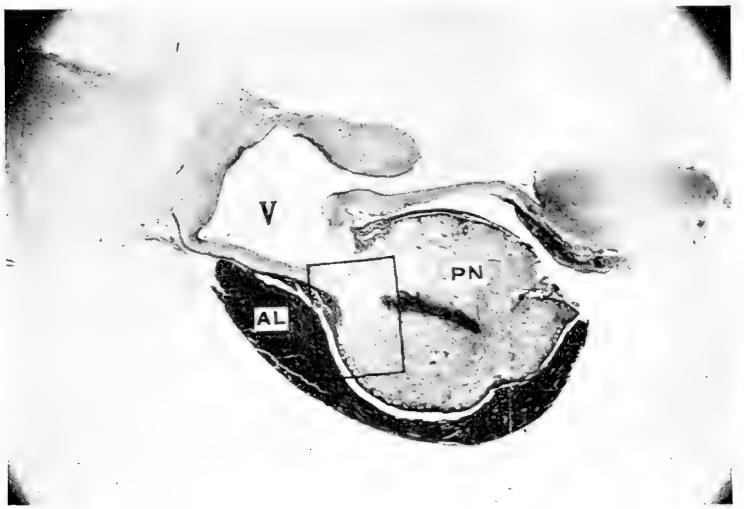

FIGURE 5. — Mesial sagittal section of gland after injection into pars nervosa of India ink. Central streak in *PN* shows injection mass with some extravasation. Note layer of colloid globules arising from epithelial investment of *PN*.

Owing to the projection of the tonguelike process of the pars intermedia along the anterior part of the infundibular stalk, it is almost inevitable for a larger or smaller fragment of this portion of the gland to be left adherent to the stalk, even in what (from the anterior lobe standpoint) [10] we regard as a "total" hypophysectomy. These pars intermedia fragments undergo a definite hyperplasia (indicating a compensatory activation) (Fig. 13), even during the few days of life possible for the animal deprived of the bulk of the

[10] For a remaining, even a microscopical, fragment of anterior lobe may suffice to preserve life, whereas a "total" anterior lobe removal is invariably fatal, though a considerable portion of viable pars intermedia may remain.

gland proper, including the entire pars anterior; and in these cases one may find evidences in the tissues, serially sectioned, of a stream

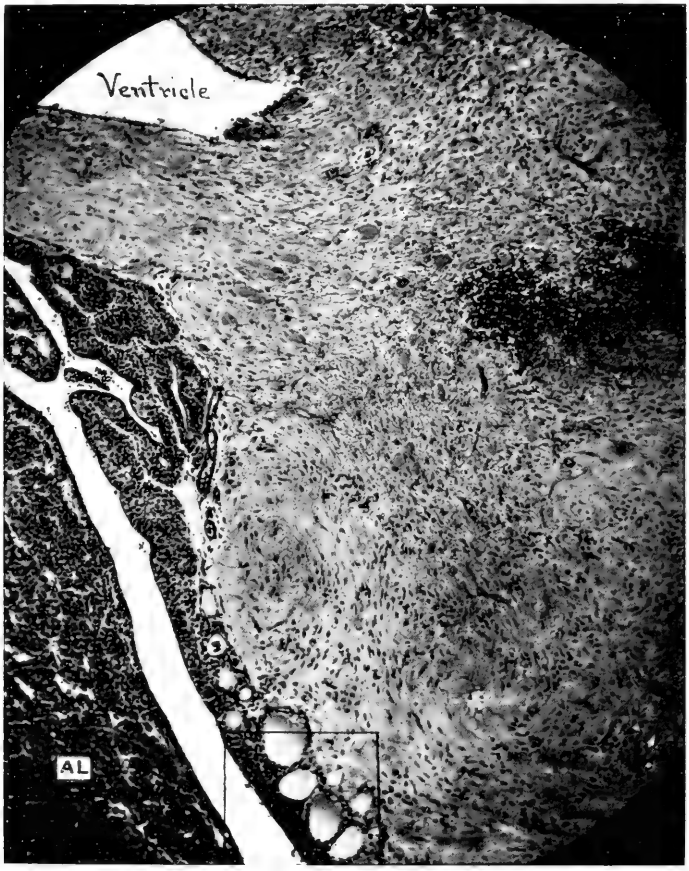

FIGURE 6. — Enlargement of squared area in Fig. 5. Showing upper edge of injection mass and many hyaline bodies streaming toward and crowded below ventricle.

of hyaline globules passing from the active epithelial fragment toward the cavity.

A protocol of such an experiment will serve in illustration.

Dog 19 (Series 1909–1910). — *Total hypophysectomy; sacrificed on twelfth day, owing to onset of acute cachexia hypophyseopriva.*

Healthy, mongrel, male puppy; weight, 3.8 kilo. Twenty-four-hour urine estimation, 180 c.c.

November 8, 1909. Operation. — Hypophysectomy without complications: presumably a total removal (confirmed by subsequent sections of removed tissue). One testis removed. Glycosuria demonstrated three hours later.

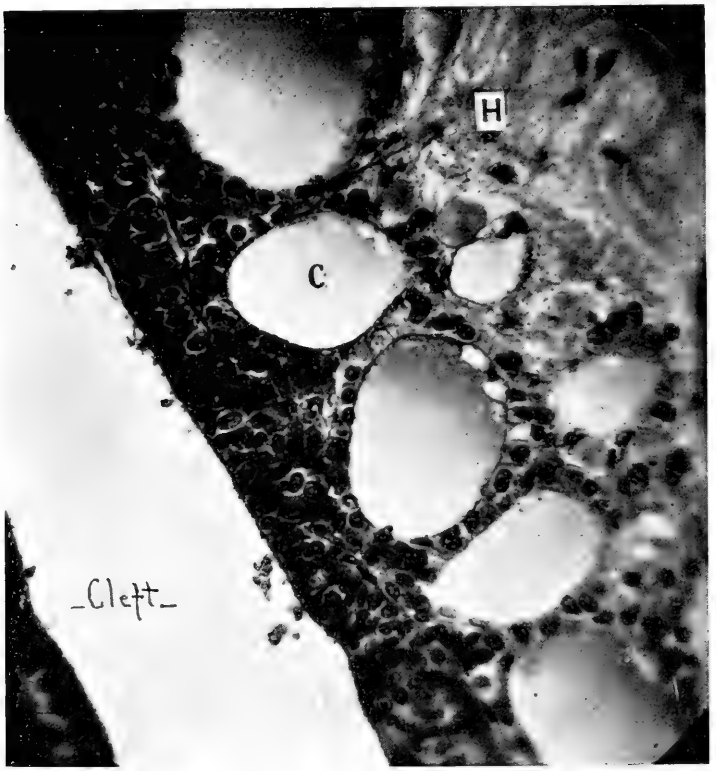

FIGURE 7. — Further enlargement of area squared in Fig. 6, showing formation of colloid by cluster of pars intermedia cells, making temporary vesicles (*C*). Wall becomes thinned and contents discharge into tissue spaces of pars nervosa. *H*, hyaline bodies.

November 9. — Eating solid food; responsive; seems well. Normal temperature (38.8° C.). Glycosuria persists.

November 10. — Good condition. Slight suspicion of reduction of Fehling's solution.

November 11 to 18. — Apparently normal in all respects. Wounds healed *per primam;* good appetite; lively and friendly. No glycosuria; no polyuria.

November 19. — First evidence of cachexia hypophyseopriva shown by sensitiveness to cold; shivering. Temperature, 38.1° C.

November 20, 11 A. M. — Pulse, 72; respiration, 14; temperature, 32.1° C., a drop of 6°. Loss of appetite. Definite ataxia; hypæsthesia; overactive reflexes; muscular twitching, etc.

5 P. M. — Pulse, 68; respiration, 12; temperature, 30.3° C. Typical and very pronounced symptoms of cachexia; no improvement on raising body temperature with external heat (electric pad). Animal sacrificed.

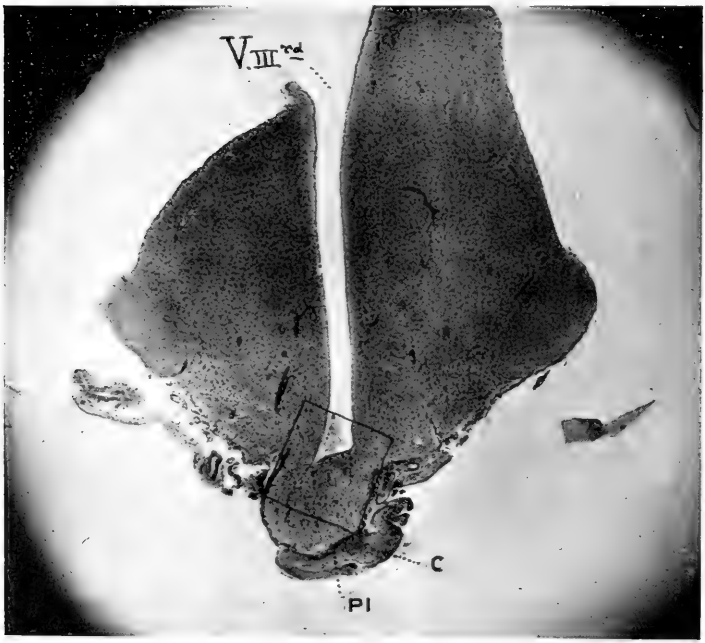

FIGURE 8. — One of a series of coronal sections of the infundibular block of Dog 19. Showing base of infundibulum capped by a small organized clot (*C*), which encloses a minute fringe of pars intermedia, from which hyaline bodies pass toward the ventricular cavity (*cf.* Figs. 9 and 10).

Autopsy immediately made. In gross, organs showed no abnormalities beyond hepatic mottling from fat deposit. Apparently total removal of hypophysis.

Microscopical. — Sections (serial) of base of brain. At tip of infundibulum is an organizing clot (Fig. 8) and, adjoining the stalk, a fragment of pars intermedia showing hyperplasia. From this fragment a procession of hyaline bodies can be seen streaming toward the infundibulum (Fig. 9). Many of the masses show what appear to be transformed nuclear remains (Fig. 10).

4. **After experimental obstruction.** — Not only excessive accumulations of colloid and hyalin, but also a great increase in the cellularity of the entire pars nervosa, was observed by Crowe, Cushing, and Homans in cases of experimental stalk division and was pictured in their report.[11] It occurred to us that the condition was

FIGURE 9. — Squared area from Fig. 8. Showing cluster of hyaline globules (near *H*) spreading from pars intermedia fragment in the direction of the ventricle (*V*), which they approach at a different level, as shown in the series of sections.

presumably due to a stasis of the products of secretion, coupled possibly with a compensatory hyperactivation of the posterior lobe, whose circulation under these conditions remains intact.

Experiments have been undertaken in our 1909–1910 series to throw further light on these changes, and in a number of animals after the usual exposure of the gland a silver "clip" has been placed

[11] *Loc. cit.*, Figs. 28 and 30, p. 151.

on the infundibular stalk, the procedure being comparable to a simple ligation of the stalk with an avoidance of the trauma, infiltration, and scar formation incidental to the earlier operative divisions. The tissues from these animals have shown the same appearances which were observed by our predecessors. The posterior lobe be-

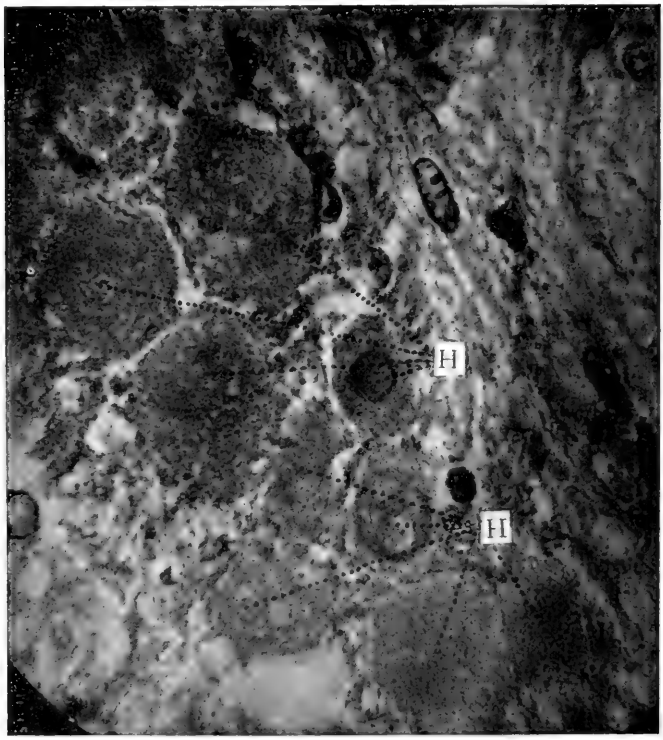

FIGURE 10. — Oil immersion enlargement of area adjacent to *H* in Fig. 9. Showing granular hyaline bodies with nuclear remains (*H*, *H*).

comes extraordinarily cellular; there is a great increase of hyalin in the tissue spaces; the former sharp demarcation between pars nervosa and the investment is largely obscured.[12]

[12] It would have been interesting to compare the physiological reactions of an emulsion of one of these obstructed posterior lobes with the posterior lobe of a normal animal for control, but the histological examination of the tissues seemed of more immediate interest. It may be noted that two out of the three animals subjected to a clean-cut placement of the clip on the stalk showed post-operative glycosuria.

A typical protocol of one of these cases may be abstracted as follows:

Dog 54 (Series 1909–1910). — *Experimental "clip" obstruction of stalk; polyuria; animal sacrificed after fourteen days.*

Healthy, white, mongrel, 4.6 kilo (10¼ lb.), female puppy, about seven months of age. Average twenty-four-hour urine, 100 c.c.

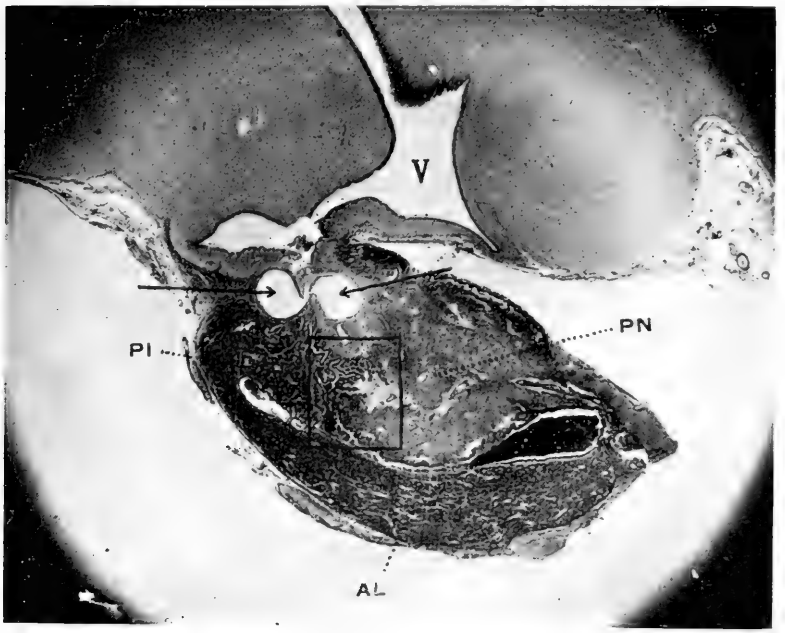

FIGURE 11. — Mesial sagittal section of gland after "clip" experiment (Dog 54). Arrows overlie scar where stalk was crushed through during placement of "clip," and circular clear spaces show the imprint of the "clip" removed after fixation. Note enlargement of anterior lobe (*AL*) from vascular stasis: very cellular condition of pars nervosa (*PN*), hypertrophy of pars intermedia (*PI*); mass of colloid and cellular debris in cleft. *PI*, pars intermedia; *PN*, pars nervosa; *AL*, anterior lobe.

April 26, 1910. Operation. — Usual approach; gland well exposed and U-shaped silver clip compressed on hypophyseal stalk, which was not broken off. No surgical misadventure; good recovery from the operation.

April 28. — No post-operative complications; no glycosuria. Playful and active. Normal temperature. Polyuria, 320 c.c.[13]

[13] In another of the clip experiments polyuria persisted for a month, on the day after the operation reaching 1750 c.c., whereas the normal for twenty-four hours had been 125 c.c.

May 3. — Slight elevation of temperature (39.1° C.) with accompanying diarrhœa; nevertheless a gain in weight of one pound since operation.

May 9. — Excellent condition; active and playful. Diarrhœa has improved. Polyuria is disappearing (135 c.c.). Animal sacrificed.

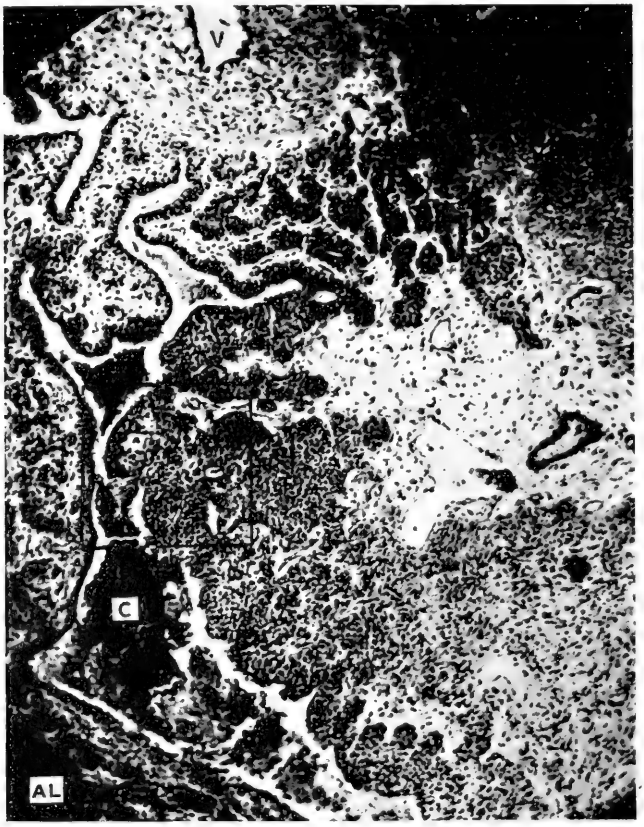

FIGURE 12.— Squared area from Fig. 11. Including tip of ventricle (*V*) which had been pinched off by clip. *C*, a mass of colloid and cellular debris in upper edge of cleft. Note hypertrophy of pars intermedia with tortuosity of processes of cleft: very cellular character of pars intermedia (section encloses the remaining small relatively non-cellular central area, *cf.* Fig. 11).

Autopsy immediately after death. In gross, organs show no change, though there is some suggestion of hyperplasia of the adrenal medulla commonly seen after hypophysectomies. Block cut as usual from base of brain, including the infundibular region with adherent gland; silver

clip seen embedded in tissues of stalk. Clip cut at the bend and extracted after fixation of tissue.

Microscopical. Anterior lobe shows no especial change other than that of engorgement. It seems larger than usual, but there are no cellular changes; no necroses.

Posterior lobe. — Holes made by clip apparent to naked eye (*cf.* Fig. 11). A fragment of tongue of pars intermedia has been crushed off above

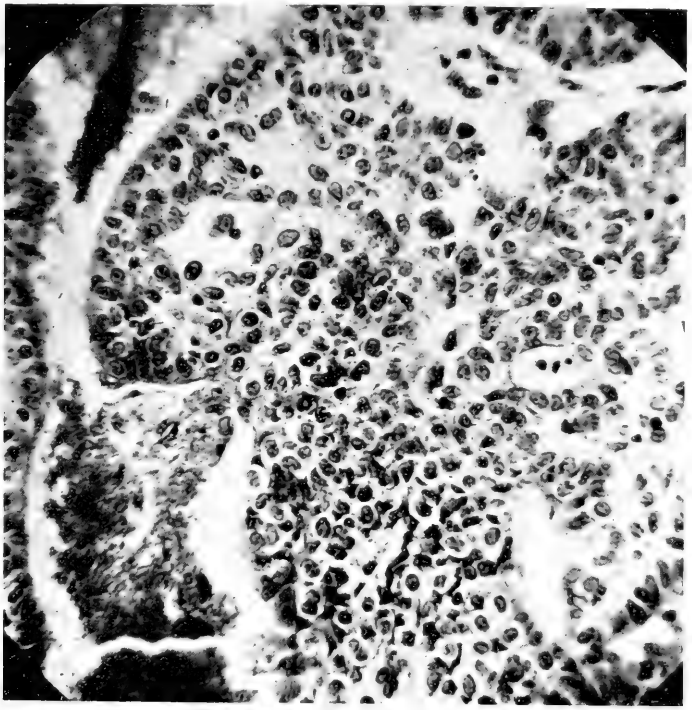

FIGURE 13. — Enlargement of area squared in Fig. 12. Showing cellular hyperplasia of pars intermedia without tendency to accumulation of colloid, though there are numerous minute vesicles in the process of formation.

site of placement of clip. Below clip pars intermedia and posterior lobe show the following extreme alterations from the normal: The entire pars nervosa is exceedingly cellular: the epithelial cells of the investment show, in addition to a marked hyperplasia (Fig. 13), a tendency to invade the pars nervosa (Fig. 12). Hyalin in large amounts is distributed thoughout the entire pars nervosa as far up as the seat of obstruction. Above this, though hyaline bodies are present, they are not more abundant than normal.

6. **In transplantations.** — Interesting too have been the evidences of hyaline secretion which has formed in posterior lobes after autoplastic transplantations into the cerebral subcortex. As mentioned in a former paper from this laboratory,[14] a number of experiments had been undertaken in the attempt to prolong the life of a totally hypophysectomized animal by immediately engrafting the extirpated gland. The more satisfactory "takes" occurred when the tissue was planted in the cerebrum, necessarily exposed during the course of the operation. Unaware at the time how minute a fragment of anterior lobe would suffice to maintain life, the tissue grafts, when studied histologically, were not looked upon as very successful, though in all of them clusters of viable cells were seen. Of interest, however, to our present subject is the fact that in a number of instances typical posterior lobe hyaline bodies were present in the brain substance adjoining the partly organized graft, showing the same appearance and possessing the same staining reactions as the hyalin to be seen in the channels of pars nervosa or infundibular stalk under normal conditions.

The hyaline bodies of Herring, which we have chiefly considered, differ in many respects from the colloid masses often seen encysted in the pars intermedia, especially where it adjoins the anterior lobe. Here these accumulations may at times assume large proportions and occupy large cysts visible to the naked eye, which may even break into and fill the cleft itself. This substance has a different staining reaction from hyalin proper and resembles more the appearance of the colloid in chronic goitre — the residual stage of earlier periods of hyperthyroidism, according to Marine.

Hypophyseal colloid, furthermore, does not seem to contain the active principle, whatever it may be, so easily demonstrable in the posterior lobe and presumably present in the hyaline bodies. Our experiences coincide with those of others in this respect. For example, we have variously injected an emulsion of colloid taken from cysts of the pars intermedia and from the cleft of the fresh gland of the pig and ox without eliciting any reactions worthy of note. Whether colloid represents the same basic substance as hyalin but

[14] Crowe, Cushing, and Homans: Quarterly journal of experimental physiology, 1909, ii, p. 389.

which has become desiccated and stored in spaces once lined by actively secreting cells, is purely a matter for conjecture. The hyaline bodies have been spoken of as thin colloid, but it would seem almost better to designate colloid as thickened and encysted hyalin. However this may be, colloid does not give the reactions of the posterior lobe which we are inclined, with Herring, to ascribe to the hyaline bodies. Such fresh colloid, if one wishes to call it colloid, as may be secreted by the newly formed acini of the pars intermedia, (as pictured in Fig. 7), may be a precursor of hyalin and may possibly be active.

These new-formed vesicles, as has been described, may be found in certain experimental conditions distributed in great number about the circumference of the pars nervosa. The pars intermedia cells apparently group themselves in a cluster and become separated by the secreted material, until an acinus lined by a single layer of cells is formed. The contained material takes a feeble hæmatoxylin stain, whereas the substance immediately on its discharge into the channels of the pars nervosa upon rupture of the acinus takes on eosinophilic properties. It would be idle to speculate at present upon the reason for the activity of hyalin and the inactivity of colloid, both substances obviously being products of secretion of pars intermedia cells. The pars nervosa presumably may play a part in activating the secreted masses during their passage towards the infundibulum and thus serve as something more than a mere channel of exit. It is not impossible too that the neuroglial and also the ependymal cells possess some additional secretory function quite apart from that of the pars intermedia.

Now, largely as a result of the early investigations of Howell, and of Schäfer and his co-workers, it has long been known that the so-called active principles of the pituitary body — that is, such principles as are demonstrable by the usual injection of extracts — are found only in the posterior lobe. An emulsion of this part of the gland, when introduced intravenously or subcutaneously, has a marked effect on arterial tension, a pressor response usually predominating,[15] produces diuresis through distention of the renal

[15] There appear to be two substances, one having a pressor, the other a depressor effect.

vessels, stimulates the smooth muscle fibres of bladder, intestine, and uterus, and dilates the frog's pupil. These responses are sufficiently characteristic and delicate to identify the substance and distinguish it from adrenalin — a substance the reactions of which simulate in many respects those of the hypophyseal extracts.

This posterior lobe substance, which may conveniently be called "pituitin" to distinguish it from extracts of the anterior lobe, "hypophysin," is soluble in water, glycerine, or alcohol, and does not lose its activity on boiling. It apparently does not exist, at least in an active state, in more than scant amounts in the pars intermedia, according to the observations of Schäfer and Franchini, with which the observations made in this laboratory and to which we have referred are in accord. Furthermore, as has been stated, pituitin, at least in an active form, is not present in the cystic accumulations of colloid which often are found in this part of the gland.[16]

On a physiological basis, therefore, we are in possession of definite facts in regard to certain reactions brought about by injections of extracts of pars nervosa. On the other hand, purely on a histological basis, Herring ventured to interpret the nature of the hyaline masses seen in this same portion of the gland as products of secretion whose destination was the ventricular cavity. This view seems to be fully corroborated by the appearances furnished by the glands which we have observed under variously modified states of activity. Obviously one step remains to be taken before the full significance of these hyaline bodies of Herring can be appreciated — the examination of the cerebrospinal fluid for the presence of a substance capable of eliciting reactions identical with those of an extract of the pars nervosa itself.

The first opportunity of making such a physiological test of the cerebrospinal fluid, after the idea had occurred to us, was afforded, in June, 1910, by a case of congenital internal hydrocephalus. During the progress of the clinical studies preliminary to a decision as to the proper method of drainage appropriate to the particular form of ventricular hydrops shown by this infant, a number of punctures were made, ventricular and lumbar, and large amounts of fluid (300 to 600 c.c.) were removed from time to time. The ventricles

[16] It is obvious from the distribution of hyalin that the infundibular wall may be as active as the pars nervosa, for it often contains a large amount of hyalin.

could be drained as readily from a lumbar as from a ventricular puncture, and the fluid from each source was clear and on the customary tests showed nothing more than the characteristics of normal cerebrospinal fluid.

The first observation was made on June 15 with some of the fluid which had been evaporated over a water bath to about one sixth its volume. Five cubic centimetres of the ventricular fluid thus concentrated were injected into the external jugular of an anæsthetized dog and of a quiet and unanæsthetized rabbit, causing a preliminary fall followed by a long-enduring rise in pressure, with slowing of pulse, amplification of beats, and an occasional dropped beat such as one often sees after injections of posterior lobe extracts. Subsequent injections of an equal dose of the concentrated lumbar fluid gave precisely similar reactions.

On the following day the experiment was repeated on another animal, with an even more marked pressor response, and a few drops of the concentrated fluid added to the normal salt solution, bathing an isolated frog's eye, caused a prompt and wide dilatation of the pupil. Positive results were obtained with a third dog on July 18 (*cf.* Fig. 14), when positive diuresis was observed. The pupillary reaction also was found to be more prompt and wider to the fluid preparation than to a freshly prepared emulsion of the posterior lobe of a dog or to an aqueous emulsion of the dried posterior lobe prepared by Armour and Company.

Again on July 20 a series of injections in a 6-kilo dog of 5 c.c. of ventricular fluid in a 30 to 1 concentration gave: (I) with *ventricular fluid*, a rise from 132 to 140, following a preliminary fall to 122, and also respiratory acceleration; (II) after an interval, with *lumbar fluid*, a particularly long enduring though low pressor response from 120 to 130; (III) after an interval, with *lumbar fluid* again, a rise similar to II, but with higher response, from 120 to 146.

Judging that the rabbit might show still more definite responses, further observations were made with other specimens of fluid from this same infant, in various concentrations; and in view of possible sources of error, not only through bulk of the fluid injected (for 5 c.c. of normal salt solution may cause a pressor response in a rabbit), but through too great concentration of its inorganic salts, we finally came to adopt a certain standard amount and concentration, namely,

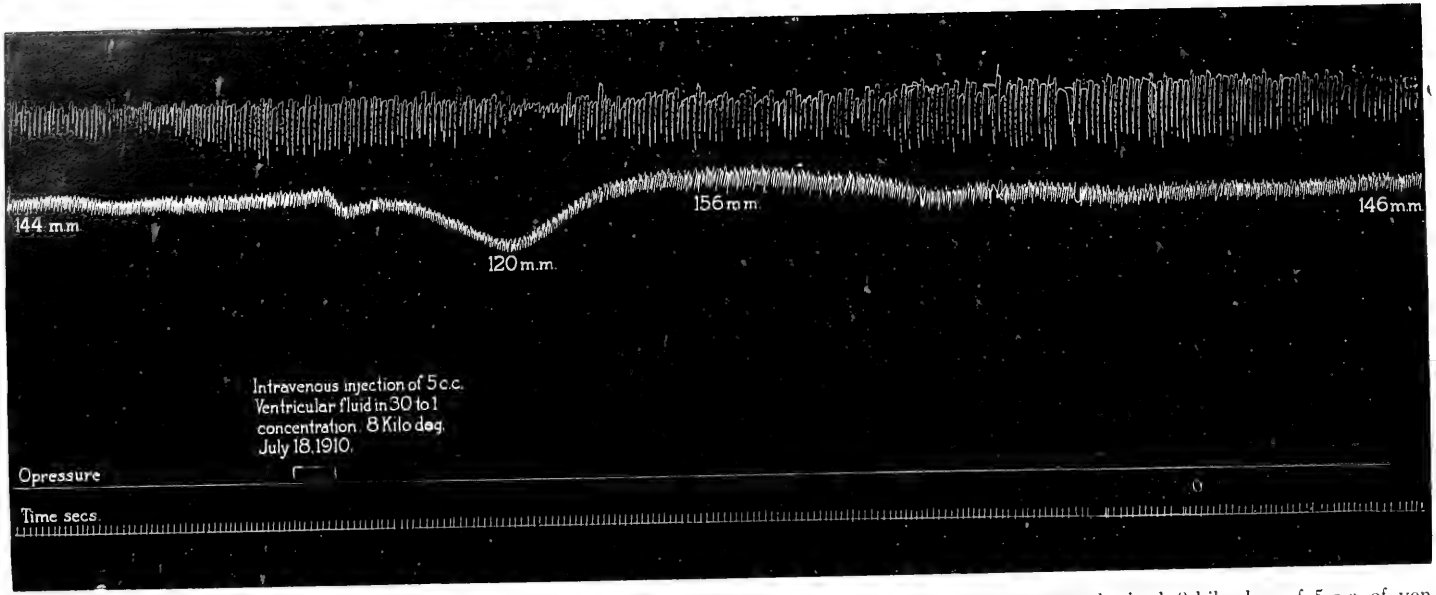

FIGURE 14. — One half the original size. Response to intravenous injection into the external jugular of an anæsthetized, 8 kilo dog, of 5 c.c. of ventricular fluid in a 30 to 1 concentration. Primary fall from 144 to 120 immediately following injection, followed by a slight pressor response to 156.

50 c.c. concentrated to 2.5 c.c. Fifty cubic centimetres of fluid is an amount which with care can usually be obtained by a lumbar puncture from man, and 2.5 c.c. of normal salt solution introduced into the external jugular of the rabbit shows practically no blood-pressure response through the amount of fluid introduced into the circulation. In quiet and unanæsthetized rabbits partly eviscerated we observed in most cases marked peristaltic effects with vigorous contractions of bladder, uterus, and intestines. In one case the animal was eviscerated, and direct injection into a branch of the mesenteric artery produced vigorous peristaltic contraction of the corresponding intestinal loop.

We have made corresponding observations with the cerebrospinal fluid obtained from eight other individuals as follows: the ventricular fluid from two patients with brain tumor causing obstructive hydrops; the lumbar subarachnoid fluid from six patients — (1) epilepsy, (1) recent trauma, (2) acromegaly, (2) hypopituitarism with adiposity. The direction taken by our clinical investigation becomes apparent from the selection of these cases. For, unless we have misinterpreted the significance of these reactions, it is anticipated that it may prove possible to determine by tests of the cerebrospinal fluid the functional activity of the physiologically important posterior lobe in conditions associated with primary or secondary glandular involvement. This naturally will require many more observations and a certain standardization of methods with more controls than we have had as yet.

All of the fluids which we have so far examined and tested on the rabbit have shown blood-pressure responses, often with the primary fall characteristic of posterior lobe extract, followed by a long-enduring rise, a slowing of pulse and amplification of the pulse wave. They have invariably dilated the frog's pupil; and diuresis with constriction of the musculature of bladder, intestine, and uterus has been commonly seen. The reactions have apparently been more pronounced in the case of the fluids from obstructive hydrocephalus than in the other conditions from which specimens have been obtained. This might be expected, in view of a continued posterior lobe secretion into the hydrocephalic ventricles with little possibility of its escape into the general circulation; and it is notable that patients thus afflicted are apt to be well nourished, as is true of ani-

mals with experimentally produced glandular deficiency. We have had occasion, too, to demonstrate post-mortem in the posterior lobes of such cases that retention (?) cysts of colloid are apt to be present and may reach an enormous size.

The fluids, on the other hand, which have given the least response have been those from the two cases of trauma and epilepsy. Fifteen cubic centimetres of normal fluid obtained by lumbar puncture in a dog and concentrated to 2 c.c. gave inconclusive reactions. It will be necessary to use a larger animal in order to obtain normal fluid in sufficient amount for further investigations.

A brief summary of two experiences may suffice:

I. **Reactions of the fluid from the congenital hydrocephalic.** — A well-nourished child, aged twelve months, with typical internal hydrocephalus producing an enormous head, 60 cm. in circumference. A number of previous tappings, both lumbar and ventricular, had been made with withdrawal of fluid, which had been found to give positive reactions (120 to 146 mm. of Hg) in strong concentrations both in a dog and a rabbit.

Inasmuch as animals of the same weight and species differ greatly in their reactions to a measured dosage of carefully prepared posterior lobe extract, the primary depressor response in some individuals being pronounced, in others inconspicuous, an injection of the concentrated cerebrospinal fluid was followed after an interval by an injection of 1 30 gm. of posterior lobe extract (Armour and Company) dissolved in an equal bulk of fluid.

Experiment, July 22, 1910. — A few drops of a 25 to 1 concentration of ventricular fluid (250 c.c. reduced to 10 c.c.) promptly and widely dilated an isolated frog's pupil at rest in dim light in normal salt solution.

A rabbit prepared for the injection was eviscerated, the urine examined, and when at rest the blood pressure registered 62 mm. (*cf.* Fig. 15). Two cubic centimetres of the fluid in its 25 to 1 concentration were injected into the external jugular. There was a momentary struggle, with slight irregularity of the blood pressure, followed by a slow, progressive rise (100 mm. at its highest point), which was long maintained. Active peristalsis was started up by the injection, even visible in the usually quiet large gut, and this was followed by a copious evacuation. The previously emptied bladder rapidly filled. The urine, previously negative, showed slight reducing properties.

This injection after an interval was followed by 1/30 gm. of dried

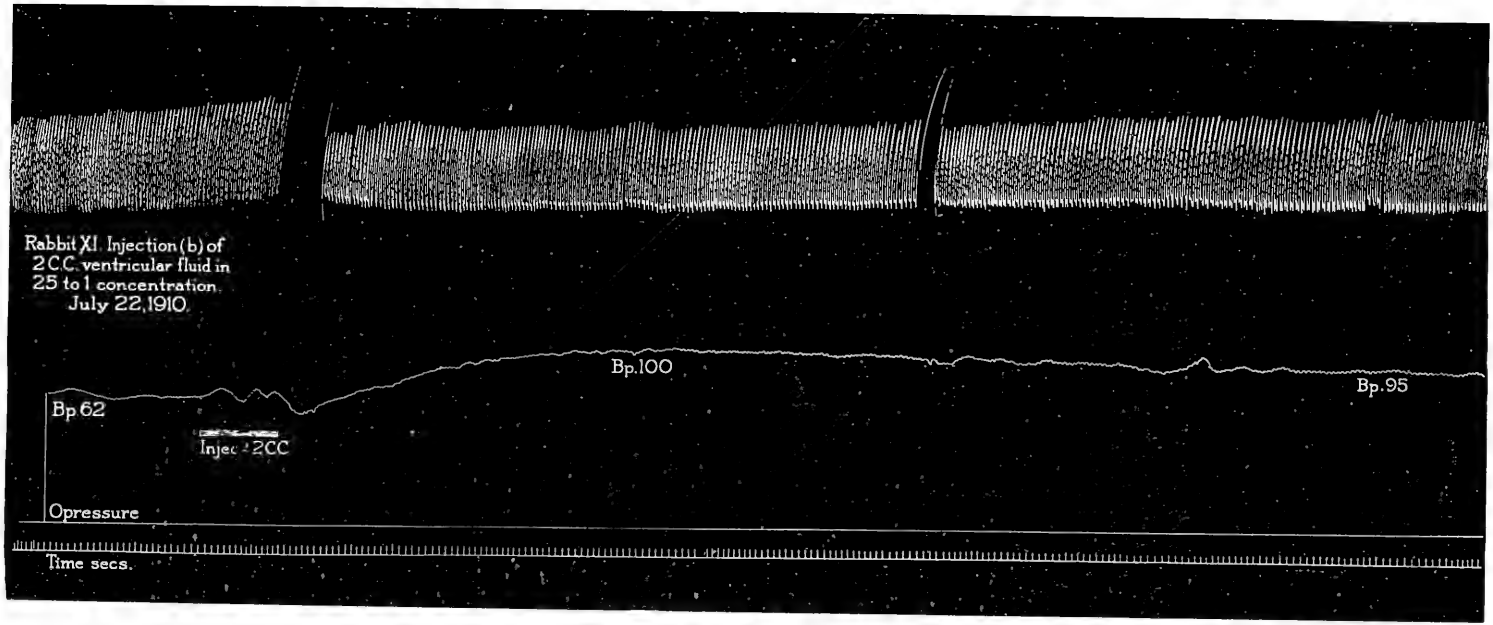

FIGURE 15.— One half the original size. Injection of 2 c.c. of ventricular fluid in a 25 to 1 concentration in external jugular of an eviscerated unanæsthetized rabbit. After slight primary fall, long pressor response from 62 to 100 mm. at highest point.

posterior lobe extract dissolved in 2 c.c. of normal salt solution. This solution dilated the isolated frog's pupil, and when injected gave almost exactly the same response, raising the pressure without preliminary fall from 66 mm. to 106 mm. with a very slow return after about eight minutes to its previous level.

II. **Reactions from the ventricular fluid obstructed by tumor.**— A patient with a cerebellar tumor and obstructive hydrocephalus producing the usual outspoken general pressure phenomena characterizing these cases. A ventricular puncture was made August 10, 1910, at Kocher's point of election, and 60 c.c. of clear fluid primarily under great tension were removed. This fluid was evaporated over a water bath to 3 c.c. — a 20 to 1 concentration.

Experiment, August 11, 1910 (Fig. 16). — Pupillary dilating property not tested. Rabbit with bladder alone eviscerated. Urine negative for reducing substances. *Injection 1.* — Into the external jugular 2.5 c.c. of the (20 to 1) fluid was injected, producing the usual momentary struggle and prompt rise from the preceding level at 84, to 114 mm., with slowing of pulse and amplification of beats; respiration shallow and rapid. Very slow return of arterial pressure to previous level. Effect on peristalsis unobserved as intestine was not exposed, but the bladder first contracted strongly under the injection and then subsequently filled; the urine showed no reducing substance.

Injection 2. — For comparison with this response after an interval a control injection was made (and recorded on the same drum raised 9 mm.) with an equal bulk (2.5 c.c.) of normal salt solution, with no obvious results beyond a momentary rise of a few millimetres. Subsequent injections in this same animal with other 2.5 c.c. emulsions of pars nervosa, etc., gave positive reactions similar to the one shown.

If our interpretation of the responses which we have obtained proves to be correct, and they are actually due to the presence of posterior lobe secretion in the cerebrospinal fluid, an excellent opportunity will be offered for the chemical isolation of the active principle of pituitin. Some preliminary observations have already been made in this direction. From the fluid of the case of idiopathic hydrocephalus the ash was separated by Dr. John King, and we found that a solution of the inorganic salts thus isolated dilated the pupil, but produced a marked depressor response without a subsequent rise in pressure — an effect possibly due to potassium salts in

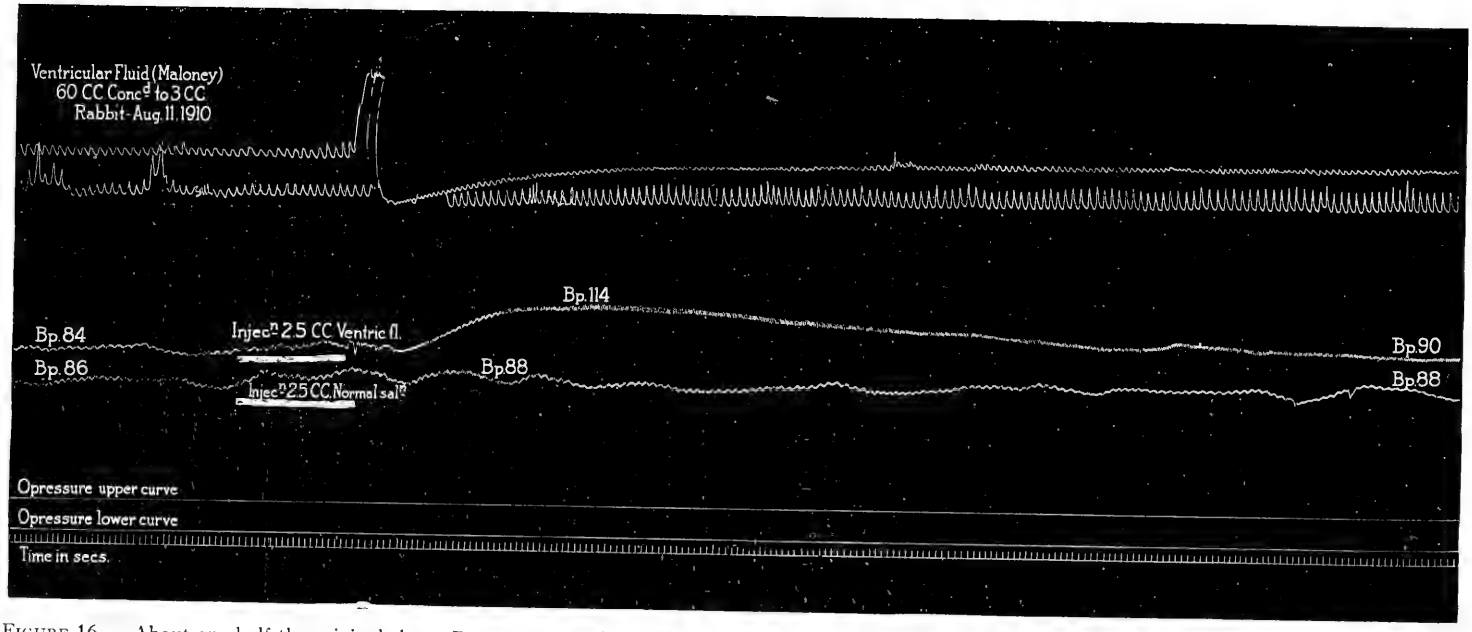

FIGURE 16. — About one half the original size. Response to an injection in a rabbit of 2.5 c.c. of a 20 to 1 concentration of ventricular fluid from case of obstruction by tumor. Long-enduring pressor response from 84 to 114 mm. For control on same drum a later injection of an equal bulk of normal salt solution.

concentration. It is conceivable, however, that we may be able to separate the pressor and depressor substances which are evidently present in posterior lobe extracts and which exist in widely different proportions in the extracts variously prepared by the commercial laboratories. Some specimens of pituitin which we have tested contain the depressor substance alone.

It is obvious that there are many controls which must be instituted, particularly in regard to the presence in the fluid of such substances as choline. This substance, however, is said to constrict the frog's pupil and, as Halliburton has shown, it apparently exerts a depressor effect — responses unlike those of pituitin. It is to be remembered too that cerebrospinal fluid normally contains a trace of proteid (globulin) and also a substance which reduces Fehling's solution and which Nawratski identified as glucose, though it rarely gives a fermentation test.

Summary.

The object of this communication is to call attention to the presence of a substance in the cerebrospinal fluid which gives the same reactions as extracts of the pars nervosa itself, indicating in all probability that the active principle long recognized as being confined to this anatomical subdivision of the gland is actually secreted into the ventricular cavity.

This would seem to establish the theory that the hyaline bodies of the pars nervosa, regarded by Herring as products of secretion of the posterior lobe — a view supported on experimental grounds by ourselves — actually discharge, as their histological appearance suggests, into the third ventricle and represent the source of the active substance resembling pituitin in the cerebrospinal fluid.

OBSERVATIONS ON AURICULAR STRIPS OF THE CAT'S HEART.[1]

By JOSEPH ERLANGER.

[*From the Physiological Laboratory of the University of Wisconsin.*]

IT has long been known that strips of the sinus venosus and, in some hands, of the auricle, as well as the entire excised cold-blooded heart, continue to beat long after preparation, the only necessary condition being the prevention of desiccation. It has also long been known that strips of the cold-blooded ventricle can be made to beat for hours or even for days merely by immersing them in certain simple salt solutions. On the other hand it would appear that the continued or prolonged activity of the excised warm-blooded heart, or of a (ventricular) strip thereof, has heretofore been attainable only through perfusion of its blood vessels.

While the author was engaged in a problem with which we are here not concerned, he had occasion to perfuse mammalian hearts in the usual manner and then to cut into them in various ways. In the course of one of these experiments it was noticed that a bit of the right auricle that remained in connection with the atrio-ventricular junction by only a narrow pedicle continued to beat vigorously, although it was clear that it could not have had any functional or vascular connection with the rest of the heart. It then occurred to him that it would be interesting to determine whether the strip cut altogether free of the heart, but immersed in the Locke's solution that was pouring from the open heart, would continue to beat. To his surprise it did; indeed it continued to beat long after it had been completely severed from the heart and even after it had been connected with a recording lever and immersed in a bath of Locke's solution. Eventually, however, it ceased beating. Then it was found that prolonged series of beautiful

[1] A preliminary report of the earlier experiments of this research appeared in the Proceedings of the American Physiological Society, This journal, 1909, xxiii, p. xxxiii.

beats could be obtained repeatedly by stimulating the strip tetanically from time to time. In this way the beat was maintained, excepting certain interruptions, for almost seven hours after preparation.

Methods in general. — The method that has been employed more or less generally throughout this research was briefly as follows: Under ether anæsthesia the animal was rapidly bled to death through both carotids. The heart was excised, perfused with Locke's modification of Ringer's solution, and, after it had begun to beat, strips were prepared in various ways and as they were required for study. In some experiments, before excising the heart, a thread was passed through the auricle by way of the two cavæ so as to make these vessels clear in the collapsed heart. In other experiments the veins were marked before excision by "S" hooks which were passed through each vein close to its opening into the auricle. The whole of the intrapericardial venous region was always removed with the heart and usually a part of the extrapericardial portions of the cavæ also. The strips in most of the experiments were made successively; that is, one was made, mounted in the Locke's solution and studied often as long as two or more hours, when another strip was prepared from the heart which in the meanwhile had been constantly perfused. Some of the more common positions of the "S" hooks as well as the positions of the strips most commonly employed are shown in Fig. 1. In other experiments several strips, as many as four, were prepared all at the same time and mounted in one and the same vessel. The strips were fixed below; the upper ends were connected with counterpoised levers. The beaker containing the Locke's solution was arranged so that it could be quickly slid up and down a rod and so cover the strip with Locke's solution or uncover it. The volume of solution was so large that its temperature changed but slowly. In some experiments the temperature was kept practically constant throughout by means of a burner properly adjusted under the beaker. In most of the experiments the strip was so connected with the lever that the contraction of the whole length was recorded; in some, so that the contractions of either or both ends could be recorded, a selected central point being fixed below, the free ends extending upward to the recording levers. As a rule, the stimulating current was led through the full length of the strip; not infrequently, however, the electrical connections were so made through switches that either one half or the other, or the whole

strip could be stimulated. In a few experiments the strip was stimulated through a pair of platinum electrodes applied by the hand of the operator to selected spots or regions. Where it was desired to study the effect of simultaneous stimulation of several strips under the same conditions they were connected in series in one circuit. As a rule, the strips were stimulated through and through with a tetanizing current developed in a Harvard induction coil driven by one Edison-Lelande cell, type S. The strength of the stimulus employed is expressed by the distance in centimetres of the secondary from the primary coil. Some idea as to the strength of stimuli employed may be formed with the knowledge that the threshold stimulus with the electrodes applied to the tongue was obtained when the coil was at thirteen and rotated through 20°. Unless it is otherwise stated, the strips were stimulated in air usually as soon as possible after they were raised out of the solution; they were reimmersed usually immediately after cessation of stimulation. Cats alone were used. The results herein recorded were obtained from a study, often extending over many hours, of thirty-nine hearts.

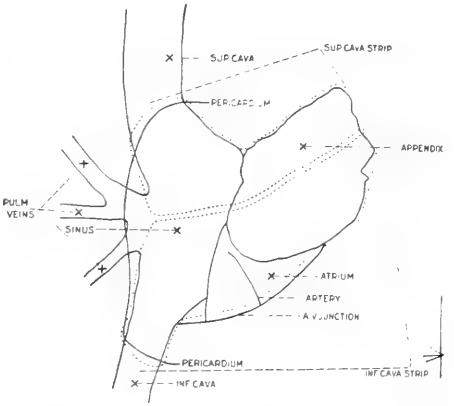

FIGURE 1. — Outline of the right auricle distended with blood, showing the three regions of the outer wall, the commonest positions of attachment, and the strips (areas included in dotted lines) most carefully studied, namely, the horizontal superior cava strip and the horizontal inferior cava strip.

RESULTS.

Parts that can be revived by stimulation. — It was found early in the course of this research that certain parts of the auricles can be made to beat when treated as has been outlined above, whereas others cannot. One of the first problems therefore was to determine as exactly as possible the limits of the responsive area or areas. This proved to be a rather difficult matter, for the reason that in the empty heart, and especially in suspended strips, the anatomically and development-

ally different parts cannot be clearly recognized. Furthermore, the descriptions of this part of the heart in the books at our disposal have not been clear enough for our purposes, nor do they agree in their terminology. The recent article by Keith and Flack [2] has not materially helped us in this regard. Therefore, rather than to employ terms and limits that may not withstand the test of time, we have attempted to plot our results on the supraventricular parts divided into regions that can be easily distinguished by means of gross differences in appearance.

In the outer wall of the cat's heart distended with blood (Fig. 1) the auricular appendix is recognized without difficulty. The left or posterior edge (heart suspended from aorta and viewed from behind) of the right appendix is usually perfectly clear. To the left of the lower half of this edge is seen a more or less triangular area which in some ways resembles in appearance the appendicular region and also that part of the supraventricular region lying between the appendix above and the atrio-ventricular junction below and with which it is continuous along the lower part of its anterior edge. This triangular area we have, for the sake of convenience, termed atrium. It is probably coextensive with the trabeculated parts of the region usually termed atrium. To the left of the right appendix above and of the atrial area below is found what we have termed the sinus region. In the distended heart it resembles in appearance the walls of the cavæ, and includes all of the outer wall of the right auricle, including the intrapericardial parts of the cavæ, not accounted for above. The sulcus terminalis is clearly marked only on the upper edge of the auricle and has therefore been of little assistance to us as a landmark. We have attempted to determine the position of the sino-auricular junction by means of the arterial circle described by Keith and Flack, but have not succeeded in satisfying ourselves in many cases of its existence as an easily recognizable entity even in injected hearts. There is, however, one very constant artery which has served us as a landmark, namely, one which ascends from the atrio-ventricular groove somewhat posterior to the appendix over about the middle of our atrial area (shown in Fig. 1). After ascending a short distance, the main branch curves to the left in the direction of the inferior cava, smaller branches going in various directions.

[2] Keith and Flack: Journal of anatomy and physiology, 1907, xli, p. 172.

By passing ligatures through the distended auricle at various points along the boundary of the sinus and atrium, as above described, the limits of the sinus as viewed from within can be accurately determined. It is thus seen that the sinus includes practically all of the non-trabeculated parts of the outer wall of the auricle. The so-called crista terminalis (corresponding with the sulcus terminalis) is apparently a very broad structure in the cat's heart. Running over it toward the superior cava from the right outer edge of the mouth of the inferior cava is a grayish fold of tissue which is probably a remnant of the venous valve. This fold lies considerably to the caval side of the right edge of the sinus as we have described it.

Other regions we have arbitrarily distinguished require no especial delimiting, namely, in the left auricle, the pulmonary region and the appendix; the right and left vaults (anterior or aortic surface of the auricles); and, common to both auricles, the septum and the coronary region (including the region of the node of Tawara and the atrio-ventricular bundle). In how far these regions will be found to correspond with those now distinguished by embryologists and comparative anatomists we have not been able to exactly determine. We shall, however, have something more to say in regard to this subject later.

The attempts to revive strips that have visibly ceased beating have met with success only when they have contained the whole or parts of the sinus, atrium, coronary, or septal regions, and only when these parts were stimulated. There has been only one exception to this rule: upon one occasion a strip composed presumably of only the right appendix gave two spontaneous beats when immersed after stimulation in air. With this single exception strips composed only of right appendix, vault (exclusive of septal attachment), pulmonary, and left appendicular regions have never been revived.

Furthermore, in the case of strips that are beating spontaneously or have been made to beat by previous stimulation, an *increased rate of beat subsequent to stimulation* is obtained only when they contain the whole or parts of the sinus, atrium (probably), coronary, or septal regions and only when these parts are stimulated. Nevertheless, as will be made clear later, stimulation of the right appendix, vault, pulmonary, and left appendicular regions is not without effect.

The method that has been employed to determine more exactly the anatomical limits of these two areas has been to excise practically the whole outer wall of the right auricle and to suspend it from three

points, namely, (1) from the atrio-ventricular groove at about the place where arises the artery that has already been described, (2) from near the mouth of the superior cava or actually from that vein, and (3) from the tip of the appendix. The first point was fixed immovably; from it the sinus region upon one side, the appendicular upon the other, extended obliquely outward and upward to the other two points of fixation which were connected with counterpoised levers. By means of this arrangement the strip was spread wide open so that a pair of platinum electrodes could be applied to any desired point while at the same time the contractions of the two ends of the strip could be recorded separately. In every case a sketch was made of the strip as it appeared when viewed through the sides of the beaker, that is, magnified in the horizontal axis, and the points stimulated were drawn into the sketch.

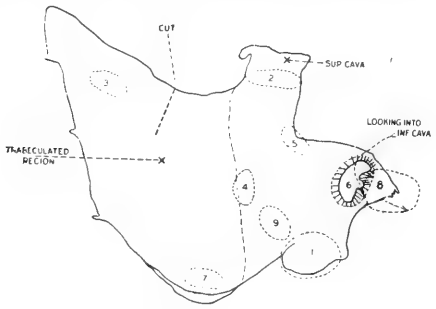

FIGURE 2. — Outline of the strip used in Exp. 29 (Table I) showing some of the points stimulated.

The results, selected at random from a typical experiment, are presented in tabular form (Table I, columns 1, 2, 3, and Fig. 2).

These, and many other experiments in which the same as well as other spots were stimulated, show that the accelerating after-effect is almost invariably obtained when the region immediately about the mouths of the great veins is stimulated. Possibly it is not so frequently obtained when the stimuli are applied midway between the veins, and even less frequently further to the right. It can, however, be obtained, sometimes in marked degree, as far to the right as the trabeculated part of the auricular wall. Indeed, on one occasion, and one only, a slight acceleration resulted when the appendix was stimulated after it had been separated from the sinus end by means of a cut parallel to, and a slight distance to the right of, the thick trabecula that lies just to the left of the left border of the appendix. The accelerating after-effect of stimulation certainly does not diminish below the inferior cava.

Other experiments show that to the left of the veins the accelerating after-effect extends into the auricular septum. It does not, however, extend beyond the junction of the right with the left auricle.[3]

The veins themselves do not seem to beat, and stimulation, even close to the intrapericardial parts, has, if we except certain very doubtful cases, seen only when the superior cava is stimulated, been entirely without any after-effects.

The area, therefore, that responds with increased rate of beat subsequently to stimulation and that can be made to beat spontaneously, may be described as including the whole of the area we have termed sinus, the interauricular septum, and to a less degree the atrium. This area will probably be found to be coextensive with the area, as determined by Adam,[4] that responds to local warming with an increased rate of beat. It seems to be somewhat larger than His' sinus reuniens (including here the coronary sinus),[5] but on the right edge only. Whether or not it is coextensive with the region Keith and Flack believe contains tissue derived from the sinus venosus we have been unable to definitely determine. It would, however, appear that it extends considerably further to the right than their sinus, to include a part of the region Keith and Flack term the auricular canal.[6] It certainly is more extensive than the sino-auricular "nodal tissue," since, according to Keith and Mackenzie,[7] "in the mammalian heart the sino-auricular nodal tissue is concentrated on the right auricle, chiefly along the sino-auricular junction and in front and on each side of the superior vena cava." The statement of these authors to the effect that "the excitable regions of the heart are those where the nerves come into a specially direct connection with the specialized tissue which we have termed nodal" is not, therefore, borne out by experiment.

Relative rate of beat of the different parts of the spontaneously rhythmical region. — The relative rate of beat of strips each made up in part of a portion of the spontaneously rhythmical region of the auricle,

[3] It is to be regretted that the great length of the specimen tracings illustrating most of the points made in this paper precludes their reproduction here.

[4] Adam: Archiv für die gesammte Physiologie, 1906, cxi, p. 618.

[5] His: Beiträge zur Anatomie des menschlichen Herzens, Leipzig, 1886.

[6] Keith and Flack: *Loc. cit.*

[7] Keith and Mackenzie: Lancet, Jan. 8, 1910.

TABLE I.

Showing Some of the Effects of Stimulating Selected Ppoints, Exp. 29 (see Fig. 2).

Point stimulated.	S. rate before. Beats per 10 sec.	Max. S. rate after. Beats per 10 sec.	S. ampl. before. In mm.	Max. S. ampl. after. In mm.	A. Ampl. before. In mm.	Max. A. ampl. after. In mm.	Remarks.
2	5.0	7.5	0.5	1.0	Not beating	Not beating	.....
3	4.7	5.0	0.7	0.8	Not beating	6.0	
1	5.0	10.2	0.7	2.0	3.5+	4.5	
1	4.2	10.5	0.7	1.3	0.7	2.0	
3	3.8	4.2	0.6	0.7	Very small	4.0	.....
1	4.0	10.2	0.7	1.5	1.6	3.0	
2	....	11.0	0.5	1.5	0.5	2.3	
4	....	....	....	....	....	....	No increase.
4	7.8	8.0	0.5—	3.0	Not beating?	Not beating?	Temp. raised to 30½° after long rest.
1	6.0	12.7	2.5	3.0	Very small	2.4	
3	5.2	5.0	0.5	0.6	1.0	6.5	
5	6.0	6.0	No increase		No increase		.....
2	5.5	16.2	1.0	2.0	1.5	3.5	
1	7.0	12.6	Very small	0.6	Very small	1.0	After long rest and after warming to 31°.
2	6.2	17.0	Very small	1.0	0.5	2.8	
8	....	....	....	....	....	....	No change.
8	....	....	....	....	....	....	No change.. Long rest.
2	5.0	7.6	0.4	0.8	1.0	1.6	
2	5.0	7.6	0.5	2.5	1.4	2.5	
1	4.2	9.0	1.4	1.7	1.5	3.0	.....
3	4.3	4.2	0.7	0.8	1.5	8.5	
All over Ap.	....	....	....	....	....	....	About same as preceding.
2	3.6	10.2	0.6	3.0	1.0	4.0	

9	....	....	....	....	....	....	Only change is slight increase in S. ampl.
9	3.6	4.3	1.0	1.8	0.8	1.0	
1	4.0	5.0	1.5	1.3	0.8	1.0	
2	3.5	6.0	1.0	2.0	0.8	2.0	
....	....	....	....	....	....	....	.....
....	....	....	....	....	....	....	Long interval, then warmed to 36½°, when strip behaved magnificently.
3	10.2	9.3	small	No increase	1.5	9.5	36.5
2	9.3	14.3	0.5(?)	6.0	5.0	6.8	
1	7.5	15.7	0.4(?)	0.6(?)	1.0	2.0	
2	7.0	13.3	0.4(?)	0.7(?)	0.8	1.3	
3	6.3	7.0	0.4(?)	0.4(?)	0.8	7.5	
4	7.3	15.3	0.4(?)	1.0	5.5	4.5+	} Temp. falling.
6	....	....	....	....	....	....	
5	5.3	10.8	Very small	Sl. incr.	0.5	1.0	No change.
2	4.8(?)	6.5	Very small	Sl. incr.	No change		
4	5.7	10.3	Very small	2.3	0.5	1.0	
....	....	....	....	....	....	....	31.5
3	9.7	9.5	....	0.7	2.0	10.8	Long interval. Then warmed to 37.5°
4	9.5	17.2	0.7	3.7	8.7	6.7	Irregular } Temp. falling.
All over Ap.	8.0	6.4	Very small	No. incr.	0.6	8.0	
Around 4	6.4	15.2	Very small	2.0	7.5	7.5	
3	7.2	7.0	Very small	No incr.	0.6	5.2	
2	7.0	15.7	Very small	4.0	4.5	5.3	
1	9.3	17.4	0.5	0.5	Very small	0.7	
2	7.4(?)	16.5	Very small	0.7	0.5	0.7	After warming to 37° } Temp. falling.
4	....	....	....	....	....	....	
Around 4	5.7	12.0	0.5 —	2.5	Small	No incr.	Marked slowing
Around 4	....	....	....	....	....	....	.....
All over Ap.	....	....	....	....	....	....	Response very irregular.
2	5.3	15.5	1.0	1.5	8.0	6.5	Usual result.
At and about 1	4.5	11.0	1.3	1.0	8.5	8.0	.....
2	6.7	9.2	Very small	Sl. incr.	2.5	2.0	.....

that is, the region that can be made to beat spontaneously through electrical stimulation, when subjected as nearly as possible to the same conditions, is shown in Table II. The maximum rate of beat developing subsequent to stimulation has been used in compiling these statistics. The strips were stimulated through and through.

There can be no doubt but that to a certain extent the great variability of results shown in the table is due to the fact that the stimuli cannot be applied to the different strips in exactly the same way, manner, and place. And since the effect subsequent to stimulation depends upon the density of the stimulus and the place to which it is applied, it follows that the results obtained with different strips in the same experiment and with the same strips in different experiments are not exactly comparable. By way of illustrating this point the following experience may be cited. With a strip consisting of the whole outer wall of the right auricle, it was found that under conditions that were otherwise exactly the same, stimulation "through" resulted in a beat at the rate of 54 per minute, whereas applied directly to the sinus with platinum electrodes, the stimulation resulted in beats at the rate of 90 per minute.

Viewing in the light of this difficulty the results tabulated above, the only warrantable conclusion seems to be that the three regions of the auricle most carefully studied, the superior cava, the inferior cava, and the coronary sinus regions, possess approximately the same grade of rhythmicity.

This conclusion is borne out also by the results obtained from local stimulation of the outer wall of the right auricle, which have been referred to above. Whenever acceleration is obtained the resulting rate of beat under a given set of conditions is approximately the same (see Table I, columns 1, 2, and 3).

Discussion of foregoing results. — The results detailed above are of considerable interest in view of the physiological importance that has been attributed, mainly by histologists, to the so-called sinus and auricular nodes. These two structures have such a peculiar and characteristic appearance histologically that their discoverers have been tempted to designate them the motor centres of the heart. This suggestion has been accepted by some physiologists and clinicians almost as a demonstrated fact.

The most important experimental evidence favorable to this conten-

tion is furnished by Hering.[8] (1) In the first place this investigator, finding that a cut carried through the auricle so as to presumably inter-

TABLE II.

SHOWING RELATIVE RATE OF BEAT OF STRIPS UNDER APPROXIMATELY THE SAME CONDITIONS.

Exp. No.	Nature of strip.	Temp.	Rate per min.	Remarks.
4	(*a*) Sup. and Inf. cavæ and intervening tissue . .	?	138	Under exactly the same conditions.
	(*b*) Septum including cor. sinus and A-V bundle . .	?	114	.....
5	(*a*) Sup. cava	?	96	Under exactly the same conditions.
	(*b*) Inf. cava	?	76	.....
	(*c*) Coronary sinus	?	102	
6	(*a*) Sup. and Inf. cavæ and intervening tissue . . .	30—	84	Under exactly the same conditions
	(*b*) Vertical strip just to right of (*a*)	30—	114	
7	(*a*) Sup. cava—horizontal	33	144	.....
	(*b*) Inf. cava "	33	132	
9	(*a*) Sup. cava "	?	72	
	(*b*) Inf. cava "	?	84	.....
10	(*a*) Sup. cava "	33+	74	
	(*b*) Inf. cava "	33+	96	
11	(*a*) Sup. cava "	30	84	.....
	(*b*) Inf. cava "	30	78	
12	(*a*) Sup. cava "	34?	102	
	(*b*) Inf. cava "	34?	72	.....
13	(*a*) Inf. cava "	33	72	
	(*b*) Sup. cava "	33½	114	
14	(*a*) Inf. cava "	34	138	.....
	(*b*) Sup. cava "	34?	108	

sect the sinus node, and that a cut 1 cm. long in the "furrow which runs from the apex of the angle formed where the superior cava joins the

[8] HERING: Münchener medizinische Wochenschrift, April 27, 1909. A review of Hering's earlier work on this subject will be found here.

right auricle" suffices in many cases to bring the supraventricular parts of the heart to a standstill, "has no doubt but that normally the impulses of the mammalian heart (inclusive of man) arise in the sinus node described by Keith and Flack."

While there is every reason for believing that a cut that stops the beat of the heart acts upon the motor centre, this action need not be directly upon the centre. The effects of stimulation, particularly of the heart, are not limited to the point of their application, — witness general fibrillation from local stimulation. Furthermore, in Hering's hands a cut made as described by him has not always stopped the heart, while cuts made in other parts of the sinus region may stop the heart.[9]

(2) A second method that has been used to locate the place of origin of the normal cardiac excitation wave has been to determine in the dying heart the part last to beat. Many investigators have shown this to be the region of the great veins. More recently Hering[10] located the *ultimum moriens* (*a*) in the mouth of the superior cava in the vicinity of the sinus node of Keith and Flack and (*b*) in the coronary region in the vicinity of the auricular node of Tawara. The logic of this method of determining the normal cardiomotor centre is not, however, clear. It serves only to locate the most viable parts of the heart, not those possessing the highest rate of rhythm, which, after all, determines the seat of the pacemaker of the heart.

This argument suggests another and perhaps the oldest way of determining the region of the heart whence originates the cardiac impulse, namely, by locating the part of the heart endowed with the highest rate of rhythm. In so far as the mammalian heart is concerned, this was attempted by Erlanger and Blackman.[11] By dividing in various ways the auricles of the perfused heart by means of cuts and crushes they found that the region of the great veins possesses the highest grade of rhythmicity but that the rhythmicity of this part exceeds but little that of the coronary sinus region. The method used, as was pointed out by these authors, is open to the objection that the disturbance of the supply of the perfused fluid to the several parts of the auricle may have altered their normal rhythmicity. This objec-

[9] Erlanger and Blackman: This journal, 1907, xix, p. 125.
[10] Hering: *Loc. cit.*
[11] Erlanger and Blackman: *Loc. cit.*

tion does not hold in the case of the present experiments. None of the specimens was perfused; they were merely suspended in Locke's solution. It is true, however, that this method has its objections also, the main one being the different permeability of parts of unequal thickness; the thicker parts, it might be assumed, are less under the influence of the surrounding medium than the thinner parts. In view of the fact, however, that the heavy strips made from adult animals survive just as long and manifest just as high a grade of rhythmicity as the thinner strips from young animals, it would seem that this objection is not a valid one.[12]

In conclusion we may repeat that present physiological conceptions permit us to assume that any region possessing the function of determining the heart beat must possess the highest rate of rhythm. If either the sinus node or the auricular node is to be considered the cardiomotor centre, it must be shown that it beats decidedly more rapidly than other parts of the heart. Experiments herein recorded show that this is not the case. They show that the region of the inferior cava and the region to the right of the mouths of the veins, neither of which contains any of the tissue of the nodes as now delimited, possess a degree of rhythmicity that is distinctly exceeded neither by the part of the heart containing the sinus node nor the part containing the auricular node.

Behavior of strips during stimulation. — The behavior of a strip while it is being stimulated through and through, owing to circumstances that are not altogether clear, is rather variable. The response to tetanic stimulation of the beating strip is usually a fairly high initial extra contraction, sometimes two or three, followed by small, rapid, and irregular beats. Not infrequently the initial contraction is followed by complete relaxation; it may, however, be that this quiescence is only apparent and is actually due to responses so fine and rapid as to be imperceptible. It would appear therefore that there is almost every gradation of response to stimulation between large irregular contractions and complete quiescence of the strip. Therefore there seems to be no good reason for believing that the quiescence is the

[12] Very recently Lewis (Heart, 1909–1910, i, p. 262) has called attention to a slight difference in the time of appearance of the action current in different parts of the auricle as determined with the string galvanometer. He is inclined to believe that this difference is such as to indicate that the heart beat normally arises in the vicinity of the superior cava.

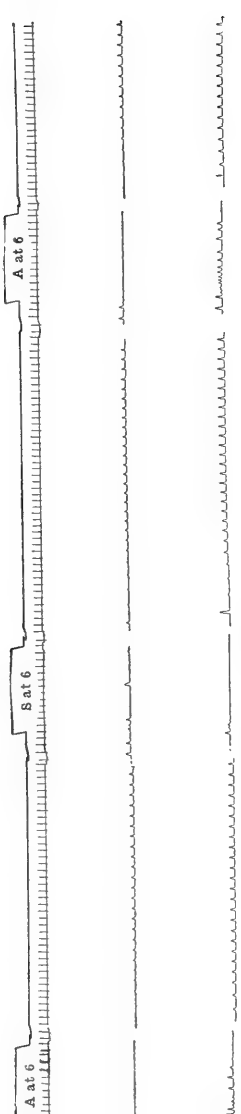

FIGURE 3 (one half the original size). — Showing the characteristic behavior of the sinus end (*S*) and the appendicular end (*A*) of a strip when one or the other end is stimulated through and through. From Exp. 27.[13]

result of stimulation of an inhibitory mechanism.

In the case of strips so mounted that the movements of the appendicular and sinus ends may be recorded simultaneously, stimulation of the sinus end through and through while the whole strip is beating results, as a rule, in complete cessation of the sinus end, so far as visible beats are concerned, the appendicular end usually stopping also or showing a few small beats; while stimulation of the appendicular end through and through results in apparent cessation of its beats, or in rapid irregular beats which rarely are of considerable amplitude, more commonly exceedingly fine; whereas the sinus almost invariably beats rapidly and very irregularly (Fig. 3).

These rather variable results can be brought into more or less harmonious accord if we assume, and apparently justifiably, (1) that the apparent cessation of beat during stimulation is not an inhibition properly so called, but rather what might be termed a complete tetanus in relaxation, the intermediate stages, showing irregular contractions, then being incomplete tetani; (2) that the more irritable (or better, perhaps, the more rhythmical) tissue of the sinus responds to such stimulation at a more rapid rate and consequently falls into a more complete tetanus than the appen-

[13] GENERAL. —All records read from left to right. Time in seconds. Where stimulations are not indicated by the signal (uppermost line) they are incated by irregularities of record. The lowering and raising of the beaker to expose and cover the strips are indicated by the elevation and depression of the record respectively.

dix, and (3) that the sinus, being more irritable than the appendix, can respond more accurately to the slower impulses emerging from the tetanized appendix than the more sluggish appendix can respond to the rapidly recurring impulses sent out from the tetanized sinus. Then it becomes clear why, during stimulation of the sinus, while this part may or may not be quiescent, the appendix almost invariably is quiescent; whereas during stimulation of the appendix the sinus almost invariably beats.

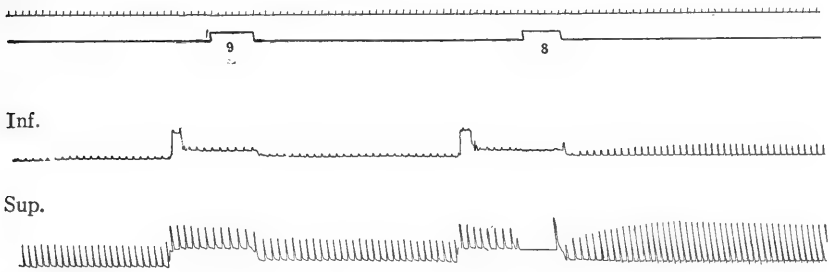

FIGURE 4. — Showing that a stimulus (coil at 9) too weak to call forth irregularities determines no subsequent improvement of beat. Two strips (superior cava and inferior cava) mounted in series. From Exp. 10.

The strength of stimules required to produce these effects is relatively great. As a rule, the rhythm of the beating strip is not disturbed, nor are beats induced in the quiescent strip until the secondary coil has been carried to a point within 10 cm. of the primary. Frequently no effect is obtained until this distance is reduced to 7 or even 6 cm. The manner in which the terminals are connected with the strip, as well as their location, affects the threshold somewhat.

After effects of stimulation on amplitude. — The character of the beat subsequent to stimulation is altered or a series of beats is started only when the stimulus has been sufficiently strong to produce one or the other of the disturbances in rhythm mentioned above (Fig. 4). In that event the behavior of the strip depends, to repeat, upon its nature and upon the point of application of the stimulus.

Strips composed of the left auricle only, or of any part thereof (excepting the interauricular septum), show no detectable after-effects. They never beat excepting during stimulation.

Strips of the right auricular appendix only, with but a single excep-

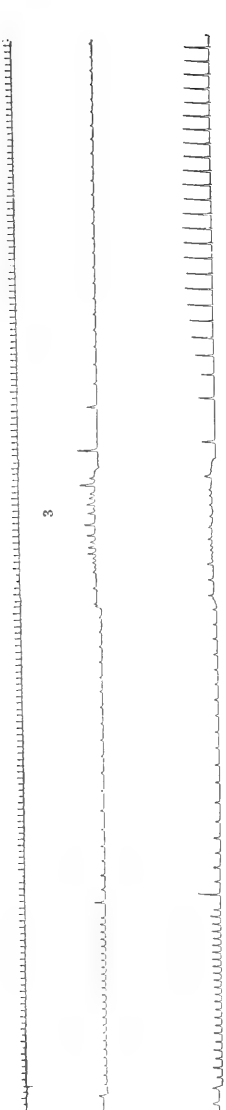

FIGURE 5 (about one half the original size). — Showing the effect of stimulation of the sinus region (1 in Fig. 2) and the appendicular end (3) of a strip composed of the entire outer wall of the right auricle arranged to record with separate levers the movements of the sinus end (*S*) and of the appendicular end (*A*). From Exp. 29.

tion with which there is some doubt connected, have contracted only during stimulation.

Strips composed of the vault only, either right or left, likewise do not beat subsequent to stimulation. This statement, in so far as it concerns the right vault, is made with some reserve, since only two experiments have been made on this part of the auricles.

Stimulation of strips composed only of septum or coronary sinus or atrium may start a series of beats in the case of the quiescent strip or *may increase the rate and* to some extent the *amplitude of an existing* beat.

Usually, however, in this investigation strips have been so made as to include two or more of the supraventricular parts; as a rule, they include some part that would upon stimulation yield a series of beats and extend out from this to include parts not spontaneously rhythmical, — to the right or left appendices or to the vault, for instance.

When such strips are stimulated through and through, a series of beats usually results, or if the strip was previously beating, both the rate and amplitude of beat are markedly increased (see Fig. 4). The same result is obtained in certain cases when only the spontaneously rhythmical part is stimulated. Stimulation of the non-rhythmical, *e. g.*, appendicular, end only at a time when no part of the strip is beating, is without effect; in case, however, the whole strip is beating the *amplitude* of beat of the non-rhythmical part may be markedly increased (Fig. 5); and if only the sinus is beating stimulation of the

non-rhythmical end may result in the extension of the beat into the latter.

It would seem therefore that under the conditions of our experiments there exists in the rhythmical regions of the supraventricular parts a mechanism which, when stimulated, brings about an increase in rate mainly, to a certain extent, however, an increase in amplitude of beat of that part also; whereas stimulation of the non-rhythmical parts, either directly or through the medium of the rhythmical parts, causes only an increase in amplitude of beat or puts them in a position to respond with beats to impulses previously impotent. In other words, the rhythmical regions are provided with a chronotropic mechanism mainly, the non-rhythmical with an inotropic almost exclusively. There is obviously a division of labor and, it would appear, a division very well adapted to the different work of the two parts.

The results obtained in this connection through local stimulation of the outer wall of the right auricle, excised and suspended as described on a previous page, confirm in every respect those just mentioned, and demonstrate in addition that all parts of the rhythmical region upon the one hand and all parts of the non-rhythmical region upon the other respond to stimulation in practically the same way. The supraventricular parts which contain the nodes behave the same in this respect as the other rhythmical parts of the auricles.

The carrying power of sinus impulses. — Attention was called above to the fact that in the case of the compound strip stimulation of the sinus, when it alone is beating, may cause the whole strip to beat, or when the whole strip is beating, may greatly increase the amplitude of the appendicular beat. By some, such results would be explained by assuming that the stimulus has altered the conducting power of the non-stimulated regions. We, however, believe that this apparently dromotropic influence is due entirely to an altered carrying power of the sinus impulse, not to any alteration in conductivity. The feebler impulses, we assume, do not reach all of the muscle fibres, while the stronger ones have a wider influence; hence the varying amplitude of contraction. There is no need of assuming here any shortcoming of the "all or none" law.

At times the impression is gained in experiments with local stimulation that impulses emanating from certain parts of the sinus are par-

ticularly effective in increasing the amplitude of the beat of the non-rhythmical parts. This is strikingly seen in the case of impulses started by local stimuli applied just below the mouth of the superior cava. These seem to spread most readily into the appendix and to elicit there the highest contractions. Such a result might be explained as being due either to the relative strength of impulses emanating from, or to the proximity of, this particular part of the sinus to the appendix. That the latter interpretation is probably the correct one is indicated by the fact that in an experiment in which stimulation of the superior cava region was regularly inducing higher contractions of the appendix than stimulation of the inferior cava region, a cut made so as to increase the distance between the superior cava and the appendix, but without injuring the superior cava region, at once did away with the unequal influence (see Fig. 2).

At this place attention should be called to the fact that the two points selected for stimulation in this experiment were on the thick tissue of the sinus region and were probably close to the gray line of the venous valves. In our experience these places above all others have responded to stimulation very constantly. In one experiment the only part of a strip consisting of the entire outer wall of the right auricle that could be seen to beat was the gray band described on page 91, and which probably represents the remnant of the venous valves. The beat in this case started in the vicinity of the superior cava and traversed the gray band to the inferior cava.

Description of typical series. — This is scarcely necessary after the discussions and figures of the foregoing sections. Briefly, however, a rhythmical strip may or may not begin to beat while in air after stimulation. Should it beat then, it is usually with decreasing amplitude. When the strip is immersed immediately after stimulation, the beats, as a rule, are small at first and infrequent, but, presumably as the strip takes on the temperature of the bath, they rapidly increase in amplitude and rate, reaching the maximum usually within ten to twenty seconds. As a rule, this is not maintained very long, but almost at once amplitude and rate diminish, much more slowly, however, than they increased, and reach the original height and frequency usually within thirty to sixty seconds (see Figs. 6, 7). Sometimes the beat remains improved over a very long period of time. This long-continued improvement is seen most impressively in the case of

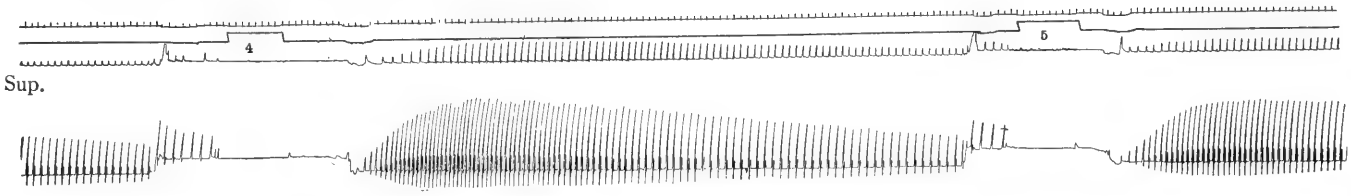

FIGURE 6 (about one half the original size). — Showing the greater subsequent effect on rate and amplitude of stronger (coil at 4) than weaker (coil at 5) stimuli. Superior and inferior cava strips mounted separately. From Exp. 12.

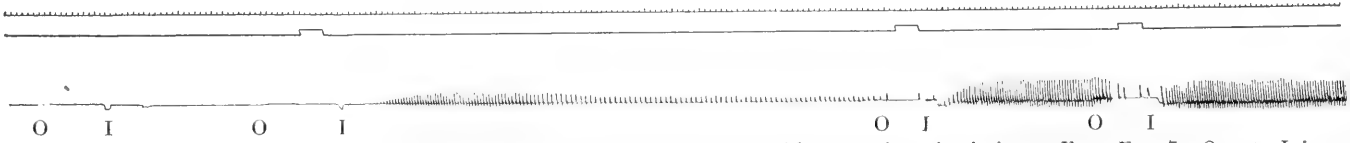

FIGURE 7 (about one third the original size). — Showing the improvement with successive stimulations. From Exp. 7. *O*, out; *I*, in.

strips which, after excision and suspension, fail to beat at all until they have been stimulated, and then beat without further treatment, it may be, as long as a half hour or more. Departures from these, perhaps the normal, responses will be considered later.

Some conditions influencing the response of strips. — (1) The *influence of the strength of stimulation* has been studied quantitatively only for the purpose of determining threshold values. In this connection it will be said merely that with the apparatus we have employed the first improving effects, as a rule, were obtained when the secondary coil was from 10 to 9 cm. from zero. This is clearly shown by the specimen record reproduced in Fig. 4. The strength of a sufficient stimulus is not without effect, the stronger currents increasing to a certain extent the amplitude as well as rate of beat (Fig. 6).

(2) *Influence of duration of stimulation.* — On account of the cooling of strips exposed for stimulation quantitative results on the subsequent influence of tetanic stimulations of varying durations have not been obtained. It is, however, obvious that the length of the subsequent series, but probably not the rate of subsequent beat, varies in the same direction as the duration of stimulation. This is certainly true for stimuli lasting as long as twenty-four seconds. Brief stimuli of different durations, one to three seconds, also have an appreciable effect.

(3) *Influence of repeated stimulation.* — Often, when a single stimulation followed by immersion is without effect, repeated stimulation under exactly the same conditions will eventually start a series. Then, or at any time while the strip is not beating with maximum amplitude, repeated stimulation up to the number of three, four, or even five may successively increase the response (Fig. 7).

(4) *Fatigue and recovery with rest.* — Although it is possible in most experiments to obtain series after series by means of stimulation, it is found in some cases that sooner or later the strip fails. Then a rest varying in duration from some minutes to many hours may so change the condition of the strip that responses to stimulation equal to those first obtained may again be elicited. It would seem, therefore, that under the influence of stimulation the strip eventually fatigues and that a long rest serves to remove these fatigue effects.

The longest time it has been found possible to keep a strip beating by our method, allowing rests for recovery from fatigue effects, has been nineteen hours.

(5) *Temperature.* — The best responses to stimulation are obtained at temperatures lying between 29° and 36° C. The maximum temperature has not been determined. An active strip when gradually cooled usually stops beating at 25.5° to 24.5° C. Upon warming, it may begin to beat spontaneously at from 27.5° to 28.5° C. Strips cooled to 26.5° C. have given beautiful series subsequent to stimulation. A study of the exact relation between temperature and rate of beat has not been included in this research.

(6) *Is perfusion essential?* — As a preliminary to practically all of our experiments the heart was perfused with Locke's solution. This was done for two reasons: first, because it was discovered while working with the perfused heart that strips could be made to beat; and, secondly, by washing the heart tissue free of blood the fluid bathing the strip remained clear, so that the behavior of the strip could be noted with the eye as well as with recording instruments. It should be added, however, that this preliminary perfusion does not seem to be essential to the obtaining of series of beats, although the unperfused strips do not seem to behave quite so well as the perfused.

(7) *Influence of certain substances upon the strips.* — A few experiments were made for the purpose of studying the influence of certain substances in the bath upon the behavior of the strips.

Oxygen, it was found, is not essential to the success of an experiment, at least in amounts over those ordinarily held in solution by freshly distilled water exposed to atmospheric air. In larger amounts, however, such as can be added to a solution by bubbling the pure gas through it, oxygen improves decidedly the beat of active strips.

A non-rhythmical strip, such, for example, as one made of the right appendix, and which could not be made rhythmical by stimulation, could not be made rhythmical either by substituting CO_2 for the O_2 or by treating it with a 0.9 per cent solution of sodium chloride even when stimulated tetanically. In pure sodium chloride solution such strips slowly lose their irritability, in CO_2 saturated solution rapidly, but they can usually be revived by reimmersion in pure Locke's solution, provided they have not been exposed too long to the unfavorable conditions. The irritability of rhythmical strips is affected by CO_2 and sodium chloride in the same way as is that of the non-rhythmical, and if they are beating the amplitude diminishes gradually to complete disappearance. The rate of beat is not certainly altered by such treatment.

It is interesting to call attention here to the fact that although the non-rhythmical terrapin's ventricle [14] and limulus heart muscle [15] can be made to beat spontaneously by treatment with sodium chloride, non-rhythmical mammalian auricular tissue cannot be made rhythmical in the same way.

(8) *Effect upon strips of sudden elevation of temperature.* — In the vast majority of our experiments we have had to deal with two important factors influencing the response of strips. It will be recalled that the strip is taken out of the bath to be stimulated: it is, in other words, taken out of the warm bath and temporarily exposed to room temperature. The question therefore suggests itself, how much, if any, of the effect subsequent to stimulation is to be attributed to the sudden elevation of temperature upon immersion and how much to tetanic stimulation? The conditions of the experiment have been varied in order to determine the relative values of these factors.

As a result, it has been shown that immersion alone may or may not exert some influence. Strips quickly prepared from a perfused heart when immersed in the bath sometimes beat, usually feebly, very rarely with considerable vigor, and, as a rule, such beats soon fail. When the strip has stopped beating, or if it has not contracted at all, exposure to room temperature and reimmersion has rarely if ever inaugurated a series of beats (see Fig. 7). If the strip is beating spontaneously or has been made to beat through tetanic stimulation, exposure to room temperature followed by reimmersion but without stimulation may improve the beat somewhat, never however as much as tetanic stimulation followed by immersion (Fig. 8, *a* and *b*).

It should here be noted that the beneficial influence of stimulation is preserved a very long while in the unimmersed strip. If, for instance, a feebly beating strip be stimulated tetanically immediately after removal from the solution and then be kept exposed to the air, it may or it may not suffer some improvement immediately after stimulation, but when it is immersed a long while, it may be many minutes, subsequent to stimulation, the amplitude and rate may be increased by much more than from simple reimmersion.

Stimulation out of the bath without subsequent immersion may, as has been noted above, increase the amplitude of subsequent beats, even

[14] For literature, see MARTIN: This journal, 1904, xi, p. 103.

[15] CARLSON: This journal, 1908, xxi, p. 11.

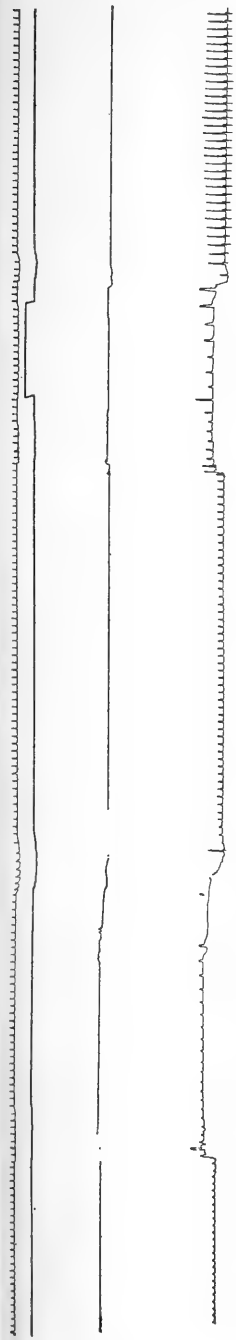

FIGURE 8 (one half the original size). — Showing (*a*) slight improvement of beat consequent upon temporary exposure to room temperature and (*b*) the much greater improvement consequent upon exposure plus stimulation. From Exp. 12.

when the strip is kept at room temperature, but such conditions cannot be continued any great length of time owing to the excessive cooling of the strips.

In an attempt to eliminate this difficulty strips were mounted and studied in a moist chamber, but it was found that strips so treated gradually shorten and at the same time lose their irritability. Curiously enough, this shortening of the strips is accelerated during tetanic stimulation. When a strip that has lost its irritability in a moist chamber is reimmersed in Locke's solution, its irritability returns, and the beats eventually become as large as those of a companion strip from the same heart, but not exposed in a moist chamber.

On the other hand, attempts to revive the strip by stimulating it in the bath have yielded unsatisfactory results. Despite the fact that care was taken to insulate the connections up to the points of attachment to the muscle, so much of the current was shunted by the solution that at best only minimal stimulating effects could be obtained.

It has therefore been impossible to determine quantitatively the relative potency of stimulation and of immersion with elevation of temperature in the initiation of a series of beats. It is evident, however, that both are factors, and that of the two tetanic stimulation is by far the more potent.

The cause of improvement of beat. — It would be of the very greatest interest if it could be determined how tetanic stimulation effects the improvement in rate and force

of beat of auricular strips. We were inclined to believe at first that the phenomenon was related in some way to the treppe process, that the stimuli and resulting contractions, by acting upon an otherwise completely or relatively inactive strip, remove resistance to conduction and heighten rhythmicity. A few experiments made for the purpose of testing this hypothesis soon showed, however, that it was untenable. Thus repeated induction shocks, although eliciting contractions showing a beautiful treppe, have never resulted in any improvement of subsequent beat, nor started a series, despite the fact that the strength of the single shocks has in some of the trials been very much greater than that of the tetanus.

On the other hand, the quiescence or apparent quiescence of the strip that usually obtains during tetanic stimulation might lead one to believe that both this quiescence and the subsequent improvement of reactivity of the heart tissue are affected in some way through the same mechanism that stops the intact heart and subsequently improves its beat when the peripheral end of the vagus nerve is stimulated.

In view of this suggestion it was deemed advisable to determine what effect atropin might have upon the response of strips to tetanic stimulation. We give the protocol of such an experiment:

Time in min.	
0	At this time when the horizontal inferior cava strip was reacting constantly and beautifully to tetanic stimulation, coil at 9½, and while the strip was beating, enough atropin was added to the Locke's solution bathing the strip to make it a 0.5 per cent solution.
4	After making five contractions the strip stopped beating.
6	Not beating. Stimulation, coil at 9½, gives initial contraction only. Stimulation, coil at 9½, gives initial contraction and is followed by a series of slow irregular beats in Si: A block.
12	No further improvement with stimulation coil at 9, at 8½, at 8, nor at 7½.
22	Strip immersed in pure Locke's solution (free of atropin).
23	Stimulation with coil at 7½ gives decided improvement of beat through several successive stimulations. Later, improvement was obtained with coil at 8½, but not at 9½. The beats, however, were never as good as those obtained before atropin was administered.

98 Strip was immersed in another beaker of fresh Locke's solution and again, upon stimulation with the coil at 8, a better beat was obtained, but the beats obtained were at no time as fine as the splendid series of the pre-atropin stage of the experiment.

This experiment, as well as others of the same kind, demonstrates that in the case of strips prepared by our method atropin seems to have no other action than to diminish the ability of the strip to respond with a series of beats after tetanic stimulation. It is possible, although difficult to demonstrate clearly, that the strip treated with atropin is more apt to respond with contractions while being stimulated tetanically than the strip not so treated, But even if this could be demonstrated beyond peradventure it would be quite as justifiable to conclude from it that it is due to decreased irritability through atropin action as to the setting aside of an inhibitory mechanism. We have therefore reached no conclusion as to the way in which tetanic stimulation acts to initiate a series of beats and to improve both the rate and amplitude of beating strips.

It should be added here that the galvanic current, within the strengths that have been used (2 volts, 0.75 milleamp.) have been without appreciable after-influence upon the strips.

Irregular types of response. — The after-effect of stimulation of automatically rhythmical parts of a strip usually consists of a perfectly regular series of beats such as has been described above. Not infrequently, however, and especially when a small part rather than the whole of the strip is stimulated, atypical series of beats are obtained, Such atypical responses for the sake of convenience are considered under two heads.

(1) The first of these groups is characterized by a sudden change in rate of beat, consisting usually of (*a*) a decrease, rarely of (*b*) an increase.

(*a*) In the case of the former the series shows the usual initial increase in amplitude and rate. When the beats have attained their maximum or have passed it and are beginning to decrease, there occurs an abrupt slowing of rate. This new rate may be constant from the moment of its onset (Figs. 5 and 9); more commonly, however, the slow rate is developed more or less gradually out of complete stoppage of variable duration (see Fig. 10).

(*b*) The sudden increase in rate, which has been seen only occasion-

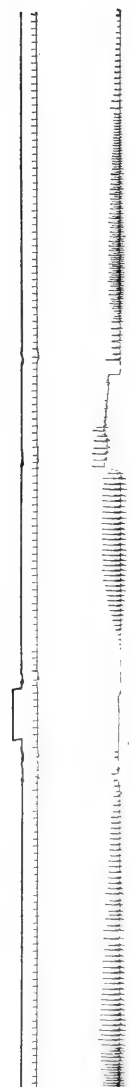

FIGURE 9 (one half the original size). — Showing some of the irregular types of series commonly obtained, including (*A*) sudden decrease in rate and (*B*) sudden increase in rate, in this case with alternating beat. From Exp. 17.

ally, usually appears while the amplitude of beat is increasing subsequently to stimulation and persists a variable, though usually a short, time, when there occurs an abrupt return to a slow rate which is approximately the same as that recorded prior to the sudden increase (see Fig. 9).

We have never succeeded in discovering the mechanism of the above-mentioned variations from the usual response. Their resemblance to pictures often seen in experiments on heart block is very striking. Thus the sudden stoppage with subsequent development of a slow rate is identical with the result of suddenly and completely severing the functional connection between a less rhythmical and a more rhythmical part of the heart. And the sudden acceleration with equally sudden return to the former slow rate resembles what is seen when a partial block suddenly disappears and then reappears. Still, with certain exceptions to be mentioned below, we have never succeeded in obtaining with the eye or with recording instruments any evidence of block at these times. It should be added that in those instances in which there is no stage of development associated with the change in rate, the two rates do not bear to each other the exact aliquot relation that obtains in changing degrees of partial heart block.

The sudden decrease in rate, sometimes preceded by stoppage and gradual development of a new rate, is satisfactorily accounted for, amongst other ways, upon the assumption that the part of the strip that has had its rhythmicity increased by stimulation and which for that reason has been setting the pace of the strip, suddenly loses its rhythmicity almost completely, or in some way loses its influence over the rest of the strip, and that then the next most rhythmical part assumes the function of pacemaker. This it may do with rate

of beat fully developed at the outset or, owing to the fact that its rhythmicity has been dominated for a while by another part, it may gradually acquire its usual rate, much in the same way as happens when the ventricles are suddenly liberated from the influence of their normal pacemaker, the auricles, or from an artificial pacemaker, for example, single induction shocks.[16]

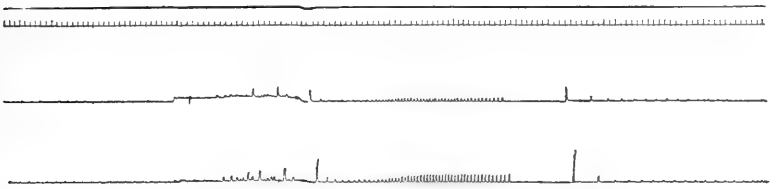

FIGURE 10 (two fifths the original size). — Showing the development of a slow rate of beat out of stoppage subsequent to a series of rapid beats induced by local stimulation.

It is more difficult to account for the sudden increase in rate. Possibly, however, the stimulus in these cases starts beating rapidly a limited area, the impulses from which fail for some unknown reason to reach the rest of the strip. When they do become potent, the rate of beat of the whole strip is suddenly increased.

These changes in rate resemble those which are commonly observed in cases of so-called paroxysmal tachycardia, and I have no doubt, as has very recently been suggested by Lewis [17] and others, but that certain of the paroxysms in man are started in much the same way as in the case of our strips: they are determined by some stimulus acting either directly or possibly indirectly upon some part of the spontaneously rhythmical region of the heart. Our experiments would seem to indicate that the part acted upon need not be the so-called nodal regions, as has been assumed by some authors, but any part of the right auricle inclusive of the septum, but possibly exclusive of the right appendix and vault.[18]

[16] ERLANGER and HIRSCHFELDER: This journal, 1906, xv, p. 153.

[17] LEWIS: Heart, 1909–1910, i, p. 262.

[18] LEWIS (*Loc. cit.*) maintains, mainly as a result of his observations on a case of paroxysmal tachycardia in which the paroxysms were preceded by ectopic auricular extrasystoles, that "there is no essential distinction between extrasystolic and paroxysmal beats." If I understand Lewis correctly, I do not believe his statement is justifiable. I am inclined to believe that there is a distinct difference between occasional extrasystoles, paroxysmal acceleration, and fibrillation

In this connection a case of paroxysmal tachycardia recently described by Lewis [19] is of some interest in that there seems to be every reason for believing that the auricle in this case behaved quite like some of our strips. Not alone were there the paroxysms of tachycardia, but in addition these paroxysms ended abruptly and were succeeded by a pause before the heart resumed its normal slow rate.

(2) In the second category of atypical series may be included all of the variations which without doubt are due to the blocking of impulses.

The types of block and the consequent departures from the typical series of beats have been very varied. Some of the blocks undoubtedly were entirely artificial and due to injury inflicted while preparing the strip. There is, however, every reason for believing that most were natural and were due either (*a*) to the natural difficulty the impulse experiences in traversing heart tissue or (*b*) to the existence of natural blocking points possibly at the junctions of developmentally different segments of the heart.

(*a*) The former variety of block is frequently seen in strips beginning to beat subsequently to immersion in the bath *per se*, or subsequently to stimulation and immersion. It is then seen that only the venous end of the strip begins to beat. Gradually, however, and without further treatment of the strip, or as a result of repeated stimulation, the contraction wave penetrates further and further until the strip beats throughout its entire length. This phenomenon is probably identical with that observed when strips of terrapin's ventricle, in which there is no reason for believing there are any natural blocking points, are

in that these probably represent three distinct results of irritation of the heart. The relation between them may be represented as follows: When a constant but weak stimulus acts upon a heart whose irritability is gradually increasing, or when a gradually increasing stimulus acts upon a heart with constant irritability, the first response may be occasional extrasystoles without any alteration of rhythmicity. Then the stimulus may alter for a time the rhythmicity of the heart tissue and thus cause the paroxysmal accelerations. Eventually fibrillation, the nature of which is not understood, may ensue. For this a very strong stimulus or a highly irritable tissue is necessary. In the present research we have not met with fibrillation, probably for the reason that under the conditions of our experiments the strips possess a very low grade of irritability. If these three conditions are the same and extrasystolic in nature, the proof that such is the case is still to be furnished.

[19] Lewis: Heart, 1909, i, p. 43.

taught to beat by applying stimuli to one end or by immersion in certain salt solutions. The blocks in this case are almost invariably complete and pass away gradually by the progressive increase in the extent of the part of the strip that beats.

(*b*) The second variety of block, namely, that occurring at what may perhaps be considered natural blocking points, may be either partial or complete. The records that have been obtained of these blocks leave no doubt as to the correctness of our interpretation (see Figs. 11 and 12). The blocks appear and disappear abruptly, and therefore determine a very abrupt and unmistakable change in the appearance of the record. As to the cause of these changes in the degree of block, it would appear that almost invariably they are referable to changes in the strength of the impulse; only rarely they can be explained upon the basis of altered irritability. Thus, in case the conditions for the occurrence of block are present, it may come on while the strength of the impulse is presumably decreasing and disappear when the strength of the impulse is presumably increasing. A glance at the figures (Figs. 11 and 12) will make clear the basis for this statement, in that it is seen that the block disappears while the amplitude of beat is increasing subsequently to stimulation and reappears as the amplitude of the beat diminishes.

On the other hand, a block between the pacemaker and its dependent part may disappear when only the dependent part is stimulated, and its irritability, presumably, thereby increased.

More rarely a block may appear suddenly and without apparent cause in the midst of an otherwise perfectly typical series of beats.

Position of blocking point. — Every effort has been made to determine macroscopically the exact position of the blocks that may be termed natural. For the sake of safety we have used for this purpose only those instances in which the block was partial, for the reason that localization is difficult in the case of complete block.

The vast majority of such blocks were seen in horizontal inferior cava strips, and in these strips they appeared to be at or near the junction of the parts we have designated sinus and atrium, perhaps a bit nearer than this to the venous end of the heart.

Blocks have also been seen some distance to the left of the superior cava in horizontal superior cava strips, but upon the whole it would seem that the impulse is conducted more readily from the region of the superior cava than from that of the inferior cava.

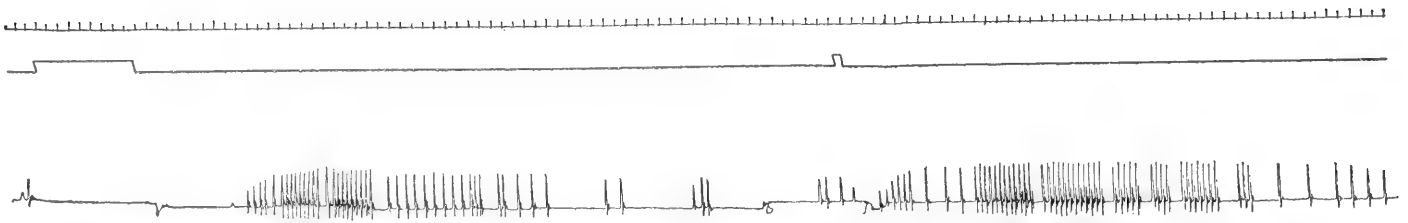

FIGURE 11 (seven eighths the original size). — Inferior cava strip showing complete and several grades of partial block presumably between sinus and atrium. The movements of the lever due to sinus contractions are barely perceptible. From Exp. 7.

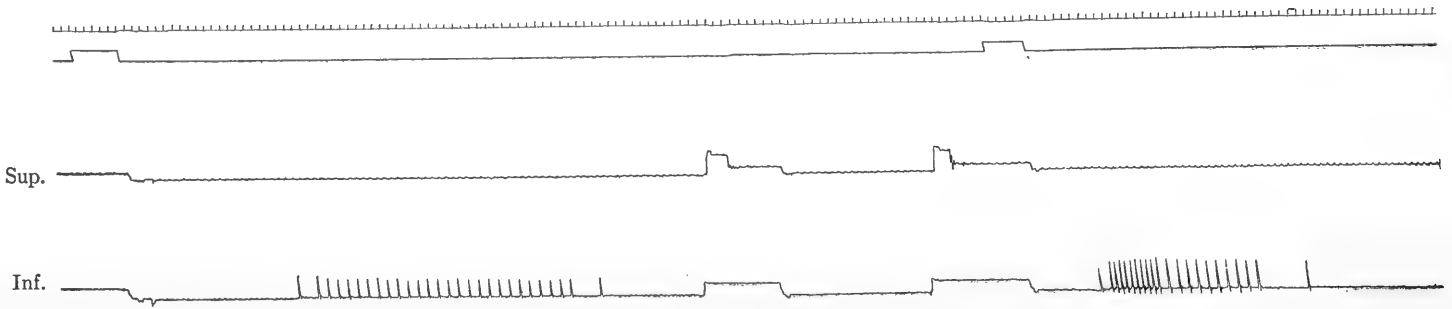

FIGURE 12 (seven twelfths the original size). — Superior and inferior cava strips, the latter showing complete and partial block presumably between sinus and atrium. The movements of the lever due to sinus contractions are barely perceptible. From Exp. 10.

Admitting, for purposes of discussion, the existence of a natural blocking point along the whole length of the sino-atrial junction, this result may be accounted for by the difference in the thickness and arrangement of the tissue surrounding the superior and inferior cava regions. About the former it is thick, while about the latter it is thinner and trabeculated, the tissue between the trabeculæ being especially thin.

Spontaneous blocks have been observed, though much more rarely, between the inferior cava region and the left auricle, between the superior cava and the right vault, and between the auricle and the right appendix. A more exact localization of these positions could not be made on account of the absence of clear landmarks.

That the blocks we have seen may have been the result of mechanical injuries cannot of course be positively precluded. There seems to be little reason for believing, however, that such was the case, while the frequent appearance, in different specimens, of block at about the same place would bespeak nothing but a natural resistance to the passage of the impulse at that place.

Viewed from the practical standpoint, these results serve to indicate that in the normal heart there is located somewhat to the left of the opening of the cavæ (especially of the inferior cava) into the auricle a place of relatively high resistance to the passage of the impulse, but that evidences of this resistance can be brought to light only when the conductivity of the auricular tissue has been greatly reduced by subjection to unfavorable conditions, such as have obtained in our experiments, and by narrowing the conducting tissues. Whether there are other such regions of low conductivity cannot be positively affirmed until we shall have the results of a larger number of experiments at our disposal.

There is hardly any need of adding that a more exact localization of these "natural" blocking points macroscopically but especially microscopically would be of the greatest interest, and should be attempted. It will not, however, be an easy investigation owing to the fact that the blocks cannot be produced at will, and even when obtained they are usually evanescent and in places rendered inaccessible by apparatus or folds of tissue.

Summary.

An arbitrary division of the cat's heart is made into sinus, coronary, septal, atrial, appendicular (right and left), vaultal (right and left), and pulmonary regions. When treated according to the method herein described, strips composed wholly or in part of the sinus, septal, coronary, and (or) atrial regions can be made rhythmical, and the beats have been maintained with intervals as long as nineteen hours. Under the same conditions the rate of beat developed in any part of these four regions is practically the same. This property as well as that of rhythmicity in general is therefore not dependent upon the presence of nodal tissue. Stimulation of the rhythmical regions in the main increases the rate of beat and the strength of impulse, whereas stimulation of the non-rhythmical regions increases the force of their beat, which must of course be determined from without, or renders them capable of responding to impulses from without.

The behavior of strips during stimulation and the subsequent response of rhythmical strips with regular series of beats and irregular series of beats, the latter including sudden accelerations and retardations resembling those seen in certain cases of paroxysmal tachycardia, and blocks of various kinds and at various places, but especially at or about the sino-atrial junction, are described and discussed.

A COMPARISON OF THE TOTAL NITROGEN EXCRETION OF EITHER KIDNEY IN NORMAL INDIVIDUALS DURING VARYING PERIODS OF TIME.

By THEODORE B. BARRINGER, Jr., and BENJ. S. BARRINGER.

[*From the Laboratory of Clinical Pathology, Cornell University Medical College, New York.*]

IN estimating the functional capacity of a diseased kidney more or less attention has been given of late to a comparison of the nitrogen excretion of the two kidneys, chiefly as shown by the urea output.

If the ability of a kidney to excrete the end products of protein katabolism is to be made a basis for the estimation of that kidney's functional ability, it would seem more rational to determine the excretion of the sum of the end products as shown by the total nitrogen, rather than by any one constituent as urea, which Folin has shown may form between 61 and 87 per cent of the total nitrogen.

As a basis for the urea comparison, Albarran[1] has reported his findings in a series of normal persons. Although on *a priori* grounds the total nitrogen excretion of either kidney might be expected to show the same variations as those of its most important constituent, urea, only isolated determinations have yet been made in normal cases as far as we can ascertain.

Our work was carried out on eleven young men, all in good health. In collecting the urine the method of Albarran was employed.

The flute-tipped ureteral catheter, with two lateral openings, was used. As large a catheter as possible — in a majority of cases 7 F. — was introduced to a depth of 15 cm. into the ureter. In most of the cases one ureter was catheterized, a bladder catheter collecting the urine from the other kidney. Before the collection of urines 1.5 c.c. of indigo carmine was injected into the ureteral catheter to determine if extra-catheter flow were present. No cases were included in the series in which blue appeared from the bladder catheter after this

[1] Albarran: Exploration des fonctions renals, 1905, p. 329.

injection. In one case the ureteral catheterism was bi-lateral, in which a bladder catheter controlled the question of extra-cather flow. In one case the urines were obtained by the Luys Separator.

As a rule, the urines for the first half hour following instrumentation were not collected. If, however, the urine began to run freely from each catheter after instrumentation, and there were no signs of reflex oliguria, the collection was begun immediately.

The catheterization occurred at intervals of from one to seven hours after eating, and the urine was collected for periods of time varying between twenty minutes and two hours.

In the following table our results are shown in detail:

TABLE I.

Normal Cases.

	Right Kidney.			Left Kidney.		
	Quantity.	Total nitrogen.	Urea-N + Amm.-N.	Quantity.	Total nitrogen.	Urea-N + Amm.-N.
B.	c.c.			c.c.		
Oct. 5	27.5	.291	.205	32.	.278	.215
E.						
Oct. 28	27.5	.368	.264	27.5	.36	.213
F.						
Oct. 28	15.		.127	20.		.132
W.						
Nov. 19	10.5	.102	.097	12.	.119	.113
M.						
Dec. 6	6.25	.101	.08	5.25	.096	.08
R.						
Dec. 6	7.75	.087	.056	7.5	.081	.053
L.						
Dec. 9	13.	.128	.107	13.9	.132	.09
F.						
Dec. 12	11.5	.125	.083	12.25	.133	.111
B.						
Dec. 15	12.75	.117	.092	11.4	.099	.074
L.						
Dec. 20	24.	.1444		31.	.1457	
M.						
Jan. 6	8.	.0996		7.5	.1081	

As regards the quantities secreted by each kidney during the same length of time: Once they were equal. Six times they varied by less than 10 per cent. Four times they varied by between 10 and 20 per cent.

Albarran found in twenty-six examinations that: Twice the quantities were equal. Ten times they varied by less than 10 per cent. Fourteen times they varied by between 10 and 30 per cent.

As regards the total nitrogen.: In one case the quantities were equal. In seven cases they varied by less than 1 gm. per litre. In two cases they varied by between 1 and 2 gm. per litre.

The nitrogen-urea plus ammonia-urea showed in three cases a variation of less than 1 gm. per litre and in six cases a variation of between 1 and 2 gm.

Albarran found in a series of thirty-nine cases that the *urea* itself differed by less than 1 gm. per litre in twenty-nine cases. In ten cases there was a difference of between 1 and 2 gm. per litre. Obviously no basis exists for a comparison between these results and our findings of the excretions of urea-nitrogen plus ammonia-nitrogen.

SOME OBSERVATIONS ON THE PRODUCTION OF LIGHT BY THE FIREFLY.

By JOSEPH H. KASTLE and F. ALEX. McDERMOTT.

Introduction.

IN connection with certain investigations on the oxidizing ferments and biological oxidations, the attention of one of us (Kastle) was attracted to the subject of light production by the common firefly as early as 1901. It was found that aqueous extracts of the luminous organ of the firefly gave no color with fresh tincture of guaiacum. With guaiacum containing small amounts of hydrogen peroxide, however, a faint blue coloration was obtained, and, as is the case with such a great variety of living tissues, the hydrogen peroxide was decomposed. In other words, these preliminary observations pointed to the absence of an oxidase in the luminous organ of this insect, and to the presence of small amounts of peroxidase and catalase. During the summer of 1909 the opportunity presented itself for making some additional observations on the production of light by this insect. For the most part these were made before we had had the opportunity to thoroughly familiarize ourselves with the extensive literature of the subject,* and hence include much that had already been done before. We have been able, however, to add a little here and there to the great wealth of observations on this interesting phenomenon, and to confirm much of the older work on the subject. In what follows we propose to consider the production of light by the luminous

* During the past year one of us (McD.) has attempted to compile a complete bibliography of the literature of this subject, and already over eight hundred references have been obtained, dating back in some instances to the times of Aristotle and Pliny the younger.[24] Obviously anything like a complete survey of this extensive literature is beyond the scope of the present communication, and hence only such references have been made use of as seemed to us necessary for an understanding of our present knowledge of the subject.

organs of the common firefly (Photinus pyralis Linn.), under the influence of mechanical, physical, and chemical stimuli.

Mechanical and Physical Stimuli.

Mechanical stimulation. — It has long been known that the production of light by living things is greatly influenced by mechanical and physical stimuli. Thus Spallanzani [36] observed that light production by the Lampyridæ is greatly intensified by scratching or stabbing the luminous organ with a needle. Kölliker [23] also found that light is emitted by the luminous organ if it be divided or crushed, and also if it is pulled to pieces or subjected to slight pressure. Similarly Faraday [12] found the luminous material of the glow-worm to glow actively on being pressed with a knife. Humboldt [20] also observed that a luminous medusa which had been bound to a tin plate by a strip of metal actively lightened as the result of any motion or disturbance of the plate. Macartney [26] also observed the luminous medusæ to emit light when the water containing them was subjected to agitation. As the result of his studies on this subject, Heinemann [16] finds mechanical stimuli to be the most active of all stimuli to the production of light in living forms. Artaud [1] also showed that luminiferous sea water at rest lightens again when it is disturbed. That such is the case is evident from the production of light in tropical seas, following in the wake of a boat or other object moving through the water. Peters,[31] from his work on the phosphorescent Ctenophores, concluded that the combined action of darkness and agitation was one condition which would result in light production by these organisms, but that neither alone could be relied on as a positive stimulus to the photogenic function. On the other hand, Kölliker [23] found, in the case of the higher phosphorescent animals, that the normal movements of the animals themselves are apparently without influence on the production of light. According to Agassiz [43] phosphorescent medusæ are sensitive to shock, and according to Todd [39] all mechanical and chemical stimuli which ordinarily produce pain cause the luminous organs of the firefly to lighten. Pflüger [33] observed that when one cuts off the head of a Lampyris the light is extinguished, but that when after a time the motions of the rump begin again, the light reappears, although weaker than at first.

According to Tilesius [38] and Meyen [27] repeated stimulation exhausts the light-producing power of photogenic organisms, and Macaire [25] found that sudden noises have the power to darken the light of the firefly.

The following observations of our own on the effect of mechanical and physical stimuli on the production of light by the detached organs of the common firefly (Photinus pyralis Linn.) are also of interest in this connection. The luminous organs were removed from the insects by means of a delicate pair of scissors. As a general thing, these luminous organs at once burst into glow as the result of removal from the insect. In some cases this glow spread uniformly over the whole surface of the luminous organ; in other instances it was confined to those portions of the luminous organ nearest to the line of the cut. If allowed to remain undisturbed, these detached luminous organs gradually ceased to glow and remained quiescent practically indefinitely. They were found to retain their power to glow actively, however, for several hours after removal from the insect, in spite of the fact that they had dried out considerably in this time, and in one instance a luminous organ which had been preserved in a 1 per cent trikresol solution was found to glow strongly eight hours after removal from the insect. Squeezing between the thumb and finger, a sudden blow with a match-stick, tapping with the head of a pin, or pricking or scratching with a needle or pin, dropping through a distance of three or four feet to the floor, and plunging into ice water were all observed to cause these quiescent, dark, luminous organs to burst actively into a glow.

As a matter of fact, it has been found that percussion furnishes us with a simple test for judging of the photogenic activity of the luminous organ of the firefly. Thus we have observed repeatedly that the moist luminous organ of the firefly which does not emit light on percussion cannot be made to lighten by any other means.

Temperature. — The production of physiologic light is also greatly influenced by temperature. Thus Macaire [25] observed the light of Lampyridæ to be extinguished above 52° C. and under 12° C., and that heat which is far below that of boiling water destroys the light-producing material of the firefly. He also states that the light of the Lampyridæ is extinguished at 10° R. (12.5° C.). At 22° R. (27.5° C.) he found these animals to lighten again. At 33° R. (41.3° C.) the light

was strongest, and at 46° R. (57.5° C.) it was extinguished. He also found it to be impossible to restore the power to lighten in those insects which had been heated to 47°–50° R. (58°–63° C.) He found further that the free luminous organs of the Lampyridæ glow most actively at 33° R. (41.3° C.); at 42° R. (52.5° C.) the light was extinguished and the organ itself took on the appearance of cooked egg. This author also observed that heat cannot cause the emission of light in the luminous organ which has been kept *in vacuo*, but that it lightens as soon as air is admitted. Jousset de Bellesme [21] also found that increasing warmth stimulates the Lampyridæ to lighten. This author, however, considers this to be an indirect phenomenon, due to the fact that heat excites the animal. He also observed that if the animal be killed by exposure to a high temperature, the light is extinguished forever. Bongardt observed that up to 40° C. the light of fresh organs of the firefly increased somewhat with the rise of temperature, whereas above this temperature it became weaker, and ceased at 68° C. This author also observed that below 23° C. these animals do not lighten. At 48° C. also they seem to be dead, but still lighten again occasionally; at 59° C. their power to lighten is permanently destroyed. Kölliker [23] also investigated the effect of temperature on this phenomenon. According to this author, heating the luminous organs to 40° to 60° R. (50° to 75° C.) produces a constant bright light. He also found that cooling to −3° to −5° R. (−3.75° to −6.25° C.) caused the organ to lighten, but only rarely and not so certainly. He also observed that sudden changes of temperature, such as that caused by removing the insect from a piece of ice to the hand, nearly always react upon the organism, causing it to lighten.

According to Pflüger,[33] these temperature effects are characteristic of all light-producing material of animal origin. Thus with molluscs (Pholaden) light is produced by moderate warmth and is destroyed by boiling. This author observed that the light emitted by putrid fish disappears at 0° C., but reappears on warming and is permanently extinguished by boiling. Artaud [1] also observed that luminous sea water lightens best at 43° C., and according to Michaelis [28] the water of the Ost See cannot be heated above 24° R. (30° C.) without extinguishing its luminosity. Peters [31] observed that the phenomenon of phosphorescence in Ctenophores was produced at

temperatures between 9° and 37° C., the optimum temperature being about 21.5° C., the temperature of sea water.

Electricity. — Similarly, most observers are agreed that light is produced by the luminous material of photogenic life forms as the result of electrical stimulation. Thus Macaire [25] found the Lampyris (Luciola italica) to emit an intense light as the result of electrical stimulation. He also observed that the clear, translucent light organs of this insect emit light under the influence of the electric current as long as the current is passing, but that dead, opaque organs no longer respond to the electrical stimulus. So also he observed that the electric current is powerless to produce light in living organs which are kept *in vacuo*, but that on the admission of air the current at once causes such organs to glow. Jousset de Bellesme [21] also found the electric current to be an exciter of phosphorescence in the Lampyridæ, but whether a primary excitation or a secondary one due to a general irritation of the animal could not be determined. Kölliker [23] also found that electrical stimulation of the light organ of the firefly resulted in the production of an intense light. Pfaff [32] observed that phosphorescent sea water appears full of light when an electric current is passing through it, the light apparently emanating from minute particles in active motion. Heinemann [16] has also devoted considerable attention to the study of the electrical stimulus on phosphorescent animals, and Pflüger [33] has called attention to the fact that neither the contraction of the muscles of lower animals nor the emission of light by photogenic life forms is brought about by the electric spark or shock, but only by the passage of a continuous current.

Vacuum. — The effect of the vacuum on the production of light by photogenic life forms has also been investigated by different observers with different results. Thus Carradori [4] observed that the Italian luciole continues to shine in a barometric vacuum. On the other hand, Pflüger [33] cites Heinrichs [17] to the effect that the light emitted by living things disappears *in vacuo* to reappear again on exposure to air. Similar results were obtained by Macaire [25] with Lampyris (Phausis) splendidula. He observed that *in vacuo* neither heat nor the electric current could cause the production of light in the luminous organ of this species, but that they glowed at once on the admission of air. So also it is said that the light of decaying fish is extinguished in a vacuum, and Dubois [8, 9] observed the lighting of Pholas and Pyrophorus to be suspended therein.

Our observations on the effect of drying *in vacuo* on the photogenic process are also of interest. A number of live fireflies were placed in a large petri dish, in a vacuum desiccator, over sulphuric acid. As soon as the desiccator was connected with the vacuum pump (giving a vacuum of 27.5 inches) the insects began to show signs of distress, as manifested by greatly increased activity. At the same time they began gradually to elongate from head to tail, to such an extent that the luminous organ protruded almost or quite its entire length beyond the elytra, which ordinarily cover it over completely on the dorsal side. At the same time the insects began to lighten vigorously and rapidly, and in a short time the entire luminous organ of each insect was glowing brightly and continuously, whereas ordinarily, as is well known, light production by this species is an intermittent phenomenon. The detached luminous organs of the insect showed essentially the same conduct so far as the production of light *in vacuo* is concerned. On exposing a number of them to the vacuum they began glowing brightly and continuously, one after another, until all of them were in a state of glow, which lasted an hour or longer. In this connection it is of interest to note that all of the luminous material is not consumed on drying *in vacuo*, since on moistening the dry material with water it again emits light.

The production of light by photogenic life forms is also brought about by the action of chemical stimuli. In the literature accessible to us we have been able to find references to the action of no less than sixty-eight different substances. These include various gases and vapors; also liquids and solutions of acids, alkalies, salts, and alkaloids.

Chemical Stimuli.

In order to test the conduct of the luminous organ of the firefly towards various gases the following mode of procedure was adopted: The luminous organs of the insect were removed by means of scissors, and kept upon a watch-glass until they had become quiescent and had ceased to glow. They were then placed in short glass tubes open at both ends, about 2 cm. in length by 0.5 cm. in diameter, and stopped loosely at both ends by means of glass wool. The receptacle for the gas consisted of a test tube with a gas inlet tube sealed into the bottom. The bottom of the tube was loosely stopped with glass wool, and the

upper end of the test tube was closed with a stopper carrying an open capillary tube. The gas whose effect was to be observed was passed in at the bottom of the tube and allowed to escape at the top. When the tube was filled with the gas, the rubber stopper carrying the capillary tube was removed as quickly as possible, and the short glass tube containing the luminous organ of the insect was dropped into the larger tube containing the gas, and the stopper again inserted. In this way the conduct of the luminous organ in a current of any given gas or in a quiet atmosphere of the gas could be observed at will.

In studying the effect of vapors such as chloroform, ether, etc., a small amount of the volatile substance was placed in a small test tube. A loose plug of glass wool was then inserted above, but not touching the liquid or substance, and the test tube closed with a cork. After the tubes had stood for a sufficient length of time for the vapor of the substance to diffuse into the upper part of the tube, the small glass tube containing the luminous organ of the firefly was introduced, and the test tube again closed with the cork. For hydrofluoric acid a lead tube was employed, and the luminous organ was suspended in the gas in a little basket made of platinum wire, and the conduct of the luminous organ observed by looking down into the tube from above. In testing the effect of solutions of acids, salts, alkaloids, etc., the solution was either injected into the live insect by means of a very fine hypodermic needle, or the cut surface of the excised luminous organ was brought in contact with the given solution on a test plate.

The following are the principal points of interest regarding the action of chemical stimuli on the biophotogenic process.

Air. — The production of light by living things takes place in atmospheric air. Indeed according to many observers oxygen is essential to its production, as manifest by the abundant supply of trachea in the luminous organs of insects, and by the fact that the light is intensified by an increase in the amount of oxygen supplied the tissue. Faraday [12] observed the luminous material of the glow-worm and firefly to glow on exposure to air, such exposure always causing a fresh emanation of light. According to Milne-Edwards,[29] physiologic light is produced only under the influence of oxygen. Dubois [8] found even the dry luminous organs to glow in air at a pressure of 600 atmospheres. On the other hand, according to Bongardt,[3] a stream

of air or a current of an indifferent gas seems to retard the lighting process. He concludes that in hydrogen or carbon dioxide it is not the indifferent gas itself which causes the light to diminish, but the current of gas. Still other references to this subject are given under oxygen. It has also been our experience that atmospheric air at room temperature is no direct stimulus to light production by the luminous organ of the firefly, unless the organ has been previously immersed in an atmosphere of an indifferent gas such as nitrogen, or some reducing gas such as hydrogen sulphide. Thus luminous organs of the firefly were frequently kept in the air for an hour or longer under the conditions already described on page 127, without showing any luminosity, and yet at the end of this time the organ glowed brightly on percussion. On the other hand, a luminous organ which had ceased to glow in hydrogen sulphide glowed strongly on being brought out into the air, and this phenomenon, namely, alternate extinction of the light in hydrogen sulphide and glowing in the air, could be repeated several times on the same luminous organ.

Oxygen. — According to Bischoff,[2] oxygen is absorbed and carbon dioxide evolved in the glowing of certain luminiferous rhizomorphs. According to Macartney,[26] Spallanzani[36] observed that in oxygen glow-worms shine more brilliantly than in air. Forster[13] found the light of certain living things to be more intense in oxygen than in common air. Jousset de Bellesme[21] observed the Lampyridæ to lighten intensely in oxygen. Dubois[10] found that Pyrophorus conducts itself in oxygen just as in air, only the intensity of the light seemed to be increased. According to Pflüger,[33] the lighting of dead fish first takes place with the absorption of oxygen and the evolution of carbon dioxide. He also states in another connection that the lighting of dead fish, etc., takes place only in respirable gases. This author states that dead fish glow in water containing oxygen, but not in freshly boiled water; they glow, however, in the latter medium also on the admission of air. Watasé[41] claims to have proved that the lighting of the firefly is directly due to oxidation ; on crushing the luminous organs on a glass slide and immersing in carbon dioxide, the light was extinguished; when the slide was removed from the carbon dioxide and placed in oxygen, the light reappeared. According to this author, this phenomenon could be repeated several times in succession on the same specimen. Miss Townsend[40] has also found that the photogenic tissue of

Photinus marginellus responds definitely to the action of oxygen. The light emitted by this tissue increases in brilliancy when placed in oxygen, and tissues, the light of which has been wholly extinguished in carbon dioxide, become instantly luminous when placed in oxygen. This author also observed that during life all the tracheoles are filled with air. On the other hand, Kölliker [23] found oxygen to have no real exciting action on the resting luminous organ of the firefly, and that the live insects lightened in oxygen only after immersion therein for an hour or longer. However, they then lightened very brilliantly. Giesbrecht [14] also concludes that the lighting of marine animals occurs without the aid of free oxygen, and Bongardt [3] also found the intensity of the light of certain fireflies to diminish in a current of pure oxygen, so that at the end of forty minutes only two insects were emitting light, and these but feebly, whereas, after shutting off the current of gas, the insects lightened intensely after one and six hours. Macartney [26] cites Sir Humphry Davy as authority for the statement that the light of the glow-worm is not increased in oxygen or chlorine, nor diminished in hydrogen. Carradori [4] considered that oxygen could have no effect upon the luminosity of the Luciola italica, since this insect continued to shine when immersed in oil, which he claimed could contain no free oxygen.

We found the luminous organs of the common firefly to glow in oxygen, but not very brilliantly. The glow persisted for some time, finally dying out. On immersion in hydrogen the organs which had lost their glow in oxygen did not glow. Such organs, however, were found to emit light on percussion at the conclusion of the experiment. A live insect placed in oxygen glowed feebly, but with no sudden and intermittent flashes * of light such as characterize the light emission by this insect under normal conditions.

* As is well known, the light of our common firefly (Photinus pyralis Linn.) is emitted intermittently at more or less regular intervals in distinct and definite flashes. In other words, the emission of light might almost be said to partake of the nature of a luminous explosion or coruscation. As we shall see, this sudden and intermittent emission of light can also be brought about by means of certain chemical stimuli. Usually, however, the light production as brought about by chemical stimuli is not of this character, but shows itself as a distinct and persistent glow which slowly and gradually spreads over the luminous organ and which continues without interruption for a considerable interval, sometimes for an hour or even longer.

Nitrogen. — Bischoff [2] observed that certain luminous rhizomorphs did not entirely lose their luminosity when brought into an atmosphere of nitrogen. Macaire [25] states that the light of the living organism is extinguished in indifferent gases. According to Spallanzani,[36] no living organism produces light in non-respirable gases. Jousset de Bellesme [21] observed the same conduct of Lampyridæ in nitrogen as in carbon dioxide, and Pflüger [33] states that the light of dead fish is extinguished in nitrogen and reappears on the admission of air. In our own experiments we found that the luminous organ of the firefly did not glow in nitrogen during a period of fifteen minutes; such organs, however, were found to emit light on percussion after removal to the air.

Hydrogen. — According to Bischoff,[2] the light of luminous rhizomorphs is entirely extinguished in hydrogen. Jousset de Bellesme [21] observed the same conduct of Lampyridæ in hydrogen as in nitrogen and carbon dioxide. Pflüger [33] also calls attention to the fact that the light of dead fish is extinguished in hydrogen, but reappears on the admission of air. On the other hand, Bongardt [3] found Lampyris noctiluca to lighten in hydrogen; the light gradually became weaker and ceased to be emitted after about fifty minutes. After four hours the insects lightened after shutting off the supply of gas and then lightened again on turning on the gas the next morning. We observed the luminous organ of the firefly to glow in hydrogen, but not very brilliantly. However, the weak glow persisted for some time, finally dying out. These organs glowed again when placed in oxygen.

Carbon dioxide. — Jousset de Bellesme [21] found Lampyridæ not to lighten in carbon dioxide. If, however, they were kept in this gas several hours and were then placed in a current of air, they lightened intensely. Bongardt [3] observed that Lampyris noctiluca was not killed by carbon dioxide, but that it lightened less intensely. According to Pflüger,[33] the light produced by living organisms is extinguished in carbon dioxide. He also pointed out that the light-producing substances of dead fish and decaying wood are destroyed on immersion in water saturated with carbon dioxide. Watasé [41] found carbon dioxide to extinguish the light of the crushed luminous organ of the firefly, and Miss Townsend [40] found the luminous tissue of Photinus marginellus to be extinguished in this gas.

We observed that when the luminous organs of the firefly are brought

into carbon dioxide, they do not glow at first; after some time, however, they glow faintly; this glow persists for some time and finally dies out. After an hour's exposure to the gas they glowed on percussion in the air. In certain of our experiments with inert gases results were obtained which seemed to indicate that the emission of light by the luminous organ in these gases was more the result of a mechanical stimulus due to variations in temperature and gas pressure than to the chemical action of the gas. In fact, our results show a distinct analogy to the influence of air currents and slight changes of temperature on the strychninized frog.

Carbon monoxide. — Bongardt [3] found Lampyris noctiluca to lighten in carbon monoxide after a few minutes' exposure. At the end of ten minutes the insect ceased to lighten. After three hours in the gas the insect was apparently dead, but after sundown those exposed to carbon monoxide still lightened. In carbon monoxide we observed the resting luminous organs of the firefly to glow dimly and the glow to be slow in appearing.

Chlorine. — Macaire [25] found the light-producing material of living organisms to be permanently destroyed by chlorine. On the other hand, Kölliker [23] found chlorine to act as a stimulus to the lighting of the firefly. We observed that the quiescent luminous organs of the firefly glowed faintly in chlorine and then went out. The organs exposed to this gas were found to be almost dead to percussion after a short exposure, whereas a control which had been kept in the air for the same length of time glowed very brightly on percussion.

Nitrous oxide. — According to Macaire,[25] "le gaz oxide d'azote" * produces nearly the same effect as oxygen on the production of light by Lampyris splendidula, namely, a brighter light than air. We have observed that on placing a live firefly in nitrous oxide it lightened once. The luminous organ then became quiescent. Later it emitted a very bright, steady glow, which was maintained for some time. When brought out into the air, it made only a few irregular movements with its legs during the first ten minutes, but later recovered completely and was finally lost sight of. The detached luminous organs were found to glow slowly but brightly in nitrous oxide, the light emitted being more yellow in color than that ordinarily produced.

* One can scarcely be sure from this name that MACAIRE meant nitrous oxide. — J. H. K.

Nitric oxide. — In nitric oxide Bongardt [3] found live insects to move about unquietly, and after half an hour no more lighting was observed. In four minutes after closing the tube three of the insects lightened intensely. On conducting the nitric oxide again through the tube the emission of light ceased in eleven minutes. We observed the luminous organs of the firefly to glow feebly when placed in this gas and then die out. They glowed again on being brought into the air.

Nitrogen tetroxide. — We have not been able to find any references in the literature to the action of nitrogen tetroxide. We have found that the conduct of the luminous organs of the firefly in this gas is similar to that in nitric oxide, only the glow is somewhat stronger.

Ammonia. — In ammonia we observed the luminous organs to glow brightly after a short delay.

Hydrogen sulphide. — According to Macaire,[25] the luminous organ of the firefly is entirely deprived of its light-producing power by hydrogen sulphide. Pflüger [33] also states that the light-producing substances of decaying fish and wood are destroyed by solutions of hydrogen sulphide. Jousset de Bellesme [21] observed that hydrogen sulphide extinguished the light of Lampyris immediately, leaving the photogenic cells intact in form, but robbing them of their photogenic function. In one instance we found that the luminous organ of the firefly did not glow in hydrogen sulphide, but did so on being brought out of this gas into the air. In another instance we found a luminous organ to glow feebly in hydrogen sulphide and then to die out; it also glowed again on bringing it out into the air. This phenomenon could be repeated at will several times, the organ ceasing to glow in hydrogen sulphide but glowing again on exposure to the air.

Suphur dioxide. — Carradori [5] found sulphur dioxide to extinguish the light of the luciole; Dubois [8] made the same observation on the cucuyo (Pyrophorus noctilucus), and Macaire [25] on the glow-worm. Of all the substances which we have thus far studied sulphur dioxide has been found to be the most quickly poisonous to the light-producing function of the firefly. When the detached luminous organs are placed in sulphur dioxide, they do not glow, and if glowing when placed in it they are immediately extinguished. Luminous organs which had been exposed to sulphur dioxide were found not to glow in oxygen, and were found to be dead to percussion when removed from this gas. These phenomena were repeated several times on different luminous

organs, the light of the glowing, luminous organs going out almost instantly on exposure to this gas.

Carbon bisulphide. — Kölliker [23] found carbon bisulphide to have no effect upon Lampyris. Heinemann [16] found the light organs of Mexican cucuyos to be killed in carbon bisulphide after a short time. We have observed that the resting luminous organs of the firefly glow very strongly in the vapor of carbon bisulphide. The glow is slow in appearing, but very bright. One of the tubes containing carbon bisulphide and the luminous organ was accidentally dropped upon the floor; the glowing, luminous organ broke into a number of pieces, each of which glowed very strongly, showing distinct coruscations. Of all substances which we have thus far examined, carbon bisulphide is probably the most powerful exciter of luminosity in the luminous organ of the firefly, and gives rise to phenomena which in a darkened room are very striking and beautiful.

Cyanogen. — We found the luminous organs of the firefly to glow in cyanogen gas. They soon went out, however, turned brown, and were found to be dead to percussion after a short exposure to the gas. The experiment was repeated several times with like results.

Hydrocyanic acid. — According to Bongardt [3] the luminous organs of Lampyris noctiluca are killed by exposure to hydrocyanic acid, and the live insects do not lighten in this gas, but five hours after apparent death one of these thus exposed did lighten again intensely, and another three hours after apparent death in the gas. Heinemann [16] found that on bringing a moistened piece of potassium cyanide in close proximity to the glowing organ of the firefly, it goes out in a short time and is apparently dead. Weakly luminous organs were found to be killed by such an exposure without showing any preliminary stimulation. According to Michaelis,[28] ten minutes' exposure to hydrocyanic acid weakens the luminescence of glowing sea water, and after thirty minutes the light is entirely extinguished.

We found that the resting luminous organs of the firefly glowed strongly in hydrocyanic acid gas and then died out, only, however, to glow again strongly. Certain of the organs glowed for some time, and after a few minutes' exposure to the gas were found to glow on percussion after they were taken out into the air. Such organs were observed to smell strongly of hydrocyanic acid, indicating that a certain amount of the substance had been actually absorbed.

Iodine cyanide. — In the vapor of iodine cyanide the luminous organs were found to glow faintly and for some time, after which they died out. Such organs were then found to be colored light brown, and did not glow on percussion.

Hydrofluoric acid. — In hydrofluoric acid gas we found the luminous organ of the firefly to give a slow but distinct glow. After remaining in the gas for some time it glowed brightly, and immersion in the solution of the acid failed to extinguish it.

Ether. — Heinemann [16] observed that ordinary ether acts as an irritant towards the luminous material. It kills the luminous organ, however, in four to five minutes, converting it into a solid, heavy mass. Kölliker [23] also found ether vapor to stimulate light production. Pfaff [32] observed sea water to lighten on the addition of ether. According to Macaire,[25] ether destroys the light-producing material of the firefly.

We etherized a firefly which was not lighting. A short time after being placed in the ether vapor it showed one short but vivid coruscation, and then one or two faint flashes of light, after which the luminous organ burst into a fine, steady glow which lasted for some time, dying out after several minutes. The detached resting luminous organ of the firefly in ether vapor also gave one or two preliminary minute flashes, and then burst into a full, steady glow lasting for some minutes. These interesting observations were repeated a number of times, always with a like result.

Chloroform. — Kölliker [23] found chloroform vapor to act as a stimulus to light production by the Lampyridæ. Heinemann [16] also found it to act as an irritant, similarly to ether. In order to determine the effect of chloroform on the luminous activity of the firefly, an active insect was placed in the vapor of chloroform. It at once showed loss of ordinary bodily activity; one or two bright flashes of light were emitted, and then the luminous organ burst into a strong, steady glow which persisted for half an hour. At the end of this time both the insect and the luminous organ were apparently dead. This observation was repeated a number of times.

The resting luminous organ of a firefly was placed in the vapor of chloroform; in a short time it showed a few faint flashes, and then burst into a full, steady glow, dying out more quickly than in ether. This phenomenon could be repeated at will. Luminous organs from

fireflies, one of which was still alive after twenty-four hours' captivity in a plain glass bottle, and the other dead, were placed in chloroform vapor; both glowed, but the glow was slower in developing and fainter than in the case of luminous organs from freshly captured, active insects.

Carbon tetrachloride. — When placed in the vapor of carbon tetrachloride, the luminous organs of the firefly glowed quickly and strongly, the glow being followed by a series of brilliant and repeated coruscations, and with the production of bright points and pulsations of light throughout the entire luminous organ, persisting for a considerable time. This substance is one of the most powerful exciters of luminosity in the firefly that we have thus far met with.

Ethyl chloride. — The luminous organ of the firefly was found to glow after some delay in the vapor of ethyl chloride. It soon went out, however, and showed no luminosity on being brought out into the air, and responded only feebly to percussion.

Ethyl bromide. — In ethyl bromide the luminous organ of the firefly glowed feebly after remaining in the vapor for some time.

Bromoform. — In the vapor of bromoform the luminous organ of the firefly glowed slightly after remaining in the vapor for some time.

Iodoform. — In the vapor of iodoform at room temperature the luminous organ of the firefly glowed very faintly after remaining in the vapor for some time.

Ethylene bromide. — In the vapor of ethylene bromide the luminous organ of the firefly was found to glow brightly and persistently for a long time.

Ethyl alcohol, — Giesbrecht [14] states that ethyl alcohol acts as an irritant to the luminous material. Heinemann [16] found that absolute alcohol instantly destroys the power of the Mexican cucuyo to lighten. Köliker [23] found alcohol of 45 per cent and higher concentration to act as a stimulus. Pfaff [32] observed sea water to lighten on the addition of alcohol, and Artaud [1] obtained similar results. On the other hand, Macaire [25] found alcohol to destroy the light-producing material of the firefly. According to our own observations, the detached luminous organs of the firefly glow brightly in the vapor of ethyl alcohol, finally showing a succession of brilliant luminous pulsations, and then dying out.

Methyl alcohol. — The results obtained were similar to those obtained

with ethyl alcohol. With both of these alcohols the luminous effects were very striking.

Amyl alcohol. — In the vapor of amyl alcohol the glow obtained with the luminous organ of the firefly was faint and slow in developing; it then became somewhat brighter, and then died down.

Allyl alcohol. — In the vapor of allyl alcohol the luminous organ was slow in glowing. It then began glowing in one corner of the luminous organ; after a time, however, the glow spread over the entire organ, which then glowed uniformly and brightly for some time.

Acetone. — In the vapor of acetone the luminous organ of the firefly glowed brightly after a short exposure and then died down.

Formaldehyde. — According to Giesbrecht,[14] formaldehyde excites certain luminous marine forms to luminescence. We have also observed that the luminous organs of the firefly glow steadily in the vapor of formaldehyde, but only for a short time.

Illuminating gas. — The luminous organs of the firefly were found to glow feebly but persistently in illuminating gas.

Acetylene. — In acetylene the luminous organs of the firefly glowed faintly and then went out; they then glowed again faintly and again died out; glowing intermittent. The organs glowed brightly on removal to the air and also on percussion.

Benzene. — In benzene Heinemann [16] found the light of the Mexican cucuyo to be unchanged after half an hour; they were killed, however, in three quarters to one hour. In benzene we found the luminous organs of the firefly to glow brightly and for some time, the glow finally dying out.

Petroleum. — Heinemann [16] observed that in petroleum the light of the Mexican cucuyo continued to be given out for some time. We have found that in the vapor of petroleum ether the luminous organ of the firefly glowed quickly and showed a succession of distinct light flashes.

Phenol. — In the vapor of phenol the detached luminous organ of the firefly showed a slight glow in five minutes; this continued dimly for some time, when a few bright spots appeared which gradually spread over the entire organ, and in forty-five minutes the entire organ was in a bright and steady glow. At the end of one hour and forty minutes the glow had died down.

Ortho-cresol. — Kölliker [23] found creosote to act as a stimulus to the lighting of the luminous organs of the firefly. In the vapor of ortho-cresol we observed that a few faint bright spots had made their appearance in fifteen minutes; in forty minutes the entire organ was in a bright, steady glow, which was maintained for an hour and forty minutes, when the light went out.

Para-cresol. — In the vapor of this compound the detached luminous organs of the firefly showed a faint glow after half an hour; then a succession of bright points appeared, which gradually spread until the entire organ was in a state of bright, steady glow, which was still bright at the end of one hour and forty minutes.

Amyl nitrite. — In the vapor of amyl nitrite a live firefly glowed strongly for five to ten minutes, without the appearance of any separate or distinct flashes or coruscations. When removed from the nitrite vapor, this insect was apparently dead, and the luminous organ failed to emit light on percussion, indicating that the amyl nitrite acts as a specific poison to the luminous material. The detached luminous organs were also found to glow strongly in the vapor of amyl nitrite, and on percussion after removal from the vapor they showed only faint luminosity.

Mononitrobenzene. — With nitrobenzene some very interesting results were obtained. When placed in the vapor of this substance, luminous organs of the firefly glow faintly after a short time; the glow gradually becomes brighter, and the organ then shows a succession of brilliant flashes, after which it settles down into a bright, steady glow, which in some instances lasted as long as seven hours. The luminous flashes which are observed when the luminous organ of the firefly is placed in the vapor of this compound do not cover the entire organ at any one time, but appear to run over it rapidly, reminding one of the spread of combustion through a mass of moist gunpowder.

Essential oils. — The conduct of resting luminous organs of the firefly was also tested towards the vapor of a number of essential oils, with the following results. In oil of wintergreen the organ showed a slight glow, confined to small areas and soon dying out; it gave a strong glow on percussion after removal from the vapor. Similar results were obtained with oil of cloves, oil of cinnamon, oil of bergamot, and oil of rosemary. In the vapor of oil of eucalyptus the organ exhibited a strong glow, slow in developing. The detached organs

failed to emit light in the vapor of oil of peppermint, but exhibited a fairly strong glow on percussion after removal from the vapor. With oil of lavender beautiful phenomena were observed; in the vapor of this oil the organs exhibited a strong glow, slow in developing, and also a series of flashes or distinct light pulsations, appearing irregularly in spots over the luminous organ, and gradually spreading over the entire surface, lighting it up very brightly in patches, similar in general effect to the spread of a conflagration or the setting off of a number of explosives by means of a common fuse.

In addition to these gases and vapors, whose effect on the photogenic process has been described in the foregoing, the effect of a large number of other substances, in the liquid condition or in solution, has been investigated by ourselves and others. These include aqueous solutions of various acids, alkalies, and salts, and also alkaloids and such liquid substances as glycerine, etc. As might be expected, the results obtained by different investigators are in many instances conflicting. These differences are doubtless to be accounted for by reason of the fact that the various substances tested were employed at different concentrations or are due perhaps to some idiosyncrasy on the part of the particular photogenic organism studied. Thus Giesbrecht [14] states that glycerine is a stimulus to light production, whereas, according to Heinemann,[16] it is exceedingly toxic in its action on the luminous organs of the Lampyridæ.

Lack of space prevents any detailed account of these observations. It is perhaps sufficient to say in this connection that, in addition to those substances whose effects on the photogenic process have already been described, the following have been found to stimulate the production of light by various luminescent life forms: strong acids, such as hydrochloric, nitric, sulphuric, chromic, etc., and various salts, such as the halogen and neutral salts of the alkali metals and those of the alkaline earths. Also sodium carbonate, sodium sulphate, and the diphosphate, potassium hydroxide, potassium carbonate, potassium nitrate, potassium cyanide, potassium ferrocyanide, potassium permanganate, calcium chloride, silver nitrate, mercuric chloride, and even chemically indifferent substances such as cane sugar. All of these have been found, by various observers, to act as stimuli to light production. Dilute, aqueous ammonia has also been found to be a powerful stimulus; indeed, according to Watasé,[42] a true lumi-

nous tissue will glow in dilute ammonia when all other stimuli have failed. According to Kölliker, all nerve stimuli cause the emission of light when allowed to act on a luminous organ, and conversely all substances which prevent the transmission of the nerve impulse act as poisons towards the luminous organ. Among the substances which have been found to be either toxic or without action on the photogenic process may be mentioned the following; certain strong mineral acids, acetic acid, salts of certain of the heavy metals, such as those of copper, etc., very dilute solutions of sodium chloride, ammonium carbonate, magnesium sulphate, conine, and strychnine. According to Macaire [25] and also according to Pflüger,[33] all chemical agents which coagulate albumin or bring about its chemical decomposition destroy the light-producing material of luminous plants and animals.

Without entering into the details of all of our own experiments, it may be said that strychnine, adrenalin, hydroxylamine sulphate, hydrazine sulphate, the nitrites of sodium, potassium and barium, sodium hydroxide, sodium acetate, sodium bicarbonate, disodium phosphate, sodium fluoride, and sodium sulphite, all acted as powerful stimuli to light production, whereas pure water, sodium chloride, sodium bromide, sodium nitrate, sodium benzoate, potassium nitrate, potassium iodide, barium chloride, etc., showed but little or no effect. Strychnine, hydroxylamine sulphate, and the nitrites were especially remarkable in their effect upon the light organ of the firefly. When a drop of a solution of strychnine sulphate was injected into a live, active firefly, it was observed that invariably the insect gave one or two strong flashes of light, after which the luminous organ became quiescent and the insect apparently dead. In a short while, however, the entire luminous organ began glowing, and a great many bright scintillations or bright points of light followed one another in rapid succession over its entire area. This phenomenon was repeated a number of times with other specimens of the insect. Hydroxylamine sulphate, adrenalin, and the nitrites of sodium, potassium and barium, also acted as powerful stimuli. Injections of these substances into the live insect, or the placing of the detached luminous organs in their solutions, invariably resulted in the production of a strong, steady glow, the nitrites especially causing the emission of light from the luminous organ when other chemical stimuli had failed.

So far as their effect on the luminous organ or the photogenic ma-

terial is concerned, it seems that substances are roughly divisible into four groups, namely, (1) those substances which distinctly stimulate the light production, called by some authors "exciters" (*Erregern*); (2) those substances which distinctly inhibit luminescence, probably by poisoning the luminous organ or photogenic substance (poisons); (3) inert substances, such as nitrogen, hydrogen, etc., which inhibit luminescence only so long as they are present in excess in the atmosphere surrounding the insect or photogenic substance, but which are without any well-defined toxic action on the photogenic substance (non-toxic inhibitors); and (4) substances which have little or no effect on the light production (indifferent substances).

It is also evident from the foregoing that no definite relationship exists between chemical composition and power to excite the living photogenic material to luminescence, nor can the effect of these various chemical stimuli be distinctly attributed to any particular group or ion, without it is the nitro group. It is of interest to note, however, in this connection that the anæsthetics and related compounds act as powerful stimuli to the photogenic process.

On the Effect of Water on the Production of Light by the Photogenic Material of the Living Organism.

Of the many substances whose effect on the production of light in the living organism has thus far been investigated, water apparently plays the most essential and interesting rôle. The fact that, in common with all living tissues, the luminous organs and light-producing cells of animals and plants normally contain large amounts of water tended naturally to preclude the recognition of the essential part played by this compound in the production of light in the living organism. Thus, according to Kölliker,[23] water has no effect on the production of light by the firefly. It came gradually to be recognized, however, that water plays an essential part in the photogenic process. Thus Carradori,[4] in 1798, mentions the fact that the light of the Italian luciole is suspended by drying, but is again revived by softening in water, though not after too long a period of desiccation. He mentions that Reaumur, Beccaria, and Spallanzani had observed the same thing with regard to the Pholas and Medusæ. Carus[6] made a similar observation in 1864, and Panceri[30] in 1872. According to

Bongardt,[3] Panceri [30] pointed out that Pholas and Phyllirhoë which had been dried out for ten days, lightened again when moistened with water. In 1887 Dubois [9] confirmed Panceri's observation on the Pholas dactylus. Jousset de Bellesme [21] found the Lampyridæ to lighten more frequently in moist than in dry weather, — even the dismembered organ after four days. Giesbrecht [14] filtered certain copepods on gauze and observed that they began to lighten as soon as water was poured over them. He also found the secretion of the luminous glands to lighten on being brought in contact with water. According to Pflüger,[33] the fact that the light-producing material can be dried and then made to glow again by moistening with water speaks in favor of the view that such material is actually living. According to this author, this phenomenon is not shown by the light-producing organs of the firefly, but only with the light-producing material of the Pholas, which is very tenacious of life. On the other hand, Bongardt [3] found that the dry powder obtained by drying and crushing the luminous organs of Lampyris noctiluca retains its power of glowing on moistening with water for over a year. This author concludes that a material is produced in the light organ of this species which lightens when it is brought to the proper degree of moisture. Miss Townsend [40] also observed that when the luminous organs of the firefly are crushed and dried and reduced to powder, the powder lightens under the influence of air and moisture, thereby proving, according to this author, that the production of light is independent of the life of the cell.

All things considered, the remarkable effect of water in exciting the dried material of the luminous organs of photogenic life forms to luminescence is certainly one of the most striking phenomena in the whole field of biochemistry, and is obviously of such interest as to warrant further investigation. It therefore occurred to us to repeat these observations on the dried material of the luminous organs of the firefly, in the hope of extending our knowledge of this particular phase of the subject.

It is known, of course, that if the fresh luminous organs of the firefly be rubbed up in a mortar, the whole mass glows actively for some time as the result of mechanical stimulation. As the mass dries, however, the light gradually dies out, but on the addition of water the glow reappears. We have found it impossible, however, to pre-

pare a luminously active dry powder by the complete drying of the luminous organs of the firefly in the air, a fact which may account for Pflüger's idea, namely, that a material of greater vitality than the luminous organ of the firefly is required for the preparation of dry, luminously active material. Luminous organs of the firefly which had dried out spontaneously in the air at summer temperature were found not to emit light when ground to a powder and moistened with water. Similarly a fresh luminous organ of the firefly was crushed between folds of Japanese bibulous paper and allowed to dry spontaneously in the air at room temperature for two or three days. The dry material thus obtained did not lighten on moistening with water. On the other hand, by drying whole insects or fresh luminous organs *in vacuo* over sulphuric acid, it has proved an easy matter to obtain dry material which glows vigorously on moistening with water, and which retains its photogenic activity for long periods of time. With the view of throwing still further light on the preparation of the dry, luminously active material, an equal number of live, active fireflies and active luminous organs of fireflies were dried in a vacuum over sulphuric acid. Attention has already been called to the peculiar and interesting conduct of the live fireflies *in vacuo*, and to the fact that under the influence of the vacuum all the luminous organs burst into a steady glow which lasts for some time. After eighteen hours in the vacuum the insects were found to be dead and as brittle as glass. The two sets of dry luminous organs thus obtained were ground to fine powders. The material obtained from the luminous organs which had been detached from the insects previous to drying *in vacuo* was labelled (1), whereas that obtained from the luminous organs of the fireflies which had been dried whole was labelled (2). On moistening small amounts of powders Nos. (1) and (2) with water, both glowed instantly, but the light emitted by (2) was feeble as compared with that emitted by (1). Both continued to glow for one hour and thirty minutes; at the end of this time both were again moistened with water. With (1) the glow was renewed and lasted for an hour longer, whereas (2) failed to glow a second time. Hence a more active material may be obtained by drying the detached luminous organs than by drying the entire insect. Taking everything into consideration, this is probably what one would be inclined to expect. The fact that the luminous organs of the firefly, whether still a part of the insect or detached,

glow so long and persistently in a vacuum, points, of course, to such an expenditure of the light-producing material as to greatly diminish the luminous power of the dry material obtained by this method, and naturally suggested the advisability of drying the luminous organs in hydrogen, or *in vacuo* and hydrogen, with the view of preventing this waste of the photogenic substance. In fact, one of our experiments, in which a number of luminous organs were being dried in hydrogen and *vacuo* with considerable diminution of the amount of light emitted, gave every promise of enabling us to overcome this waste of photogenic material, when, unfortunately, concentrated sulphuric acid was sucked over into the tube containing the luminous organs, and, occurring as it did towards the end of the firefly season, put an end to attempts in this direction.*

In the mean time a quantity of the dried powder of the luminous organ of the firefly had been obtained by drying the luminous organs *in vacuo* in the usual way. With the view of learning what we could with regard to the stability of the dry material, small amounts of it were put up in sealed glass tubes in air and *in vacuo*. These tubes were prepared on August 10, 1909. On January 4, 1910, two of the tubes were opened and the material moistened with water. Both specimens, namely, the one which had been preserved in air and that which had been preserved *in vacuo*, glowed actively for fifteen minutes. Two of the tubes were again tested at the time this communication went to press (September 9, 1910), and both specimens were again found to be active. It is evident, therefore, that, when dry, the photogenic material of the firefly retains its power to emit light through the action of water, for a period of thirteen months. It is proposed to keep the remainder of the tubes for longer periods and to test them at intervals of six months or a year, until the supply is exhausted, in the hope of obtaining further information on this phase of the subject. Already, however, certain facts of interest have been brought to light concerning the conduct of the dry material. In our first work with the powder obtained by drying the luminous organs of the firefly *in vacuo*, during the summer of 1909, it was observed that while the dry material always glowed actively on the first addition of water,

* Since the above was written, it has been found possible to prepare very active photogenic material, by drying over sulphuric acid in hydrogen, under diminished pressure.

it failed to glow again, on moistening, after drying a second time. In other words, it appeared from our first experiments that the dry material resulting from the first drying *in vacuo* contained only a certain limited amount of photogenic material which, on moistening with water, spent itself in the emission of light; or, in still other words, that the reaction between the dry photogenic substance and the water was complete so far as the emission of light energy is concerned. We have since obtained results, however, which indicate that such is not the case, but that the dry material which has once emitted light through the action of water may, after drying again, emit light a second time on moistening, and may even emit it a third time by the action of water. Thus the specimens which had glowed on moistening with water on January 4, 1910, were allowed to dry in the air at room temperature. On January 8, 1910, they were found to be quite dry, and on moistening again with water, both of them glowed freely and for about ten minutes, although not so intensely as they did the first time that they were moistened. These specimens were then allowed to dry in the air again, and on January 12, 1910, they were moistened again (the third time), when one of them, the specimen which had originally been preserved *in vacuo*, glowed quite strongly again, the glow lasting for fully five minutes. The two specimens were then allowed to dry out again, and were again moistened on January 18, 1910; neither of them emitted light. On January 10, 1910, two of the specimens which had been preserved in sealed tubes in air were moistened (1) with distilled water, and (2) with N/20 solution of sulphur dioxide respectively. The specimen which had been moistened with distilled water glowed brightly for thirty-five minutes. The specimen which had been moistened with N/20 sulphur dioxide was slower in becoming luminous, glowed only feebly, and ceased glowing in three minutes. The two tubes were then allowed to dry in the air, and on January 14, 1910, both were moistened with distilled water; (1) glowed feebly for about two minutes, and then went out, whereas (2) did not show any glow at all. The two specimens were then allowed to dry in the air again, and on January 18, 1910, they were again moistened (the third time); neither specimen showed any luminosity.

It is evident from these experiments that the latent energy of the photogenic material obtained by drying the luminous organs of the

firefly *in vacuo* is not always completely liberated by a single application of water, or, if such is the case, then a certain amount of the photogenic material is regenerated on drying at room temperature under the conditions already described. The fact, however, that the material ultimately loses its power to emit light on being brought into contact with water indicates that it is actually consumed during the process, and that it cannot be produced continuously from the other substances present under the conditions at hand. The fact that the material lightens so feebly and for such a short time when moistened with a dilute solution of sulphur dioxide, and that, when so treated, it fails entirely to glow on moistening with water after drying in the air, again illustrates the poisonous nature of this substance to the photogenic process, and agrees with all of our other observations on the effect of this substance on the light-producing power of the firefly.

In order to test the conduct of the dry photogenic material towards water, in the presence of various gases, the following experiments were carried out. Small amounts of the dry photogenic powder, obtained by drying the luminous organs of the firefly *in vacuo* over sulphuric acid, were placed in small receptacles made of a single thickness of Japanese bibulous paper. These were then placed in a tiny basket of platinum wire suspended from the end of a small separatory funnel, and so arranged that the contents of the basket could be moistened with water from the separatory funnel. The stem of the separatory funnel, carrying the basket, was then placed in a large glass tube by means of a doubly perforated stopper, through the second opening of which was inserted an exit tube for the escape of gas from the large tube. The latter was about three inches in length and was drawn out at the lower end into a smaller tube about three inches long and one quarter of an inch in diameter. The small tube served as an inlet tube for the introduction of any gas with which we desired to fill the central chamber of the apparatus in which the dry photogenic material was suspended. By means of this simple device we were able to study the effect of freshly boiled and cooled distilled water on the dry photogenic material in atmospheres of the following gases:

Hydrogen. — The dry powder showed no luminosity in dry hydrogen. When moistened with a drop or two of water, it glowed for five

minutes, and then the light died out. The admission of the vapors of carbon disulphide and carbon tetrachloride, both of which were found to greatly stimulate the fresh luminous organ of the firefly, caused no return of the light when admitted along with the hydrogen.

On shutting off the current of hydrogen, however, for about five minutes, the moist material began glowing again; on again admitting hydrogen, the light died out, and on again shutting off the hydrogen, the light reappeared. This phenomenon was repeated several times, and was doubtless due to the slow diffusion of air into the tube containing the moistened photogenic material.

Carbon dioxide. — The dry powder exhibited no luminosity in carbon dioxide. On moistening it with a drop or two of water the material glowed for four minutes, at the end of which time the light died out. Air was then admitted, when the material glowed again in two minutes.

Sulphur dioxide. — The dry powder showed no luminosity in sulphur dioxide. Even after moistening it with a drop or two of water it still did not become luminous, and the subsequent admission of air and air with carbon disulphide failed to produce a glow. The photogenic material was evidently killed by sulphur dioxide.

Hydrogen sulphide. — The dry powder showed no luminosity in hydrogen sulphide. After moistening with a drop or two of water it glowed for two minutes in this gas and then went out. Air was admitted, and after some delay the moistened material again glowed brightly.

Oxygen. — The dry powder showed no luminosity in pure oxygen. On moistening with a drop or two of water the material instantly lighted up with a bright glow which continued practically undiminished for half an hour.

Air. — As already pointed out, the dry powder does not glow in the air. On moistening it with a drop or two of water, it glowed rather dimly for an hour. This experiment served as a control on the other five, and furnished us with a basis for comparison.

Sulphuric acid. — Neither concentrated nor dilute sulphuric acid caused the evolution of light from the dried photogenic organs of the firefly. In the concentrated acid the pale yellow color of the ventral surface of the dried luminous segments of the abdomen of the firefly became first light and then dark green and finally dark blue, and a transient blue coloration was produced in the acid.

Nitric acid. — Neither concentrated nor dilute (10 per cent) nitric acid caused the evolution of light from the dry photogenic tissue. The concentrated acid stained the tissue a deep yellow.

It would seem therefore, from these experiments, that in the production of light by the firefly two substances are actively concerned, in addition to the photogenic material itself, namely, water and oxygen. In other words, the light results from the action of oxygen and water on the photogenic material. As to the precise mechanism of these changes we at present know nothing. The fact that some light is emitted when the dry photogenic material is moistened in an atmosphere of certain gases other than oxygen, such as hydrogen, carbon dioxide, etc., indicates, in all probability, that the gases contained small amounts of air, or that the water employed contained small amounts of dissolved oxygen, or that the dry photogenic material itself contained sufficient oxygen, in loose combination, to evolve a limited amount of light when the dry material is moistened in such atmospheres.

As one would naturally expect, the effect of drying the photogenic material of the firefly is to greatly increase its stability towards heat. That such is the case is indicated by the following observation: A small amount of the dry material, enclosed in an envelope of bibulous paper, was placed in a test tube. The test tube was then closed with a cork and heated for ten minutes in boiling water. When removed from the tube at the end of this time and moistened with water, it glowed quite as brightly as some of the unheated material, indicating no loss of activity by exposure to the temperature of boiling water for ten minutes.

Summary.

Our present knowledge of the photogenic process may be briefly summarized as follows:

So far as the purely chemical aspect of the process is concerned, three things seem to be concerned in the production of light by living things. These are the photogenic substance, water, and oxygen. As yet nothing is known regarding the precise chemical nature of the photogenic substance. Like other biologically active substances, it is characterized by extreme irritability. Under the influence of certain chemical stimuli, especially of such substances as ether, chloro-

form, carbon bisulphide, carbon tetrachloride, and mononitrobenzene and the nitrites of certain metals, the production of light by the luminous organ of the firefly is a continuous process, extending over considerable periods of time, whereas normally the emission of light by the firefly is an intermittent process of short duration, partaking of the nature of a luminous explosion.

It is an interesting fact that the majority of poisons act as transient stimuli, and that but few of these are so suddenly destructive in their action on the photogenic material as to prevent the production of light altogether. Of all substances thus far investigated sulphur dioxide seems to be the most toxic in its effect on the photogenic material. Greatly diminished atmospheric pressure invariably results in the emission of light both by the living firefly and by the detached, resting, luminous organ. Lastly, the photogenic material which has been dried *in vacuo* over sulphuric acid retains its power to emit light by moistening with water for a period of thirteen months and perhaps even for longer intervals.

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[42] WATASÉ: Biological lectures, Wood's Hole, 1898, pp. 117–192.

[43] AGASSIZ: Memoirs of the American Academy of Arts and Sciences, 1874, x, pp. 357, 398.

ACAPNIA AND SHOCK. — VII. FAILURE OF THE CIRCULATION.

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I. What Factor in the Circulation really fails in Shock?

FAILURE of the circulation is the commonest mode of death. When the process is judged by the arterial pulse, there appears to be a progressive weakening of the heart beat. Such a decline characterizes the approach of the end after many abnormal conditions. It often follows intense and prolonged pain. It may occur at the height of an acute infectious disease. At first the rate of the heart beat is rapid. Its amplitude diminishes, while arterial pressure is nevertheless maintained at a normal level, or even above normal. After the pulse has become "thready," arterial pressure sinks rapidly. Thereafter the heart rate may be quick or slow; but unless it is extremely slow the amplitude of the beats is small, and becomes progressively smaller. Formerly this process was regarded as consisting essentially in failure or fatigue of the contractile force of the heart. Even to-day it is generally so denominated among clinicians, — for lack of better terms and more critical conceptions of the factors involved.

The later stages of this process are spoken of by surgeons as "shock." In their usage this term might be defined as a condition of the circulation essentially similar to that induced by extreme hæmorrhage, — although there may have been no apparent loss of blood. When we consider that "shock" means literally a jolt or jar or impact, and

that it was originally used to include concussion of the brain, it is evident that in its modern sense the word is illogical and ought ultimately to be superseded by a clearer term. It is, however, convenient for the purposes of this paper to speak of the three most common conditions terminating in a so-called "failing heart" as hæmorrhagic shock, traumatic shock, and toxæmic shock. The terms imply that all three conditions are fundamentally alike in their bloodlessness.

It is orthodox at the present time to say that traumatic and toxæmic shock are not due to a loss of power in the heart itself, but that these conditions consist in a fatigue, or inhibition, or failure of some sort in the vaso-motor centres. This doctrine was crystallized in its modern form by two independent, yet nearly simultaneous, investigations, — each a classic in its own field, — that of Crile [1] on traumatic shock, and that of Romberg and Pässler [2] on toxæmic shock. These two pieces of work were nearly identical in methods of observation, in the phenomena recorded, and in the conclusions reached, although in one failure of the circulation was induced by trauma, and in the other by bacterial toxins. Thus a single statement, as above, covers the "vaso-motor failure" theory of both forms of shock. Is this theory logical and true? To me it appears half true and half false. Both investigations afford ample evidence that the heart does not fail; but the same evidence proves no less conclusively, if the view to be supported in this paper is correct, that the vaso-motor centres are equally tenacious of their functional capacity.

The problem of the circulatory disturbances in shock has usually been dealt with as if a demonstration that "the heart does not fail" were equivalent to a proof that "the vaso-motor nervous system does fail." [3] This alternative involves the assumption that there are two, and only two, principal mechanical factors in the circulation, — the contractions of the heart and the peripheral resistance of the arterioles controlled by the vaso-motor nervous system. This assumption is, I believe, an error of the greatest importance both theoretical and

[1] Crile: Surgical shock, 1899; also Blood pressure in surgery.

[2] Romberg and Pässler: Deutsches Archiv für klinische Medicin, 1899, lxiv, p. 652.

[3] The literature of toxæmic shock was recently reviewed and discussed in a paper read before the Section of Medicine of the American Medical Association, June 8, 1910, by W. G. Macallum. It will soon be published in the Journal of the American Medical Association.

practical, but an error for which neither Crile nor Romberg and Pässler were responsible. They applied to their problem that conception of the circulation which the physiology of to-day teaches. This was their sole mistake. Both Crile and Romberg and Pässler concluded (correctly, I believe) that in shock the circulation fails in the same manner as after hæmorrhage, and that the heart fails because too little blood is supplied to it through the veins. Both found that intra-venous infusion restored for a time a normal arterial pressure and heart action. And unfortunately both labelled this true picture with the misleading formula, — the only formula for it offered by current physiology, — "vaso-motor failure."

Present knowledge regarding the vaso-motor nervous system indicates that its control is exercised — mainly at least — upon the finer branches of the *arterial* system. It regulates the frictional resistance to the outflow of blood from the arteries into the capillaries. Upon the latter vessels it has, so far as known, no direct influence. A relaxation of the arterioles of any region induces indirectly a mechanical distention, not direct active dilatation, of the capillaries supplied. Information regarding a central nervous control over the tonus of veins is extremely meagre. In a later paper I shall attempt to show that no direct central nervous control of the venous reservoirs exists.[4] Now the failure of vascular tonus in traumatic and toxæmic shock is almost wholly in the *venous* system. Both Crile and Romberg and Pässler saw and emphasized this fact. It seems not to have occurred to them that they were dealing with the failure of a mechanism as yet unrecognized by physiology. In this they were not alone. For half a century physiologists have been so dazzled by Claude Bernard's discovery of the vaso-motor nervous system that they have neglected to emphasize the fact that the circulation must involve a third factor in addition to the heart and the peripheral resistance of the arterial system. Otherwise it would be as unstable as a stool balanced on only two legs. It must include a mechanism, or mechanisms, regulating the volume of the blood, and determining the venous supply to the right heart. It is this veno-pressor mechanism, I believe, and neither the heart nor the vaso-motor nervous system, which is the essential element in the failure of the circulation in shock.

[4] For a summary of the experiments on which this negation is based, see section II. on p. 161.

Crile's view of traumatic shock has been almost universally accepted by surgeons. The results of the work of Romberg and Pässler have not, at least in America, been adopted to an equal extent among internists. This has been due, however, to neglect, and not to opposition. Janeway[5] says that their "conclusions have, in my opinion, never been controverted. . . . They clearly demand that we shall in most cases abandon the idea of cardiac death at the height of acute infectious diseases, such as pneumonia, typhoid fever, diphtheria, and the septic fevers; though sudden death during convalescence may be due at least in part to the later development of lesions in the heart muscle. In place of heart failure, we must write vaso-motor failure, or collapse . . . the heart stopping only because so little blood is returned to it." Similarly Crile[6] says that "the vaso-motor centre in a state of shock may be designated as paralyzed, or exhausted. The bulk of the blood does not circulate freely through the arterial system. A large portion accumulates in the venous trunks, a state equivalent to an intra-venous hæmorrhage. The arterial system bleeds into the dilated venous system. The symptoms closely mimic those of hæmorrhage. Most clinicians concede that differentiation is impossible."

A peculiar mingling of truth and error is contained in these quotations. They represent shock as identical with hæmorrhage, and in the same sentence as identical also with failure of the vaso-motor nervous system. The expression "a hæmorrhage into the veins" has become widely current among surgeons, yet it is a complete contradiction in terms. It is illogical to speak of any condition as being "a hæmorrhage" if it involves an increased flow of blood *into* the veins, for hæmorrhage consists essentially in loss of blood *from* the veins. A diminished venous stream to the right heart, an incomplete diastolic filling of the ventricles, and a consequent reduction in the volume of the systolic discharges, — these are the immediate results of a lessening of the volume of blood in the body.[7] As hæmorrhage

[5] Janeway, T. C.: New York medical journal, Feb. 2, 1907.

[6] Crile: Shock and collapse, Keen's Surgery, 1906, i, p. 929. (The quotation is abbreviated.)

[7] A paper presenting in a lucid manner the mechanics of hæmorrhage was read before the Section of Pathology and Physiology of the American Medical Association, June 7, 1910, by Carl J. Wiggers. It will soon be published in the

progresses, the amplitude of the pulse is correspondingly diminished; but until the output of the heart becomes extremely small, arterial pressure is maintained by a compensatory constriction of the arterioles by the vaso-motor nervous system. It makes no difference whether the bleeding vessel be vein or artery, the fundamental condition from which the fall of arterial pressure finally results is the depletion of the venous reservoirs and an insufficient supply to the right heart. On the contrary, the expression "a hæmorrhage into the veins" implies an altogether different and — unless the aorta itself be severed — an erroneous conception of the mechanics of hæmorrhagic shock. It suggests that the easier egress of the blood through the cut artery is the direct cause of the fall of pressure in the arterial system. It suggests also that the condition of the circulation after hæmorrhage is similar to a general relaxation and dilatation of all the arterioles of the body, such as results from severing the spinal cord just below the bulb. In point of fact, however, when this operation is performed upon a cat, although arterial pressure may sink to 60 mm. Hg or even much lower, because of the diminution of the peripheral resistance in the arterial system, the amplitude of the pulse is not reduced. It may even be increased. The tonus of the venous reservoirs is not affected. Hence the blood continues to flow to the right heart in undiminished volume; and the heart, being thus filled to its normal capacity during diastole, discharges its full stroke during systole. Clear evidence that fall of arterial pressure after hæmorrhage is due to diminished venous supply to the right heart and not to abolition of peripheral resistance in the arterial system is afforded by the fact that no considerable fall of arterial pressure occurs if the blood be re-injected into a vein as fast as it runs out of a severed artery. There are more points of difference than of similarity between the experimental condition of "spinal shock" (*i. e.*, true vaso-motor failure) on the one hand and traumatic, toxæmic, and hæmorrhagic shock on the other.

It is so easy to record arterial pressure and so difficult to measure the minute-volume of the arterial blood stream that one is inclined to forget that the pressure in the arteries is really a phenomenon of

Archives of internal medicine. See also VON DEN VELDEN, R.: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, p. 37 (bibliography on hæmorrhage.)

only secondary importance. Because of the technical perfection of the method introduced by Ludwig we are prone to lose touch with the great doctrine of Harvey. The primary function of the circulation is the volume of blood pumped onward by the heart in unit time. It is not the pressure of the blood within its vessels which keeps the tissues alive, but the quantity of oxygen and other nutriment which the stream supplies. Above a moderate degree arterial pressure is an expense rather than an asset. Within wide limits a high arterial pressure is compatible with a diminished stream, and a low arterial pressure with a normal flow. Thus it is misleading to say that "an abnormally low [arterial] blood pressure is the essential phenomenon" of traumatic shock.[8] The essential arterial condition in shock is the greatly diminished stream which both Crile and Romberg and Pässler described. This reduced blood flow results in, instead of resulting from, lowered arterial pressure. Arterial pressure falls, not as in "spinal shock" because the peripheral resistance is diminished, but because of the fact that when the volume of the stream has dwindled beyond a certain limit the utmost activity of the vaso-motor centres fails to afford a peripheral resistance sufficient to maintain the pressure.

Both Crile and Romberg and Pässler laid great stress on the fact that stimulation of a sensory nerve fails to induce in a shocked subject that reflex rise of arterial pressure which always occurs in a normal animal. They interpreted this as clear evidence of lessened functional capacity in the vaso-motor nerve centres. I believe, on the contrary, that this observation (which I have repeatedly verified) really indicates that in shock the vaso-motor centres are in a condition of maximal activity. They are already constricting the arterioles as much as possible in the effort to compensate for the lessened output of the heart and to maintain arterial pressure. This is the normal function of these centres.[9] When a sensory nerve is stimulated in shock, no increase in the constriction of arterioles results, because they are already constricted to the utmost.

Both Crile and Romberg and Pässler found that even in profound shock arterial pressure returns to a normal level when the venous supply to the right heart is restored to normal volume by an intra-

[8] Crile: Keen's Surgery, 1906, i, p. 926.

[9] *Cf.* Porter and Quinby: This journal, 1908, xx, p. 505.

venous infusion of saline. They interpreted this (correctly, I believe) as proof that the heart is still functionally capable. But does not this experiment prove also, and with equal conclusiveness, that the peripheral resistance of the arterial system and the nervous mechanism controlling this resistance are likewise still functionally capable? If the vaso-motor nervous system were inactive and the arterioles relaxed, the blood would run out through the capillaries too easily for any pressure to be developed. Think of the tissues of the body as similar to a house afire. Think of the heart as a steam fire-engine; of its arteries as lines of hose; of the arterioles controlled by the vaso-motor nervous system as the nozzles of the hose. The engine can throw no stream upon the fire, nor maintain a pressure in the hose, even though the nozzles be constricted to the utmost, unless the supply of water from the cistern or the street water main be adequate. Similarly, arterial pressure cannot be maintained merely by the heart and the peripheral resistance of the arterial system. As well might one think of lifting a weight by means of a lever with no fulcrum! The heart can discharge during systole only so much blood as distends its chambers during diastole. The diastolic filling of the right heart depends upon the volume of the stream flowing to it through the veins and upon the distending pressure which this stream affords. *Venous pressure is, so to speak, the fulcrum of the circulation.*

The object of this paper and of others to follow is to show that shock, as surgeons use the word, is due to a failure of this fulcrum. Because of the diminished venous supply the heart is not adequately distended and filled during diastole. Hence the picture of a "failing heart" revealed by the pulse. For the same reason arterial pressure ultimately sinks in spite of an intense activity (not because of failure) in the vaso-motor nervous system, and in spite of an extreme constriction (not because of relaxation) of the arterioles. Finally the blood stream is so much diminished that it is inadequate to supply oxygen to the tissues, and death quickly ensues.[10]

The distinction between the functions of the vaso-motor and of the veno-pressor mechanisms is illustrated in Fig. 1. In this diagram a force pump (*A*) is placed at the bottom of a pit or well. These conditions correspond to the heart working under the negative pressure of the thorax. The top

[10] The fatal process is exemplified in the experiments described in section III, on p. 167 et seq.

of the pit is zero pressure. If the depth of the pit is varied rhythmically, as shown by the dotted line below the pit, the principal influence of the respiratory movements upon the circulation is thereby imitated. The pump is incapable of exerting any suction whatever. Its plunger is not drawn up actively, but is pushed up by the venous inflow during the diastolic filling of its chamber. In systole it is driven down actively by the force indicated by the arrow. An air chamber (*B*) affords the arterial elasticity. The small nozzles (*C*) at the ends of the hose pipes, or arteries, play a part analogous to the vaso-motor mechanism. The height to which the jets from these nozzles rise is an expression of the arterial pressure. A narrowing of the nozzles, or vaso-constriction, will raise arterial pressure; a dilatation of the nozzles will lower it. Dilatation of one and constriction of others will vary the distribution of the stream. But so long as the pump acts efficiently and with a full venous supply, the total volume of the aortic stream will be the same, no matter whether the nozzles be constricted to induce arterial hypertension or dilated to the utmost by vaso-motor failure. In this scheme vaso-motor failure cannot induce stagnation.

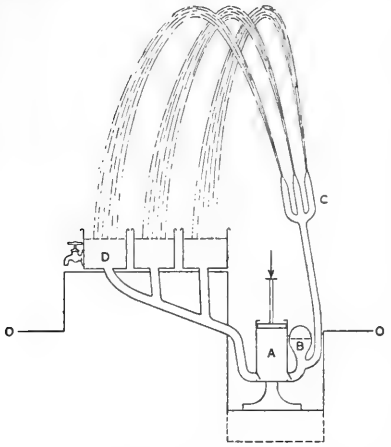

FIGURE 1. — A diagram illustrating the functions of the three essential factors in the circulation. It shows the heart (*A*) as a force pump placed at the bottom of a pit to correspond with the negative pressure of the thorax. The top of the pit (*OO*) is taken as the level of zero pressure. The pump discharges into an air chamber (*B*) affording an elasticity like that of the arteries, and against the peripheral resistance of several small nozzles (*C*), which play the part of arterioles. The vaso-motor nervous system (not shown in the diagram) controls the calibre or lumen of these nozzles, and thus regulates the arterial pressure and the relative volume of the various jets. The jets fall into tanks or reservoirs (*D*), corresponding to the various capillary areas of the body (*e. g.*, the intestine, skeletal muscles, head, etc.), from which a system of drains or veins supplies the pump. The pump exerts no suction, but is filled during diastole by the venous pressure pushing the plunger upward. In systole the force represented by the arrow drives the plunger down. For discussion see small type in text.

The jets fall into tanks (*D*),[11] corresponding to the capillaries of the various organs of the body. From these reservoirs large drains unite to form the venous system supplying the pump. A hæmorrhage may be produced in one or other of two

[11] This is the "pre-ventricular reservoir" of v. RECKLINGHAUSEN: Archiv für experimentelle Pathologie und Pharmakologie, 1906, lv, p. 476, and 1907, lvi, p. 1.

ways, — either by diverting one of the jets from its tank, thus imitating a hæmorrhage from an artery, or by opening the cock on the tank at the left, thus causing a hæmorrhage from a vein. The effects of these two procedures will be exactly the same. The volume of liquid stored in the reservoirs will be diminished. Neither the arterial pressure nor the arterial stream will be influenced until the supply in the venous reservoirs is considerably depleted. When the supply no longer affords a sufficient venous pressure to distend the heart (*i. e.*, to push up the plunger with normal rapidity and to a normal extent during diastole), then the output of the pump will be diminished. If the nozzles are now actively constricted and the peripheral resistance is thus sufficiently increased, arterial pressure may, however, be maintained at a normal height for a little while longer; but the arterial pulse will be " thready." Finally, when the limit of such vasomotor compensation is reached, and the nozzles are as small as they can be made, a slight additional diminution in the output of the pump will involve a sudden and extensive fall of arterial pressure.

After the arterial pressure has been thus lowered by extreme hæmorrhage, it may be restored by pouring liquid into the venous reservoirs. If it is restored, this fact affords proof both that the pump has not broken down and that the nozzles are not relaxed. Neither cardiac failure nor vaso-motor failure has occurred. It is evident that to speak of a " hæmorrhage into the veins because of vaso-motor failure" would be, at least in this scheme, to confuse two distinct conditions. The only conditions which will accurately simulate the phenomena of hæmorrhagic, traumatic, and toxæmic shock are such as will induce a failure in the supply and pressure from the venous reservoirs to the heart.

II. The Veno-Pressor Mechanism.

The nature of the veno-pressor mechanism is a peculiarly difficult experimental problem. From such data as I have been able to collect during the past five years it appears to consist of two sets of factors. One is tonic; the other osmotic. Both depend upon the maintenance of a normal tension of CO_2 in the fluids of the body and of a normal alkali-acid equilibrium in the tissues.[12] The respiratory centre, by regulating the CO_2 content of the arterial blood within narrow limits of variation,[13] exerts an indirect but powerful control

[12] Henderson, L. J.: Ergebnisse der Physiologie, 1909, viii, p. 254.

[13] Haldane and Priestley: This journal, 1905, xxxii, p. 252.

over the veno-pressor mechanism. Any considerable accumulation of CO_2 above normal augments the venous pressure. Excessive pulmonary ventilation tends to lower it. Acute acapnia diminishes the volume of the blood as effectively as does an extensive hæmorrhage. This condition of bloodlessness, or oligæmia, or exsanguinity results from the passage of fluid from the blood vessels out into the tissues.

The details of the experiments carried out in this laboratory upon the veno-pressor mechanism will be presented in a later paper. Neither the tonic nor the osmotic element in the mechanism is subject to any direct control by nerve centres, either spinal or bulbar. Adrenalin exerts no direct influence upon it. These statements are based upon five sets of experiments which may be summarized as follows:[14] (1) After section of the vagi, vigorous stimulation of the splanchnic nerves causes a considerable rise of arterial pressure, but produces a barely perceptible effect upon the pressure in the systemic veins. (2) Injection of adrenalin, after vagus section, has likewise an insignificant influence upon venous pressure, unless the dosage is so large as to raise arterial pressure to the point at which the heart's action is interfered with. (3) Section of the spinal cord just below the bulb causes a marked fall of arterial pressure, but no immediate drop, on the contrary sometimes a rise, in venous pressure. (4) Stimulation of the severed cord, under curare, restores arterial pressure to a normal level and maintains it at this level so long as the stimulation is continued. Simultaneously there is either no rise of venous pressure or a slight rise and immediate relapse even during continued stimulation. (5) When a cat is decapitated by the method of Sherrington[15] and is supplied with a jet of oxygen into the trachea by the method of Volhard,[16] CO_2 accumulates in the body. If the stream of oxygen is slow, a gradual rise of venous pressure develops. Arterial pressure continues unaltered; and until a high degree of hypercapnia has developed the heart rate remains unaffected. If the gas jet into the trachea consists of oxygen containing a large percentage of CO_2 the

[14] Partly quoted from a preliminary report presented by the writer before the American Physiological Society in December, 1908, This journal, 1909, xxiii, p. xxx. *Cf.* Kaya and Starling: Journal of physiology, 1909, xxxix, p. 347.

[15] Sherrington, C. S.: Journal of physiology, 1909, xxxviii, p. 375.

[16] Volhard: Münchener medizinische Wochenschrift, 1908, No. 5.

rise of venous pressure develops in a few minutes. The venous pressure falls again in the course of a few minutes if artificial respiration is supplied. It falls also, but less rapidly, if the lungs are ventilated with a rapid stream of oxygen alone or with air (as in the Meltzer[17] insufflation method). This phenomenon of hypercapnial venous hypertension affords a convincing demonstration of the existence of the veno-pressor mechanism. It appears to be a crucial experiment for this mechanism very much as section and stimulation of the cervical sympathetic nerve in the rabbit were crucial for the recognition of the vaso-motor nervous system.

The tonic element in the veno-pressor mechanism consists apparently in the tonus of the tissues rather than in that of the walls of the veins, — at least my experiments on excised veins have yielded inconclusive or negative results. In the third paper of this series[18] the powerful influence of CO_2 upon the tonicity of the intestine was described. The experiments of Lee[19] have shown a similar relation of CO_2 to the tonus (or Treppe) of skeletal muscle. The increase of tissue tonus induced by hypercapnia squeezes the blood out of the capillaries. In the intestine it can be seen to render the tissue paler. Local acapnia induced by exposure of the intestine to a stream of warm moist air rapidly results in an intense congestion and stasis. It is noteworthy that Romberg and Pässler found that animals in toxæmic shock exhibited a marked rise of arterial pressure when partially asphyxiated, — a rise in some cases equivalent to that following a liberal infusion of saline. This effect may fairly be attributed to the veno-pressor mechanism and not to the vaso-motor, since arterial pressure was scarcely at all affected by vigorous stimulation of an afferent nerve. It is a fact which doubtless many investigators have observed that when an animal has been under artificial respiration for a considerable time and the circulation has begun to fail, a marked and lasting improvement results from a period of asphyxia. The well-known effects of asphyxia upon the normal circulation are in part due to the influence of hypercapnia upon the veno-pressor mechanism.

[17] Meltzer and Auer: Zentralblatt für Physiologie, 1909, xxiii, pp. 210 and 442; also Biedl and Rothberger, *Ibid.*, p. 327.

[18] Henderson, Y.: This journal, 1909, xxiv, p. 66.

[19] Lee, F. L.: This journal, 1907, xviii, p. 267.

The osmotic element in the veno-pressor mechanism appears to be even more important than atonicity in the failure of the circulation in shock. The blood does not merely stagnate in the veins and capillaries. Ample and conclusive evidence demonstrates that both in traumatic and toxæmic shock the volume of the blood in the vessels is greatly diminished. The balance of osmotic forces, the colloidal imbibition of the proteins, and the other physico-chemical conditions determining the passage of fluid back and forth through the walls of the capillaries are upset. As a result, the distribution of water between the blood, the lymph, and the cytoplasm of the tissues is altered. In traumatic shock the excessive passage of liquid from the blood into the tissues is, I believe, initially induced by acapnia. In toxæmic shock it appears to depend either upon acapnia or upon some similarly acting disturbance of chemical conditions in the tissues (*e. g.*, the protein-alkali-acid-CO_2 equilibrium of L. J. Henderson, *loc. cit.*) involved in the febrile process.

Forty years ago H. Fischer [20] noted that the condition of the circulation in profound traumatic shock is essentially like that in the last stage of cholera. In cholera the volume of the blood is enormously diminished; and intra-venous infusions of normal saline are of very marked temporary benefit. The fluid is, however, soon lost from the blood vessels into the intestines or into the tissues. Recently Rogers [21] has demonstrated a loss of 64 per cent of the serum of the blood in the toxæmic shock of cholera. The degree of prostration of the patient he finds to be proportional to the diminution of the blood volume. In the blood remaining in the vessels the chlorides are so much reduced that intravascular hæmolysis sometimes occurs. Intravenous infusions of hypertonic saline have lowered the mortality in the shock stage of cholera by 50 per cent.

Many years ago Leyden [22] observed a relative retention of water during fever, — *i. e.*, an increase in the percentage of water as compared with the solids in the tissues. Since then a literature too large for more than a brief citation here has gathered about the topic.

[20] Fischer, H.: Ueber dem Shok, in Volkmann's Sammlung klinischer Vorträge, 1870, No. 10.

[21] Rogers, L.: Philippine journal of science, 1909, iv, p. 99; also Proceedings of the Royal Society, 1909, lxxxi, B, No. 548.

[22] Leyden : Archiv für klinische Medicin, v, p. 366.

Krehl[23] says: "Why the patient with fever fails to excrete the extra liquid in his tissues is not known, though we suspect that it is because the extra water is retained chiefly within the cells themselves and does not reach the blood or lymph. Possibly the physico-chemical properties of the cells or their secretory activities are changed." Parallel with such tissue changes a concentration of the blood has frequently been noted both in patients and in experiments on animals. Among others Sandelowsky[24] has recently noted it in pneumonia; and Oppenheimer and Reiss have found it to occur in scarlet fever. As a rule, studies upon this topic do not apply directly to the problem of fever collapse, since at the time the observations were taken the subjects were merely ill, not dying in shock. They are sufficient, however, to indicate that some degree of oligæmia is frequently, if not always, an accompaniment of toxæmia.[25]

Sherrington and Copeman[26] have shown that in traumatic shock the specific gravity of the blood is markedly increased even before the arterial pressure has fallen to a low level. Mummery,[27] who is an adherent of the "vaso-motor failure" theory, has found that in spinal shock the specific gravity is distinctly diminished.[28] The only significance which the latter observation bears for the problem of traumatic shock is the additional evidence which it affords that traumatic and spinal shock are two totally distinct conditions.

Nearly all modern writers on traumatic shock have recognized more or less explicitly the occurrence and the importance of oligæmia. It was, however, a long step forward when J. D. Malcolm[29] first boldly supported this idea to the exclusion of vaso-motor failure. He has pointed out the illogical character of the arguments for the "vaso-motor failure" theory. He bases his argument upon keen and exten-

[23] Krehl, L.: Pathologische Physiologie, fourth edition, translation by Hewlett under the title Clinical pathology, second edition, 1907, p. 422 (bibliography).

[24] Sandelowsky: Deutsches Archiv für klinische Medizin, 1909, xcvi, p. 445; Oppenheimer and Reiss, same volume.

[25] Acapnia is also an accompaniment. See Krehl, L.: Pathologische Physiologie, third edition, 1904, pp. 459 and 475.

[26] Sherrington and Copeman: Journal of physiology, 1893, xiv, p. 52.

[27] Mummery: Lancet, 1905, i, pp. 696, 776, 846.

[28] Mummery and Symes: Journal of physiology, 1907, xxxvi, p. xv.

[29] Malcolm, J. D.: Transactions of the Medical Society of London, 1909, xxxii, p. 289; and Lancet, 1905, i, ii, pp. 573, 618, 737, 922; and 1907, i, p. 497.

sive clinical observations made especially during the early days of antiseptic surgery. He has laid particular emphasis upon the point that when saline is infused into the veins of a subject in shock the increase in the volume of the blood and the improvement of the heart's action are only temporary. The fluid is rapidly transferred into the tissue spaces, into the pleural cavity, etc., and the heart is then again insufficiently supplied with blood from the veins. The first section of this paper is in part a reproduction of Malcolm's reasoning.

Seelig and Lyon[30] have recently rendered the great service of bringing forward absolute proof that in traumatic shock the arterioles are more than normally constricted, instead of being relaxed. They have shown that the strength of the impulses transmitted from the centres over the vaso-motor nerves is greater, instead of being less, than under normal conditions. In Professor Lyon's laboratory experiments have recently been carried on which demonstrate conclusively that in shock the volume of blood in nearly all of the important organs of the body is greatly diminished.[31]

As to the etiology of the exsanguinity of shock Malcolm has suggested that the intense vaso-constriction induced by pain squeezes the fluid of the blood out through the walls of the vessels into the tissue spaces. This idea is in general accord with the views regarding lymph formation expressed by so competent an authority as Starling.[32] Nevertheless I cannot agree in this simple mechanical explanation of the edematous process in shock. If the high pressure causes exudation, the low pressure of profound shock should involve re-absorption. Lymph is not a mere filtrate from the blood. The increase in the water content of the tissues in shock is probably intra-cellular quite as much as it is inter-cellular or lymphatic. I believe that it depends upon chemical rather than upon physical conditions.

The retention of chlorides in the acute infectious diseases liable to terminate in shock is probably associated with the oligæmia of these

[30] Seelig and Lyon: Journal of the American Medical Association, 1909, lii, p. 45.

[31] The results of these experiments were exhibited at the last meeting of the American Medical Association, June, 1909.

[32] Starling, E. H.: Herter lectures (N. Y., 1908) on the fluids of the body (published in Chicago, 1909).

fevers. In passing out of the blood into the tissues the chlorides carry water to dissolve them, and thus induce a diminution of the volume of the blood. Important in this connection is the single observation of Hamburger [33] recently extensively repeated and verified by Luckhardt,[34] that the CO_2 content is ordinarily less, and the chlorine content greater, in lymph than in blood. Luckhardt concurs in Hamburger's suggestion that the lymph gives up the CO_2-ions, which it has received from the tissue cells, to the blood in exchange for twice the amount of Cl-ions. This mechanism offers a simple explanation for the retention of chlorides and the development of oligæmia under the influence of acapnia. When the CO_2 content of the blood is diminished by excessive pulmonary ventilation, the more rapid passage of CO_2-ions out of the tissues into the blood involves a correspondingly increased passage of Cl-ions out of the blood into the tissues. The increased osmotic pressure thus developed in the tissue fluids withdraws water from the blood and induces oligæmia. Somewhat the same process appears to be in part responsible for the policythæmia occurring coincidently with acapnia under lowered barometric pressure.[35]

This mechanism does not, however, afford a complete explanation of that crisis in the development of shock which Crile's experiments have shown to be a characteristic feature of failure of the circulation. Up to a certain point the failure is gradual. The amplitude of the pulse diminishes, Arterial pressure may fall somewhat, or may be maintained. Finally, however, a time arrives when within a few minutes the pressure drops to an extreme low level. Up to this point intra-venous infusion of saline is retained within the circulation fairly well, and the subject is usually capable of recovery. Thereafter the subject is irrecoverable; for saline infusions pass out of the circulation into the tissues nearly as rapidly as they can be injected into a vein.

The experiments to be reported in the next section appear to afford

[33] Hamburger, H. J.: Zeitschrift für Biologie, 1893, xxx, p. 142; Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie, 1893, xiv, p. 448; and Osmotischer Druck und Ionenlehre, 1904, ii, p. 54.

[34] Luckhardt, A. B.: This journal, 1910, xxv, p. 345.

[35] See Masing and Morawitz: Hohenklima und Blutbildung, Deutsches Archiv für klinische Medizin, 1910, xcviii, p. 301. On acapnia and increased hæmoglobin, see Ward, R. O.: Journal of physiology, 1908, xxxvii, p. 378.

an adequate explanation of this crisis. They show that in traumatic shock acapnial exsanguinity develops gradually until the arterial blood stream is diminished to the point at which it is insufficient to supply the oxygen needed by the tissues. The venous blood at this time, therefore, contains little or no oxygen. The blood stream of normal life has a two thirds factor of safety; in other words, the venous blood normally contains about two thirds as much oxygen as does the arterial.[36] In the absence of an adequate supply of oxygen a condition of asphyxial acidosis rapidly develops in the tissues. The acid substances thus formed cause the proteins of the tissues to imbibe water from the blood in much the same manner that fibrin swells in dilute acids. Hence the fatally rapid transudation of fluid from the blood vessels.[37]

Thus acute oligæmia develops in two stages. The foregoing paragraphs suggest their nature. What is here said is, however, merely a superficial and approximate preliminary statement of the hypothesis of acapnial oligæmia. It is of course very far from being the whole story of the complex processes probably involved.

III. The Development of Acute Oligæmia.

Experiments were performed on twenty dogs. Some were morphinized and initially anæsthetized with chloroform. The majority were merely etherized. All were tracheotomized. Arterial pressure was recorded by means of a Hürthle manometer connected with the carotid artery. Respiration was recorded by means of a spirometer connected with the trachea for a few seconds at a time during the observation periods. Samples of arterial blood were drawn from the femoral artery. Venous samples were taken from the right heart by means of a sound inserted through the right jugular. They were in all cases 3 c.c. each and were analyzed by the method of Barcroft and Haldane.[38]

After a preliminary normal period the abdomen was opened widely

[36] The same factor of safety appears to determine circulatory failure or recovery in CO poisoning. See Ringer, A. I.: Proceedings of Society for Experimental Biology and Medicine, 1909, vi, p. 68.

[37] This idea was first suggested to me by a paper (*q. v.*) on the nature and cause of edema by Martin H. Fisher: Journal of the American Medical Association, 1908, li, p. 830.

[38] Barcroft and Haldane: Journal of physiology, 1902, xxviii, p. 234.

and the abdominal viscera handled continuously in a stream of warm moist air. In some cases one of the sciatic nerves was exposed and cut, and its central end occasionally stimulated either mechanically or electrically. An intense congestion developed in the tissues and a stasis in the veins of the exposed viscera. The mechanical irritation of the intestines induced a continual hyperpnœa, a rapid heart rate, and high arterial pressure. The amplitude of the pulse diminished gradually to extreme threadiness. Such arteries as were exposed became constricted to less than half their initial diameter. Often the cannula used to draw blood from the femoral artery at the beginning of the experiment could not be inserted after shock had developed; and a smaller cannula had then to be used. While the animal was still in fairly good condition, the withdrawal of the blood samples had no perceptible effect upon the arterial pressure; but after the pulse had become very narrow, and even before the decisive fall of pressure, the loss of even this small amount of blood produced a noticeable depression.

It usually required two or three hours to induce shock by the methods employed. After the first hour it required watchfulness and a continual irritation of some afferent nerve to prevent the subjects from relapsing into a fatal apnœa, as described in the fifth paper of this series.[39] A change in this respect was noticeable after venous anoxhæmia had developed. They then breathed rapidly even when irritation was discontinued. This hyperpnœa was probably due to the presence in the blood of the acidosis substances resulting from tissue asphyxia, since the CO_2 content of the arterial blood was only about half the normal amount.

The results of the blood gas analyses show that the arterial CO_2 content was usually diminished by the hyperpnœa to about one-half the normal, and the venous CO_2 content to about two thirds or three quarters of the normal. In some experiments the oxygen content of the arterial blood increased noticeably during the development of shock. This is probably to be explained as due to the relative increase of corpuscles and hæmoglobin in unit volume of blood induced by the passage of serum out of the vessels into the tissues. The oxygen content of the venous blood diminished progressively until it contained in some cases only a mere trace of oxygen. Assuming that the oxygen consumption of the tissues continued the

[39] Henderson, Y.: This journal, 1910, xxv, p. 395.

same as in the preliminary normal period, the degree of venous anoxhæmia affords an index of the diminution in the volume of the blood stream. The arterial pulse record underwent a corresponding diminution in amplitude, but the systolic arterial pressure was usually well maintained, until the period of approximately complete venous anoxhæmia (3 per cent of oxygen or less) was reached. From these observations it appears that up to this point an increased vaso-motor activity (*i. e.*, augmented frictional resistance in the arterioles) compensated the arterial pressure for the diminution in the stream discharged by the heart because of the failing veno-pressor mechanism.

Up to the crisis of venous anoxhæmia it was found to be possible to induce a rapid recovery of the animals by the methods described in the third paper of this series (*Loc. cit.*). They consisted in pouring saline saturated with CO_2 into the abdomen and closing the cavity, infusing saline likewise saturated with CO_2 into a vein, and attaching a bag or long tube to the trachea so that the animal partially rebreathed its expired air. It was found to be safer to use oxygen for this rebreathing, instead of air, since the subjects at this time were peculiarly susceptible to the ill effects of lack of oxygen.

After the crisis these measures were inadequate. Respiration was indeed improved; and this benefit was retained so long as the rebreathing was continued. Temporarily the arterial pressure and pulse was likewise restored to normal height and amplitude. In the course of a few minutes, however, they sank again to a mere flutter only a little above zero pressure. By repeated and increasingly liberal infusions the circulation could be maintained for a half hour longer; but sooner or later death from circulatory failure resulted. In some cases a quantity of saline equal to one fifth of the animal's weight was thus administered, — without saving the subject. At autopsy the tissues were found to be edematous, the spleen distended, the arteries empty, the veins not well filled, the left heart contracted, and the right partially relaxed.

In Fig. 2 are reproduced the graphic records obtained in one of these experiments. In the table (pp. 170–171) are summarized the analytical and other data of twelve experiments illustrating the conditions of tissue respiration under which shock does, and does not, develop. When the mixed venous blood of the right heart contains only 3 to 5 per cent of oxygen, it is fair to assume a complete venous anoxhæmia in some organs, — *e. g.*, the abdominal viscera.

TABLE I.

TABLE SHOWING THE ARTERIAL AND VENOUS BLOOD GASES, THE PULSE RATES, AND THE ARTERIAL PRESSURE BEFORE (SAMPLE I) AND DURING (SAMPLE II) THE DEVELOPMENT OF SHOCK BY THE HYPERPNŒA INDUCED BY EXPOSURE AND IRRITATION OF THE ABDOMINAL VISCERA AND BY STIMULATION OF THE SCIATIC NERVE. DOGS OF 6 TO 10 KILOS WEIGHT.

Date of experiment 1907–8.	Blood samples.	Blood gases vols. per cent.				Arterial pressure mm. Hg.	Heart rate per minute.	Notes
		Arterial.		Venous.				
		O_2.	CO_2.	O_2.	CO_2.			
July 5	I	19.6	56.3	16.3	57.6	125	75	Morphinized, anæsthesia initiated with chloroform.
	II	20.9	24.5	10.6	29.2	60	180	Early in development of shock.
July 12 . . .	I	21.4	41.6	14.1	49.1	135	100	Morphinized.
	II	24.6	24.1	1.2	52.9	115	192	Early in development of shock.
July 16 . . .	I	20.3	63.1	16.1	62.5	125	90	Morphinized.
	II	21.0	29.9	8.8	43.0	30	212	Well-developed shock.
July 18 . .	I	12.3	45.5	9.5	44.2	105	60	Deeply morphinized and chloroformed.
	II	22.8	30.4	11.0	41.9	100	206	Never breathed excessively. No shock after three hours.

July 20	I	21.2	42.6	17.7	50.4	140	72	Morphinized.
	II	27.5	11.5	7.3	29.7	50	220	Well-developed shock.
Mar. 10 . . .	I	28.9	42.6	24.9	46.2	150	90	
	II	28.6	26.4	5.8	41.7	110	200	Sudden fatal apnœa a few minutes later.
Mar. 16 . . .	I	18.5	40.6	16.8	42.0	120	120	
	II	16.0	24.0	3.0	37.6	20	180	Near death
Apr. 16 . . .	I	18.4	33.6	18.0	36.2	150	150	A vigorous animal which refused to breathe excessively, and did not develop shock.
	II	27.4	30.1	13.0	37.0	120	180	
May 7	I	....	....	....	....	130	140	
	II	....	21.4	2.5	31.0	50	200	Failing
May 15 . . .	I	....	....	....	....	140	150	
	II	19.7	20.9	2.1	27.8	60	210	Failing
May 21 . . .	I	15.9	37.4	15.2	39.4	135	120	
	II	15.8	16.1	0.0	33.1	150	180	Fatal apnœa (*Cf.* This journal,) 1910, xxv, p. 398)
May 22 . . .	I	20.2	39.9	15.2	44.9	125	70	At 3.30 see Fig. 2.
	II	25.6	32.1	5.0	49.6	100	170	At 5.10

[1] Except when otherwise stated the subjects were under ether alone.

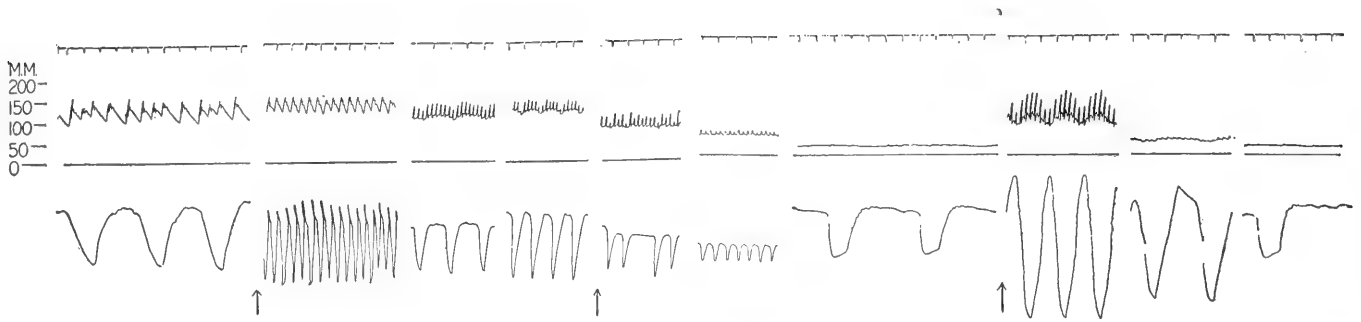

FIGURE 2. — Experiment of May 22, 1908. Dog of 7 kilos initially anæsthetized with chloroform, and afterward kept under ether. Time in seconds. Carotid pressure pulse. Respiration recorded by a volumetric spirometer, so that the relative amplitudes of the breaths are shown accurately. At the first arrow the abdomen was opened; and the viscera were handled in a stream of warm moist air for an hour and a quarter. This induced continuous hyperpnœa. At the second arrow the viscera were replaced and the abdomen closed. Note the smallness of the pulse at this time, although arterial pressure was as yet undiminished. Thereafter, however, it fell rapidly, until the pulse was a mere flutter at a pressure of only 20 mm. of Hg. Until now the respiration had continued rapid, while becoming more and more shallow. Now it changed to gasps, and the subject was at the point of death. One hundred and fifty cubic centimetres of carbonated saline were slowly injected into the jugular vein, and the subject was made to re-breathe oxygen in a paper bag. The record shows the immediate great improvement in both respiration and circulation. The benefit to the circulation was, however, very transitory. Owing to the rapid transudation of the infusion into the tissues, arterial pressure again failed rapidly.

IV. Hyper-tonus of the Heart in Shock.

It is impossible in any one paper of ordinary length to discuss all the factors concerned in acapnial shock. The behavior of the heart is, however, so important that it must be noticed here. Crile and Romberg and Pässler concluded from their experiments that the heart does not fail in shock. The only types of failure which are generally recognized at the present time are those in which the ventricles do not contract adequately. It is noteworthy that the pumping action of the heart can be abolished by excessive contractility also. If for any reason it relaxes less readily than normally in diastole, the resistance thus offered to the distention of its chambers by the venous inflow will result in a diminution of the systolic stroke.

The heart appears to differ from all other organs in that its tonus is increased, instead of being diminished, by acapnia. Rapidly induced acute acapnia augments cardiac tonus to such an extent that death from cardiac tetanus results, as was shown in the first paper of this series.[40] In a less rapidly developing acapnia, such as occurred in the experiments described in the present paper, hyper-tonus of the heart is probably a secondary but important contributing factor in the failure of the circulation. This hyper-tonus causes the ventricles to relax less readily during diastole at the same time that the force of the venous stream is diminished by the failing veno-pressor mechanism. Thus two factors work together to lessen the pumping action of the heart.

This behavior on the part of the heart itself appears to have been first noticed in animals and described by Howell.[41] Boise[42] has also observed it clinically. He regards it indeed as the essential element in traumatic shock. Jerusalem and Starling[43] have shown by experiments on the isolated mammalian heart that the CO_2 content of the blood must be maintained at a certain height if the pumping

[40] Henderson, Y.: This journal, 1908, xxi, p. 143; also 1910, xxv, p. 325.

[41] Howell, W. H.: Contributions to medical research dedicated to V. C. Vaughan, 1903, p. 51.

[42] Boise, E.: Transactions of the American Gynecological Society, 1908, p. 7; and American journal of obstetrics, 1907, lv, p. 1.

[43] Jerusalem and Starling: Journal of physiology, 1910, xl, p. 279.

action of the ventricles is to be normally carried out. In acapnia they find that the diastolic relaxation is incomplete, and the output is therefore minimal.

V. Conclusions.

Traumatic shock and toxæmic shock are in all essential features identical. In both the circulation fails in the same manner as after hæmorrhage. It is illogical to call this condition "vaso-motor failure," for the same evidence which shows that in shock the heart is still functionally capable demonstrates also that the vaso-motor mechanism is in a high degree of activity. The true vaso-motor failure of spinal shock, after a high section of the spinal cord, is an altogether distinct and different condition. The essential failing element in traumatic and toxæmic shock is the — hitherto undescribed — veno-pressor mechanism.

The veno-pressor mechanism (to be more fully described in a later paper) consists in part of the tonus of the tissues, and in part of osmotic processes. The tonus of the contractile tissues is largely dependent upon their content of CO_2. This tonus prevents stasis by compressing the capillaries. When it is diminished by acapnia, the blood stagnates in the venous reservoirs. The tension of CO_2 within the body is regulated by the respiratory centre. Thus this centre exerts an indirect but powerful control over the veno-pressor mechanism.

The osmotic element in the veno-pressor mechanism is the regulation of the water content of the blood, the lymph, the tissue fluid, and the cytoplasm of the tissue cells by the CO_2 which they contain. Acapnia upsets the normal balance of osmotic forces and induces a passage of water out of the blood into the tissues. When the blood stream has been thus diminished to the point at which it supplies to the tissues less oxygen than they demand, and the venous blood therefore contains little or no oxygen, a condition of asphyxial acidosis results. The proteins of the tissues swell and imbibe water from the blood. If saline is infused into a vein under these conditions, it passes rapidly out of the blood vessels into the tissues. Fatal oligæmia quickly ensues.

Dogs were brought into shock by exposure and aeration of the abdominal viscera and by the hyperpnœa thus induced. When fatal

apnœa vera was prevented by continual sensory irritation, all (except a few which were so deeply morphinized or were otherwise so resistant as to refuse to breathe excessively) died from failure of the circulation. During the development of shock large arteries (*e. g.*, the femoral) became constricted, not relaxed, — confirming the observations of Seelig and Lyon as against the supporters of the "vaso-motor failure" theory. The cause of the failure of the circulation was a diminution in the volume of the blood — oligæmia, or exsanguinity, — similar to that observed clinically in traumatic shock by Malcolm, and similar also to that occurring in the acute infectious diseases. Blood gas analyses and arterial pressure records show two stages in this process. The first is due to acapnia and is curable. The second, which develops from the first, is the fatal failure of the circulation quickly following (in shock as in carbon monoxid poisoning) the occurrence of venous anoxhæmia.

The heart in shock does not fail, — if by failure is meant beating more and more feebly until it sinks into prolonged diastole. On the contrary, during the tachycardia early in shock the systoles empty the ventricles more completely than normally. With the increased tonus induced in the heart by acapnia the ventricles require more than a normal venous pressure for their diastolic distention, — at the same time that the force of the venous stream is diminished by the failing veno-pressor mechanism. Thus hyper-tonus of the heart, as held by Howell and by Boise, and confirmed by Jerusalem and Starling, is an important contributing element in circulatory failure.

The essential sequence of events in acapnial shock is: (1) hyperpnœa; (2) acapnia; (3) failure of the veno-pressor mechanism (or sometimes sudden fatal apnœa); (4) venous anoxhæmia, tissue asphyxia, and acidosis; (5) acute oligæmia.

I am indebted to my colleague Prof. F. P. Underhill for valuable assistance and criticism in this work, and to Dr. T. B. Barringer and Mr. S. C. Harvey for collaboration in the related topics, here referred to, which will be published in later papers.

Note. — The following additional references may be useful to any one caring to look up the literature bearing on the topics here discussed: *Viscosity of blood increased by* CO_2, Zeitschrift für klinische Medizin, 1909, lxviii, p. 177; Müller, W.: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1910, xxi, p. 377. *Alkalinity of blood influenced by* CO_2, Henderson, L. J., and Spiro, K.:

Biochemische Zeitschrift, xv, p. 114; BOYCOTT and CHISOLM: Biochemical journal, 1910, v, p. 23. *Influence of CO_2 on colloids*, BAYLISS: Proceedings of the Royal Society, 1909, lxxxi, B, No. B, 548. *Chlorides in pneumonia*, v. WYSS, H.: Zeitschrift fur klinische Medizin, 1910, lxx, p. 183. *Water content of tissues and alterations in the circulation in acute infectious diseases*, SCHWENKENBECKER and INAGAKI: Archiv für experimentelle Pathologie und Pharmakologie, 1906, lv, p. 203, and 1909, lx, p. 166; MEYER, F., same volume, p. 208; LUSK, G.: The Science of Nutrition, second edition, 1909, p. 331; *Saline infusions in acute diseases*, LENHARTZ, Deutsches Archiv für klinische Medicin, 1899, lxiv, p. 189; *and in cholera*, NICHOLS and ANDREWS: Philippine journal of science, 1909, iv, B, p. 81.

METABOLISM OF DEVELOPMENT. — II. NITROGEN BALANCE DURING PREGNANCY AND MENSTRUATION OF THE DOG.

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THIS paper contains the results of a part of the experiments done within the past two years and a half with the object of determining what influence the development of the embryo may have on the protein metabolism of the mother dog. Starting with a chance observation made in 1907,[1] that the output of nitrogen in the urine of the fasting pregnant dog is much more profoundly influenced by a small amount of ingested carbohydrate than it is in the urine of a non-pregnant dog, the writer has since been interested both in the quantitative and the qualitative alterations in the metabolism produced by pregnancy and menstruation. The first paper of the series [2] dealt with the quantitative effects of pregnancy on the energy metabolism. The present one deals with quantitative effects of pregnancy and menstruation, and the next will take up their qualitative effects, on the protein metabolism.

Nitrogen Balance in Pregnancy.

The purpose of studying the nitrogen balance in pregnancy is, primarily, to determine the advantage or disadvantage to the maternal organism; secondarily, to determine the source and the amount of the protein materials entering into the product of conception. One would suppose *a priori* that from the moment of fertilization of the ovum, and in order to provide material for its growth and development, nitrogenous materials from the food would be retained and be built

[1] Murlin: Proceedings of the Society for Experimental Biology and Medicine, 1908, v, p. 72.

[2] Murlin: This journal, 1910, xxvi, p. 134.

up into the new protoplasms. But another possibility exists. The new growth may require protein materials not furnished by the food, or the maternal organism may not be capable of assimilating sufficient food protein to meet the additional demands. In either case nitrogenous materials would be taken from the mother's own body, and a comparison of the nitrogen intake with the nitrogen output for the entire period of pregnancy ought to reveal this fact. If, for example, the animal should remain in nitrogen equilibrium throughout the pregnancy, we should say at once that the embryos had been produced at the expense of the maternal tissues, or, if produced to any extent at all from the food proteins, had occasioned a corresponding loss from the maternal tissues to the outside world. The exact amount of the total loss could be determined by analyzing the entire birth — embryos, placenta, membranes, and fluids — and adding any extra nitrogen from involution of the uterus found in the urine immediately after parturition. If there should be a certain amount of nitrogen retained from the food, but the amount discharged at birth were in excess of this, the difference would represent the amount lost from the maternal tissues either to the embryonic tissues or to the outside world. If the amount issuing at birth together with the amount which could be accounted for by growth of the uterus and mammæ should be just about equal to the amount retained from the food, one would be justified in saying that the nitrogen retention is for the express purposes incident to the formation and growth of the embryo. Any nitrogen retained beyond this amount would be so much clear gain to the maternal organism.

Should an animal which had been in nitrogen balance before pregnancy show on the same diet, during any considerable portion of the period of pregnancy, a condition of minus nitrogen balance, one could say that the katabolic processes of the mother's body were for the time more active than in the non-pregnant condition. It would not, of course, signify that the nitrogen loss came from the embryo or from the surrounding maternal structures, since in both these situations, so long as development goes on normally, the synthetic or anabolic processes must be in the preponderance. The most natural interpretation would be that materials are being mobilized for the purposes of embryonic growth.

Should either a net loss of nitrogen from the mother's body for the

whole period of pregnancy or a condition of minus balance for any particular portion of the period prove to be invariable and characteristic of pregnancy, one would say with some assurance that the embryo depends to a greater or less extent upon the maternal tissues rather than upon freshly absorbed food protein for building materials. Such a fact would be a suggestive one from the standpoint of heredity, since, if the embryo (after fertilization of the ovum) is of necessity formed to any extent at all from the maternal proteins, a possible mechanism for the transmission of the species (not the individual) characters, supplementary to that of the pre-organization of the germ cells, is introduced.

Historical.

The first serious attempt to determine the influence of pregnancy on the nitrogen balance was that of Hagemann.[3] His experiments, two in number, were performed on dogs. When the amount of nitrogen delivered at birth in the embryos, placenta, etc., was considered,[4] it was seen, according to Hagemann's interpretation, that there was a net loss from the mother's body. Hagemann concluded, therefore, that "the formation of the fetus occasioned a loss of protein from the mother's body, even when there was abundance of nourishment" (*sehr reichliche Nährung*).

Ten years later Ver Ecke[5] reported numerous (nineteen in all) similar experiments on rabbits. His figures show that in many of them there was a net loss of nitrogen from the mother's body when the young were born. From which he draws the rather dramatic conclusion that "gestation constitutes a sacrifice of the individual for the species." Both Bar[6] and Jägerroos,[7] the only observers since Ver Ecke who

[3] Hagemann: Archiv für Anatomie und Physiologie, 1890, p. 577, and Inaugural Dissertation, Erlangen, 1891.

[4] This was not determined directly, but was estimated from Bischoff and Volkmann's figures.

[5] Ver Ecke: Mémoires courronnés, Académie Royale de Médicine de Belgique, 1901, xv, No. 7. A good digest of this paper by Magnus-Levy is to be found in Von Noorden's Handbuch der Pathologie des Stoffwechsels, 1906, i, pp. 403, 404; English translation, "Metabolism and practical medicine," i, p. 373.

[6] Bar, P.: Leçons de pathologie obstetricale, 1907, ii, p. 271.

[7] Jägerroos: Archiv für Gynaekologie, 1902, lxvii, p. 517.

have worked on animals, are inclined to discount this conclusion pretty strongly, because of the difficulties of carrying out metabolism experiments on rabbits with sufficient accuracy. My own examination of Ver Ecke's original paper confirms their opinion.

The next year Jägerroos published results of four experiments on dogs. In three out of the four — one on low, one on high, and one on a moderate amount of protein — (and in all cases an abundance of potential energy in the food) there was, according to Jägerróos, a net loss of protein from the mother's body as the result of the pregnancy. The one dog which may have shown a net gain or at least a condition of equilibrium (counting the nitrogen lost at birth) was given an excessive diet both as regards protein and potential energy and aborted two weeks before term.

At times in all of the experiments there was more nitrogen in the excreta alone than in the food. Jägerroos says: "In these periods of negative nitrogen balance the tissue protein (*Organeiweiss*) therefore must have served as building material for the embryos. In all my experiments, on the other hand, the food protein (*circulierendes Eiweiss*) has at times likewise served as building material, if a net sparing may be counted as a direct gain to the embryo. The question whether tissue or food protein is used in the formation of the fetus is therefore to be answered by saying that probably tissue as well as food protein can serve this purpose." Whether a condition of negative balance, that is, of increased protein katabolism, is characteristic of any portion of the period of pregnancy, is a question which Jägerroos leaves undecided.

Bar criticises the work of Jägerroos at two points: (1) he thinks the diet selected was not suitable, and inveighs pretty heavily against the continuous use (in Experiment I) of only cane sugar and meat. (2) Jägerroos, following Hagemann, overestimated the amount of nitrogen in the placentæ and membranes. The facts amply justify Bar's criticisms. Two of Jägerroos' experiments must be discarded entirely so far as the net result of the pregnancy is concerned, because the dogs aborted and ate the young. The other two Bar shows are scarcely demonstrative of the conclusions which Jägerroos draws.

Bar's own experiments are unquestionably the most satisfactory, all points considered, which have yet been done on the metabolism

of pregnancy in animals. In five of his experiments on dogs he determined the weights of the young at birth and, by muzzling the female, obtained also the weights of the placentæ and membranes. In four experiments the young and adnexa were analyzed. Since Bar's results have never been published in the current literature and we shall have occasion to refer to these figures again, they are presented here.

Dog.	Weights of young at term.	N in young at term.	Per cent of N in young at term.	N in adnexa.	N in adnexa per 100 of N in fetus.
No.	gm.	gm.			
1	1212.0	24.59	2.00	3.125	12.70
2	325.0	6.809	2.09	1.0	15.83
3	897.5	21.51	2.39	2.16	10.0
4	455.0	8.679	1.90	1.064	12.25
Av. . .	....	....	2.1	....	12.7

The percentage of N in the young alone agrees very well with the figure given by Jägerroos (2.17 per cent), but the relation of N in the adnexa to that in the fetus is only one fourth of the amount assumed by Hagemann (0.127 instead of 0.46).

Bar also sacrificed two of his pregnant dogs, one at the thirtieth day (middle) of gestation, the other at the forty-fifth day, in order to obtain the amount of N required by the entire ovum at different stages of the pregnancy. These data enabled him to plot a curve representing the amount of nitrogen diverted to the ovum, and, by difference, to obtain the amount lost or gained by the mother's own body at these successive stages. Similar curves for the rabbit are even more complete. His figures for the dog are as follows:

> At thirty days a single ovum contained 0.162 gm. N.
> At forty-five days a single ovum contained 2.09 gm. N.
> At term a single puppy contained 4.62 gm. N.

The mothers were covered in the three cases by the same sire, were kept on the same diet (proportioned to weight) and under the same general conditions; but the dog killed at the end of thirty days was

only about half as large as the one killed at forty-five days. The puppies analyzed at term and used in the table above were from the dog which (in the next pregnancy) was killed at forty-five days. It is possible, therefore, that the figure given for thirty days is proportionally a little too low in comparison with the other figures.

Bar's first dog gave birth to four puppies and ended her pregnancy with a net gain of 5.24 gm. Where this was deposited Bar does not attempt to say, but presumably in the uterus [8] and mammæ. His second dog gave birth to only a single puppy and ended the pregnancy with a net gain of 27.02 gm. N. The third experiment was marred by the occurrence of diarrhœa, vomiting, and loss of appetite from the twenty-first to the thirtieth days, notwithstanding which and the birth of seven puppies the pregnancy ended with a net loss of only 1.27 gm. N. Bar presents results from two rabbits which he believes are trustworthy, showing a retention of nitrogen from the beginning to the end of the period of gestation. His conclusion from all his animal experiments is that, "given a sufficient ration, the healthy mother's body can supply the needs of the developing fetus without drawing upon its own capital, even when those needs are extreme." [9]

On the basis of his own observations together with those of Hagemann and Jägerroos, Bar recognizes two periods in the nutrition of the pregnant organism, — the first extending from the time of fertilization to the middle of gestation (thirtieth day in the dog) and the second from the middle to the end. The dividing line corresponds with the beginning of active growth of the embryo.[10] The second period is characterized by a continuous and progressive retention of nitrogen up to the very day of parturition. In the first he distinguishes two phases: (*a*) one of "retention" immediately following fertilization and lasting until the second or third week (in the dog), and (*b*) one of "saturation," characterized by a diminished retention of nitrogen, a state of equilibrium, or even a loss of nitrogen, which continues until about the middle of gestation. This latter period he is convinced is not due to any diminution in the absorption of the nitrogenous mate-

[8] Bar analyzed the uterus and mammæ of the dog killed at term and found 3 gm. N in the uterus and 11.3 gm. in the mammæ. He estimates that the three dogs reported in full would require from 6 to 14 gm. for these tissues, according to weight. *Loc. cit.*, p. 292.

[9] Bar: *Loc. cit.*, p. 294.

[10] *Cf.* Rubner: Archiv für Hygiene, 1908, lxvi, pp. 180 *et seq.*

rials, but to a real increase in the processes of disassimilation. Bar offers no explanation, but mentions the possibility of proteolytic enzymes, or cytolysins produced by the embryo and acting upon the maternal tissues to produce a true mobilization of protein materials (*une véritable mobilization des albumines*).[11] This period he points out corresponds in time with the period of morning sickness in women.

Aside from this work on animals a number of trustworthy observations [12] have been made on the nitrogen balance in pregnant women, but all save one of them have been confined to a few weeks at most, and all save two to the very last weeks of the pregnancy. All have observed a retention of nitrogen varying in amount from 1.5 to 7 gm. per day, according to the stage of pregnancy and the amount of nitrogen ingested.

Summarizing all of the work reported to date, one may say that with respect to the first half of pregnancy the weight of evidence is that a period of diminished retention or of minus nitrogen balance is likely to occur in animals at about the stage which corresponds to the period of morning sickness of women [13] (see tabulated summary, p. 200). With respect to the second half there is perfect agreement that more nitrogen is regularly ingested than is excreted, provided a "sufficient" amount of nitrogen and potential energy is supplied. In Jägerroos' experiment No. IV 0.2 gm. N and 70 calories per kilo were not sufficient to maintain equilibrium except for one week (the third) out of the seven that the dog lived. In his experiment No. I 0.8 gm. N and 75 calories per kilo were sufficient to maintain a plus balance every week after the fourth. In all the other animal experiments a plus balance prevailed throughout the latter half, and in the single experiment of Hoffström on a woman it prevailed from the beginning of the fifth lunar month uninterruptedly to the end of gestation.

Whether the total amount of nitrogen retained is in all cases sufficient

[11] Bar: *Loc. cit.*, p. 288.

[12] Zacharjewsky: Zeitschrift für Biologie, 1894, xxx, p. 368; Schrader: Archiv für Gynaekologie, 1900, lx, p. 534; Sillevis, J.: Jets over de Stofwiselling der Gravida, Academie Poefschrift, Leyden, 1903, cited by Hoffström, *Loc. cit.*; Slemons: Johns Hopkins Hospital reports, 1904, xii, p. 111; Hahl: Archiv für Gynaekologie, 1905, lxxv, p. 31; Bar: *Loc. cit.*, p. 243; Hoffström: Skandanavisches Archiv für Physiologie, 1910, xxiii, p. 326.

[13] No one has yet attempted to follow the nitrogen balance in pregnant women earlier than the sixteenth week.

to cover the requirement for growth and development is quite a different matter. Hagemann and Ver Ecke were convinced by their experiments that in gestation the embryo must appropriate to itself a portion of the maternal protein materials; or, in the words of Ver Ecke, "gestation constitutes a sacrifice of the individual for the species." Jägerroos admits the possibility of this, but does not look upon it as necessary. He does not regard the embryo as a parasite, but admits that it *may* utilize maternal proteins if food proteins are not available. Bar regards gestation not as a sacrifice of the old individual for the new, but as an instance of "homogeneous and harmonious symbiosis."[14] It is not an occasion of loss, but of profit to the maternal organism as well as to the embryo.

Author's Experiments.

The experiments about to be reported were completed without any knowledge that Bar's magnificent work had been done. None of those alluded to here seem ever to have been published until they appeared in his "Leçons," and that work became known to the writer only by reading Hoffström's paper. It seems regrettable that his results should not have been given more extended circulation through the medium of some good journal. It is the more gratifying, however, to find the results reported in this paper in harmony, in the main, with those of so excellent an observer. Bar admits that he neglected the influence of menstruation because he could not predict the return of the œstrual cycle. That deficiency the following experiments will supply. It is of much more importance than the writer at first realized to have one's dog in equilibrium, or at least to know the protein condition of the animal, before the beginning of the sexual phenomena. Not only Bar, but Hagemann as well, was led astray by this lack of knowledge. Bar also neglects the influence of the total calorific content of the foods in the interpretation of his own experiments as compared with those of Hagemann and Jägerroos.

In planning the following experiments the chief improvement over those of Jägerroos aimed at was in the matter of suitableness of the diet. Jägerroos could not be certain that the periods of minus nitrogen balance were not due entirely to lack of appetite, and this in turn to

[14] Jägerroos: *Loc. cit.*, p. 298.

the unsuitable character of the diet and to confinement. With the exception of the early part of Experiment I and a day or two of Experiment V of the present series, no trouble was experienced with poor appetite, and there has been no sign of malnutrition such as the birth of dead puppies. In Experiment I only one puppy was born, but this may be due to the fact that copulation occurred only once. In all the other experiments copulation was permitted on two or three successive days, and four was the smallest number of puppies born. The cage used nearly all the time was a commodious one, permitting considerable freedom of movement by dogs of the bull-terrier breed (12 to 16 kgm. weight) such as those here employed. Bull-terrier dogs are most satisfactory for metabolism experiments both on account of their disposition and their hardihood.

The chief reason, however, for the good fortune in keeping the dogs in satisfactory nutritive condition which has attended these experiments seems to lie in the diet selected. Ground beef-heart (using only the thick ventricular wall free of both pericardium and endocardium) has been found to be a most satisfactory form in which to give protein to dogs, because the N-content is very constant [15] and because, mixed with cracker meal, lard, bone ash, salt, and enough water to make the whole mass slightly wet, it imparts a peculiarly agreeable taste. Only two dogs have been used in the experiments about to be reported, but all of the many dogs which have been given the diet have taken it greedily every day for as long as ten weeks without any sign whatever of distaste or malnutrition.

The average gestation period in the dog is just nine weeks (sixty-three days). In the nitrogen balance experiments below the excreta were collected in weekly periods and a balance was struck at the end of each week.

Experiment I. Dog A. Bull-terrier weighing about 12 kgm. was copulated in the laboratory by a strong male of the same breed on April 22, 1908. Collection and analysis of the excreta and analysis of the food were begun immediately. The urine was collected for the most part by the catheter and was separated into twenty-four-hour periods by washing the bladder. Immediately after this the dog was weighed, then fed

[15] It is only by careful selection of hearts, securing every day organs of about the same weight, and weighing the meat immediately after cutting and grinding, that one can depend on the N-content without analysis.

(only once in twenty-four hours) and taken for a walk. The urine for the day was made up to a standard volume, and after a thorough mixture an aliquot part (one fourth or one fifth) was put aside for the weekly urine. In this experiment the balance of the daily urine also was saved on two days of each week, and a fairly complete analysis was made (see following paper of this series). On the same two days the dog lived in the respiration apparatus for twenty-two out of the twenty-four hours.[16]

This dog was very cleanly (according to the standard of dogs) about her person, so that it was unnecessary to collect the vaginal discharge for analysis.

For convenience in preparing the food an attempt was made to supply protein in the form of meat powder, a commercial preparation known as "meatox." Varying quantities of this preparation with lard and cane sugar were given the first week in an attempt to suit the dog's appetite; but at the end of two weeks the dog refused to take this diet, and the change was made to beef-heart instead of the meat powder. Separate analyses of the ground ox-heart, freshly cut, showed the following N-content: 3.00, 2.98, and 3.00 per cent respectively. For the second two weeks the daily diet consisted of

275 gm.	beef-heart (3% N and 5% fat)	8.250 N and	339.2 cal.
40 "	lard	= 0.0	372
40 "	cane sugar	= 0.0	156
10 "	bone ash		
1 "	common salt		
	Total	= 8.250	867.2 cal.

At the beginning of the fifth week another change was made: 50 gm. cracker meal, containing 1.48 per cent N and yielding 196.2 cal., were substituted for the 40 gm. cane sugar. The entire diet then furnished 8.990 gm. N and 907.4 calories, of which 24 per cent was supplied by protein. This was continued every day and was taken eagerly up to July 15, seventeen days after parturition, when the experiment ended. The one puppy, born on the sixty-third day (June 26) and weighing 285 gm. as soon as dry, was apparently normal in every respect but one, namely, the absence of one eye. It was allowed to suck for a few days and then was taken away in order to reduce the dog's metabolism to the base level of entire sexual rest.

[16] See Experiment I, MURLIN: This journal, 1910, xxvi, p. 134.

TABLE I.

DOG A. FIRST PREGNANCY. NITROGEN BALANCE BY WEEKS.

Week of gestation.	Calories in food.	Nitrogen in food.	Nitrogen in excreta.	Nitrogen balance.
1908				
I.	4180.8	44.970	45.945	−0.975
April 23 to 29	(50 cal. per kg.)			
II.	5015.0	54.905	57.341	−2.436
April 30 to May 6	(58 cal. per kg.)			
III.	6152.8	54.555	56.844	−2.289
May 7 to 13	(71 cal. per kg.)			
IV.	6070.4	57.750	58.075	−0.325
May 14 to 20	(69 cal. per kg.)			
V.	6351.8	62.190	58.937	+3.153
May 21 to 27	(69.8 cal. per kg.)			
VI.	6351.8	62.930	59.882	+3.048
May 28 to June 4	(68 cal. per kg.)			
VII.	6351.8	62.930	57.316	+5.614
June 5 to 11	(66 cal. per kg.)			
VIII.	6351.8	62.930	57.216	+5.714
June 12 to 18	(64 cal. per kg.)			
IX.	6351.8	62.930	56.317	+6.613
June 19 to 25	(62.5 cal. per kg.)			
I. Post partum	6351.8	53.940	54.533	−0.593
June 27 to July 2	(65 cal. per kg.)	(6 days)	(6 days)	
July 14 to 15	66.3	8.990	4.576	+4.414
One puppy born. Weight, 285 gm.				

The nitrogen balance for Experiment I is given in Table I. In the second column of the table are given the apparent total calories of energy in the food and the calories per kilogram calculated on the basis of the average weight of the dog for the week. In the other columns are shown the total nitrogen in the food, the amount found in the excreta, and finally the plus or minus balance. It will be observed that a minus balance prevailed throughout the first four weeks, then changed to a plus balance, which increased gradually up to the day of parturition. The figures given for the nitrogen output do not include the nitrogen lost by falling off of hair or epidermal scales. The result by weeks is not thereby affected, however, for, while the analysis for this quantity of nitrogen would have made the minus balance in the early weeks a little greater, it is certain that it would not have changed the plus balance of the fifth nor any of the subsequent weeks to a minus balance.

There can be no doubt that the total supply of energy was sufficient

to maintain the dog in energy equilibrium, for the total energy production was determined for this pregnancy by calculation from the output of C and N, and at no time, not even in the last week of pregnancy, was the production from the dog's body equal to the supply from the food. (see Table I of previous paper).

The total amount of nitrogen lost from the mother's body during the first four weeks was 6.025 gm. The total amount retained for the last five weeks was 24.142 gm. — a net retention for the entire period of gestation therefore of 18.127 gm. The puppy was not analyzed at birth because it was desired for the purpose of the respiratory metabolism experiments reported in a previous paper. Figures are available from Bar's and Jägerroos' papers, however, which will enable us to calculate the amount of nitrogen it contained accurately enough for our present purpose. Taking the mean of all their determinations (2.16) as the percentage composition of the puppy in the present case, the total amount contained (weight, 285 gm.) would be 6.15 gm. Adding 12.7 per cent for the membranes, placenta, and fluids (see Bar's table, p. 181), or 0.78 gm., we have 6.93 gm. as the amount delivered at birth. The excreta on the day of parturition and for five days thereafter contained, on the average, 0.5 gm. more nitrogen than the food. This extra nitrogen, which is practically all in the urine, has been regarded by Jägerroos and others as belonging to the birth. In this dog the extra nitrogen for the first day or two represents blood, placenta, amniotic fluid, etc., ingested at parturition, and the rest of it is doubtless traceable to involution processes, *i. e.*, represents nitrogen stored temporarily in the uterus. On the sixth day after parturition in this case the extra nitrogen in the excreta had fallen to 0.06 gm. This point therefore may be taken as representing the end of the most active involution, though it is not to be supposed that the uterus has yet reached the minimal size of sexual rest. At any rate, counting 0.5 gm. a day for five days, we have 2.5 gm., at the most, to be added to the 6.93 gm., making a total of 9.43 gm. N as the final product of gestation. In this pregnancy, therefore, the mother has furnished all the protein materials to the embryo and has retained for her own body (18.127 − 9.43) 8.69 gm. nitrogen besides. In other words, the mother's body is in a better condition as regards protein at the end of the pregnancy than it was in the beginning. It does not follow, however, that the maternal tissues have not been drawn upon for build-

ing materials. For the first four out of nine weeks, without taking any account of losses through the skin, there was a condition of negative balance, which, according to the interpretation given on page 178, means that some of the material contributed to the embryo during this time probably had its origin in the mother's own tissues.

Experiment II. Dog B. A brindle-colored female with a predominant strain of bull-terrier blood and weighing about 13 kgm. was copulated by a bull-terrier male on February 1, 2, and 3, 1909. The daily routine of collecting and separating excreta was exactly the same as in Experiment I. The food given to this dog was the same in general character as that given to Dog A in Experiment I, and was designed to furnish about 70 cal. per kilogram of body weight. After a few days' trial the following diet proved to be satisfactory and was given every day from February 3 to April 4 at the same hour:

250 gm.	beef-heart	(2.73% N [17] and 5% fat)	= 6.825 N and	307.5 cal.
80 "	cracker meal	(1.48% N)	= 1.184	313.6
40 "	lard			372.0
10 "	bone ash			
2 "	common salt			
	Total		= 8.009	993.1 cal.
			20% protein calories.	

Four puppies were born on April 4, the sixty-third day from the first copulation. They were all large and well formed, weighing in the neighborhood of 350 gm. each. They were allowed to suck for about three weeks and then were taken away for the purpose of reducing the mother's metabolism to the level of sexual rest. The diet during these three weeks contained more meat than before, but otherwise was of about the same general character as above. It was not analyzed. That it was probably sufficient so far as energy is concerned, is shown by the fact that the mother dog maintained her body weight (13.1 kgm. on May 7 as compared with 13.0 kgm. just after parturition), although the puppies grew rapidly, weighing on the 20th of April 1220, 1280, 1180, and 1080 gm. respectively. On the 3d of May the original diet was resumed and was continued until the 12th.

[17] The apparently lower percentage of N in the beef-heart of this experiment was due to an error in the standardization of the acid. The same error applies to all the N determinations of this experiment, but not, of course, to the balance figures.

The results of this experiment are given in Table II. It is seen that the minus nitrogen balance prevailed in this case throughout at least six of the nine weeks of pregnancy. It is necessary to say "at least," because it is possible that the amount of nitrogen lost by shedding of hair, epidermal scales, and vaginal secretion (this dog was not so cleanly about her person as Dog A) would have exceeded the 1.801 gm., the amount apparently retained in the seventh week. Chittenden [18] reports a dog of about the same weight and the same general character of coat which lost on the average 0.21 gm. N per day through the hair. Assuming the same rate of shedding in this dog, the total loss for the week through this channel alone would amount to 1.47 gm. There can be no doubt, however, about any other week.

TABLE II.

Dog B. Nitrogen Balance by Weeks.

Week of gestation. 1909	Calories in food.	Nitrogen in food.	Nitrogen in excreta.	Nitrogen balance.
I. February 3 to 9	5382.1 (56 cal. per kg.)	53.287	63.116	−8.829
II. February 10 to 16	6851.7 (72 cal. per kg.)	56.063	60.893	−4.830
III. February 17 to 23	6851.7 (71 cal. per kg.)	56.063	62.031	−5.968
IV. February 24 to March 2	6851.7 (71 cal. per kg.)	56.063	64.508	−8.445
V. March 3 to 9	6851.7 (70 cal. per kg.)	56.063	62.594	−6.531
VI. March 10 to 16	6851.7 (68 cal. per kg.)	56.063	60.064	−4.001
VII. March 17 to 23	6851.7 (66 cal. per kg.)	56.063	54.262	+1.801
VIII. March 24 to 30	6851.7 (63 cal. per kg.)	56.063	47.042	+9.021
IX March 31 to April 3	3972.4 (61 cal. per kg.)	32.036 (4 days)	25.786 (4 days)	+6.250
2 weeks after lact. May 7 to 12	5958.6 (76 cal. per kg.)	48.054 (6 days)	40.006 (6 days)	+8.048
Four puppies born. Weight, 1400 gm.				

For the first six weeks there was a total loss by the excreta of 38.604 gm. N. For the last three weeks there was a total retention

[18] Chittenden: Nutrition of man, New York, 1907, p. 250.

over the amount discharged by the excreta of 17.022 gm. The four puppies weighed at birth about 1400 gm. and contained (2.16 per cent; see p. 181) not less than 30.2 gm. N. Taking into account the nitrogen loss by the fluids, membranes, and placentæ (12.7 per cent of N in fetus), the total amount delivered could not have been less than 34 gm., and was probably quite a little more than this. The net result thus was a loss of at least (38.604 + 34.0) − 17.0 =) 55.6. This case therefore presents conditions just the reverse of those presented by the previous case.

The contrast with this of the metabolic conditions in sexual rest is very striking. The six-day period from May 7 to 12 inclusive, five weeks after parturition and two weeks after the dog ceased nursing her brood, shows a positive balance of about the same proportions as that of the eighth week of gestation. Every trace of milk had disappeared from the mammæ by this time, and the processes of involution were presumably long since at an end. The nitrogen retained therefore must have gone to the maternal tissues themselves.

This high retention doubtless was due in part at least to the impoverishment resulting from the heavy drain upon the maternal tissues which had occurred in the pregnancy period; for while the diet during the period of lactation may have been sufficient for the demands of that period, it probably was not sufficient to enable the mother to recoup herself completely for the previous losses. The metabolism at this time therefore probably does not represent the base line of perfect sexual rest, but only an approach thereto. Unfortunately the case could not be followed further.

The results of Experiments I and II are represented graphically in Fig. 1. The continuous line in each case represents the protein condition of the mother, assuming that she was in nitrogen equilibrium (*i. e.*, that the ordinary demands of the tissues for protein building materials had been kept up) at the beginning of the pregnancy. The preliminary decline in protein condition at the beginning of Experiment I had been fully compensated for at the end of the sixth week. From this time to the end of pregnancy there was a steady gain. In Experiment II the decline continued until the end of the sixth week, and was not yet compensated at the end of pregnancy by something over 20 gm. N.

The discontinuous lines in the charts represent the amounts of

nitrogen diverted to the embryos and their adnexa at successive stages of the gestation period. These curves are based on Bar's figures (page 181) for the nitrogen content of the entire ova at the thirtieth and forty-fifth days and the end of gestation, due allowance being made for the differences in weight of the puppies. In the first experiment from which only one puppy was born it is clear that the pregnancy ends with a considerable advantage, in protein condition, to the mother herself (differences between the two curves); while in the second experiment it ends with the mother's body considerably depleted.

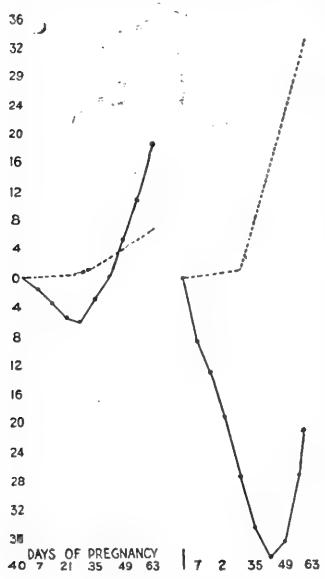

FIGURE 1. — Unbroken lines represent the protein condition of the pregnant dog at successive stages of the pregnancy, and broken lines the amount of nitrogen diverted to the embryos. Experiment I, Dog A, one puppy born; weight, 285 gm. Experiment II, Dog B, four puppies born; weight, 1400 gm.

It is highly significant, as Bar has pointed out, that the improvement in general protein condition should begin at about the time "active growth" of the embryos becomes of some consequence from a comparative standpoint. Rubner [19] also, from a consideration of the dynamic conditions in gestation, has emphasized the fact that the embryo is of no significance, relatively speaking, until about the middle (0.4) of the period. Only after the thirtieth day, in the dog, does the amount of nitrogenous materials required by the embryo become appreciable. At about the same time in Experiment I nitrogen began to be retained. In Experiment II this point is marked by a decrease in the loss (6.5 gm. N in the fifth as compared with 8.4 gm. in the fourth week). From this time the condition is progressively better, and from the sixth week to the end the rate of improvement is almost exactly that of the growth rate (*i. e.*, the two curves are nearly parallel). One would perhaps be justified in saying that all of the nitrogen retained during the last three weeks found its way to the embryos.

Since the total amount of energy in the food of the two dogs was

[19] RUBNER: *Loc. cit.*

almost exactly proportional to their weights (see tables on page 187 and 190) and the nitrogen intake was not widely different, it is significant also that the total amount of nitrogen lost up to the end of the fourth week in the two cases is nearly proportional to the weight of new-born delivered (6 : 28 :: 285 : 1400 nearly).

This itself strongly suggests that the cause of the minus balance is the presence of the embryos. We have as yet no proof of this, however, because, as already noted at page 184, we are not certain that the dog would have been in nitrogen equilibrium on the same diet just previous to menstruation. With a much higher supply of potential energy and a somewhat lower supply of nitrogen, Bar found in all his dogs a condition of plus balance in the first two weeks of pregnancy; but not having observed it himself, he did not correctly interpret the influence of menstruation. Realizing the importance of this factor, the following experiments were planned:

INFLUENCE OF MENSTRUATION ON THE PROTEIN CONDITION OF THE DOG.

Experiment III. Dog A. Third pregnancy. Having had this dog under observation through two previous pregnancies, I was able to predict

TABLE III.

EXPERIMENT III. DOG A.[1]

Week of œstrual cycle.	N in urine per week.	N in feces per week.	Total N excreted per week.	N balance per week.	N balance per day.
1909. March 20 to 26	55.268	2.901	58.163	+3.767	+0.538
March 27 to April 2	57.360	2.901	60.261	+2.669	+0.381
Last week of sexual rest April 3 to 9	57.121	3.847	60.968	+1.962	+0.280
April 10 to 16	55.209	3.494	58.703	+4.227	+0.604
Active menstruation April 17 to 23	52.102	3.453	55.555	+6.375	+0.911
First of pregnancy April 23 to 29	55.448	3.333	58.781	+4.149	+0.593
Second of pregnancy April 30 to May 6	58.790	2.631	61.421	+1.509	+0.215

[1] The food contained every day 8.990 gm. N and 907.4 calories of energy (66 to 70 cal. per kgm.).

the return of the œstrual cycle pretty exactly. The dog was accordingly placed on a measured diet about four weeks in advance of the expected menstruation. Collection and analysis of excreta began at the same time. First menstrual blood was observed on April 16, and on April 23 first copulation occurred.

The diet was exactly the same as that given during the last five weeks of Experiment I. The routine also was the same as in that experiment, except that the dog was not used for respiration experiments, and the excreta were collected for weekly periods only. Four puppies were born on June 26, the sixty-fourth day of gestation.

We see clearly that the diet, which was precisely the same as given to this dog during the last five weeks of her first pregnancy, was entirely adequate to maintain her in nitrogen equilibrium during sexual rest. It is equally clear that the minus balance which prevailed in the first two weeks of her first pregnancy (Table I) was due to the inadequate diet given at the beginning and not to anything peculiar to the early stages of pregnancy. Because of an engagement to work in another laboratory, it was impossible for the writer to follow the nitrogen balance throughout the gestation period. The experiment is sufficient, however, to disprove Hagemann's statement that a change from a plus to a minus nitrogen balance takes place at the "moment" of conception. Copulation occurred on April 23, just a week from the first appearance of menstrual blood, but bleeding did not cease until about the 27th of April.

The influence of the menstrual period is, clearly, to cause an increased retention of nitrogen. Schrader,[20] who studied the nitrogen balance through the menstrual period of several women, and Schöndorff,[21] who followed the same, quite incidentally, in a single dog, were of the opinion that this retention is a direct compensation for the loss of blood. Potthast[22] and Ver Ecke,[23] however, thought that some other explanation is necessary. The various studies on the influence of hemorrhages of other kinds on the nitrogen metabolism have given discordant results.

[20] SCHRADER: Zeitschrift für klinische Medicin, 1894, xxv, p. 72.

[21] SCHÖNDORFF: Archiv für die gesammte Physiologie, 1897, lx, p. 395.

[22] POTTHAST: Beiträge zur Kenntniss des Eiweissumsatzes, Inaugural Dissertation, Leipzig, 1887.

[23] VER ECKE: *Loc. cit.*

Hawk and Gies,[24] whose experiments were most painstakingly performed, with due regard paid to the effects of anæsthesia, state in their conclusions that "external hemorrhage equal to from 3 to 3.5 per cent of the body weight of dogs, was observed to cause the following effects: in well-nourished animals in weight and nitrogen equilibrium and fed continuously on a diet of constant composition, there was a temporarily increased output of nitrogenous and sulphur-containing products in the urine." The same was observed by Bauer[25] and Popiel.[26] Jürgensen,[27] however, in one of his experiments on a dog from which the hemorrhage amounted to only 1 or 2 per cent of the body weight, found a reduction of the N output, and Ascoli and Draghi,[28] who produced hemorrhages of from 220 to 475 c.c. in men, witnessed a temporary reduction. Hence it is possible that small blood losses up to something less than 3 per cent of the body weight may cause reduction, while larger losses may produce an increased nitrogen excretion.

It was impossible to collect the menstrual blood from the dog with any degree of accuracy for analysis; consequently the loss of nitrogen from this cause is unknown. It is not possible, however, that it could have been equal to 3 per cent of the body weight (300 gm.). Prussak[29] never found so much in the menstruation of women. It is very probable, therefore, that the diminished output of nitrogen in the urine of this dog during the three menstrual periods studied is due, to some extent at least, to the small hemorrhage. At all events, the influence of menstruation is exactly the reverse of what Bar postulated.

The most pronounced effect is coincident with the most profuse bleeding (Table III, week of April 17 to 23). The week immediately preceding also shows the effect of approaching menstruation, and the week following certainly was influenced, since bleeding did not cease until the 27th of April. The week designated as "last of sexual rest"

[24] Hawk and Gies: This journal, 1904, xi, p. 171.

[25] Bauer: Zeitschrift für Biologie, 1872, viii, p. 567.

[26] Popiel: Maly's Jahresbericht der Thierchemie, 1893, p. 505.

[27] Cited by Ascoli and Draghi.

[28] Ascoli and Draghi: Berliner klinische Wochenschrift, 1900, xxxvii, p. 1055.

[29] Prussak: Jahresbericht über Gynaekologie und Geburtshülfe, 1899, p. 162; see also Hoppe-Seyler: Zeitschrift für physiologische Chemie, 1904, xl, p. 545.

exhibits a metabolism of about the same intensity as that of the "second week of pregnancy." From this experiment, then, we may conclude that the protein metabolism immediately following conception is pitched at about the same level of intensity as immediately preceding menstruation.

This is confirmed by the next experiment done with the same dog six months later. With the thought that the minus nitrogen balance observed in Experiments I and II might be due to the fact that the protein of the beef-heart fed was too readily digested and absorbed, that is, that the adult maternal tissues have no power to long retain for the use of the embryo the nitrogenous materials, which ordinarily are converted into urea, two feedings a day were given in this experiment, and some milk protein was added in the form of a powder known as "trumilk."

Experiment IV. Dog A. Fourth pregnancy. Menstruation was expected about the last week in October. Accordingly on October 1 the dog was placed on a measured diet, which was given twice daily (at 9 A. M. and 5 P. M.) until December 5. The diet follows:

100 gm.	beef-heart	(3 % N)	= 3.00 N	and 120 cal.
55 "	cracker meal	(2.08 % N)	= 1.14 N	and 214.5
10 "	"trumilk"	(6.4 % N)	= .64 N	and 42.
10 "	lard		= 0.0 N	and 94.
5 "	bone ash		= 0.0 N	and 00.
1 "	salt		= 0.0	0.
	Total		= 4.78 N	and 469.8 cal.

The daily intake therefore was 9.55 gm. N and 936.6 cal., of which 26 per cent was supplied by protein. On December 5 albumin appeared in the urine, due to a cystitis induced by long-continued use of the catheter, and it was thought best to discontinue the experiment for a week. A change was made in the diet at the same time, to one very nearly approaching that used in the previous experiments on this dog (see p. 186). The only difference was that the cracker meal used contained more nitrogen. This change was made partly for the purpose of giving a food whose ash was more strongly acid, so as to produce a distinctly acid urine, thereby favoring the bladder condition, and partly in order to compare the corresponding stages of different pregnan-

cies on the same diet. By December 11 the albumin had entirely disappeared.

First menstrual blood was observed on November 2, and copulation occurred on November 11, 12, and 13. Five puppies were born on January 14, the sixty-fourth day.

TABLE IV.

EXPERIMENT IV. DOG A.[1]

Week of experiment.	N in urine per week	Total N in feces per week	N excreted per week	N balance per week	N balance per day
1909					
Oct. 3 to 9	56.812	5.44	62.25	+4.77	+0.68
Oct. 10 to 16	56.464	3.25	59.71	+7.21	+1.03
Oct. 17 to 23	58.62	4.10	62.72	+4.20	+0.60
Oct. 24 to 30	57.42	4.65	62.07	+4.85	+0.69
Menstruation Oct. 31 to Nov. 6	54.87	3.29	58.16	+8.76	+1.25
Menstruation Nov. 7 to 13	50.02	4.41	54.43	+12.49	+1.78
First of pregnancy Nov. 14 to 20	58.31	2.88	61.19	+5.73	+0.82
II Nov. 21 to 27	59.16	3.43	62.59	+4.33	+0.62
III Nov. 28 to Dec. 4	66.53	3.94	70.47	−3.55	−0.51
V Dec. 11 to 17	62.42	3.00 (?)	65.42	−0.39[2]	−0.05
VI Dec. 18 to 24	58.02	3.77	61.79	+3.24[2]	+0.46

[1] The food contained each day 9.56 gm. N and about 940 calories (67 to 74 cal. per kgm.).

[2] Food these two weeks contained only 9.29 gm. N per day.

The results given in Table IV present very much the same picture as in the previous experiment. Up to the week of menstruation a plus balance of about 0.6 gm. N daily prevailed. During the first week of menstruation this figure was just about doubled, and during the second week it was almost tripled, only to fall back again within the next two weeks to the level which obtained before menstruation. There can be no doubt of the influence of the menstrual processes.

The third week of the pregnancy, however, more nitrogen was

found in the urine and feces than was contained in the food. It is likely that this condition prevailed throughout the fourth week, for, on the same diet, we find it persisting into the fifth. The sixth week the retention of nitrogen had again set in, and without doubt would have continued progressively to the end, had it been possible to follow the experiment continuously. We shall return in the discussion later to the matter of the negative balance.

Meantime the question which remains is whether the same level of protein metabolism would be maintained after menstruation if the dog were not copulated. If the prompt return to the same level as before menstruation were due in any degree to conception, a duplicate experiment in which copulation is not permitted ought to reveal a difference. The literature does not contain any clear differentiation of this kind. The experiment which follows was planned for this purpose.

Experiment V. Dog A. Menstruation was expected early in May. Accordingly the dog was placed on a measured diet on the 23d of April. With the exception of a slight difference in the N-content of the cracker meal and of the milk powder used, the diet was precisely the same as in Experiment IV. Just previous to this experiment the dog had been used for a fasting experiment of eight days' duration; hence the heavy retention of nitrogen the first week. Possibly because of the previous fast, the dog did not come into "heat" at the expected time, and to hasten the onset she was liberated from the cage for a week and for several days was taken for a walk of a mile or more in the hot sun. On the 21st first signs of menstruation were observed, and the experiment was resumed. The last trace of blood in the vagina which could be obtained on a sponge of cotton was observed on the 7th of June. Free hemorrhage had ceased three days earlier.

The retention of nitrogen in this experiment is considerably greater than in the previous one. This is doubtless attributable in part to the previous fast (see protocol), which reduced the dog's protein condition considerably, and in part probably to the gradually rising temperature of the springtime (the daily temperature record in the dog room shows a gradual rise indoors as well as outdoors) as opposed to the gradually falling temperature of the autumn under which the previous experiment was performed. Taking the third week as the base

level for the metabolism of sexual rest, the relative rise due to the menstruation, however, is not quite so great as in Experiment IV on the same diet, nor so great as in Experiment III done at the same time

TABLE V.

EXPERIMENT V. DOG A.[1]

Week of experiment.	N in urine per week.	N in feces per week.	Total N in excreta per week.	N balance per week.	N balance per day.
I April 24 to 30	48.30	3.58	51.89	+13.00	+1.85
II May 1 to May 6	52.48	3.82	56.30	+ 9.22	+1.31
III May 7 to 13	54.07	3.83	57.90	+ 7.62	+1.09
IV menstruation [2] May 22 to 28	44.53	2.91	47.44	+15.65 [3]	+2.23
V menstruation May 29 to June 4	47.31	2.59	49.90	+15.62	+2.23
VI June 5 to 11	47.71	2.63	50.34	+15.18	+2.17
VII 5 days only June 12 to 16	36.63	2.47	39.10	5 days only + 7.25	+1.45

[1] Food contained each day 9.27 gm. N and 940 cal. (70 to 85 cal. per kgm.).

[2] Experiment was discontinued from May 14 to 21 (see protocol).

[3] Through a mistake dog was given wrong food at one feeding. Nitrogen intake was therefore 2.33 gm. less than it should have been.

of year but with a different diet. The return to normal, after menstruation had entirely ceased, is also not so abrupt as in both the previous experiments. Not to lay too much stress on a single instance, it nevertheless appears that the course of protein metabolism following menstruation may be influenced to some extent by conception.

DISCUSSION.

We have seen that in the first experiment the dog lost nitrogen from her body throughout the first four weeks of the pregnancy, and in the second, throughout six of the nine weeks. It is now apparent from Experiments III and IV that this condition in the first two weeks of Experiment I, and probably for longer in Experiment II, was due to the inadequate diet as regards energy supply. This, however, would not suffice to explain the negative balance of the third

and fourth weeks, because, in the first place, in Experiment III the same diet kept this dog in equilibrium under conditions which apparently were identical in every respect except that she was in complete sexual rest. In the second place, any deprivation of nitrogen due to inadequate supply in the first two weeks would predispose to a nitrogen retention in the third and fourth. Again, in Experiment IV a diet richer as regards both potential energy and nitrogen was not sufficient to maintain equilibrium in the third week. These experiments therefore fall into line with those of previous investigators in exhibiting a preponderance of protein katabolism at a time which, according to Bar, corresponds with the period of morning sickness in pregnant women. To make this point clear all the experiments which have been reported to date are brought together in the following table:

Date.	Author.	No. of exp.	Food cal. and gm. N per kgm.		N balance by weeks of the pregnancy 1	2	3	4	5	6	7	8	9
1891	Hagemann	I	82[1]	1.0[1]	−	−	−	−	−	+	+	+	+
	"	II	110	0.8	−	−	−	−	+	+	aborted		
1903	Jägerroos	I	75	0.8	+	+	−	−	+	+	+	+	+
	"	II	106	1.84	+	−	−	+	+	+	+	aborted	
	"	III	75	1.31	+	+	+	+	−	−	+	+	+
	"	IV	70	0.2	−	−	+	−	−	−	−	aborted	
1907	Bar	I	112−	0.64	+	+	+	+	−	+	+	+	+
	"	II	80−	0.66	+	+	+	±	±	+	+	+	+
	"	III	116−	0.67	+	+	−	−	+	+	+	+	+
1910	Murlin	I	70	.75	−	−	−	−	+	+	+	+	+
	"	II	70	.64	−	−	−	−	−	−	+	+	+
	"	III	70	.75	+	+	not continued						
	"	IV	70	0.8	+	+	−	−	−	+ not continued			

[1] These figures are based on the weight of the dog always at the beginning of the experiment.

There seems to be no doubt, from this showing, that there is something characteristic about the occurrence of a negative balance in the

first half of pregnancy in the dog. Even 106 calories of energy and nearly 2.0 gm. N per kilogram per day were not sufficient in Jägerroos' Experiment II to keep up a steady retention. What is the explanation of this phenomenon? Hagemann believed that it is due to the inability of the animal cell to transform one kind of protein into another without loss of nitrogen. Jägerroos thought that some of these periods in his experiments might be due to lack of appetite from the monotonous character of the diet. Bar found actual sickness (vomiting) and diarrhœa with loss of appetite in his Experiment III, but observed nothing of the kind in any of the others, and in the experiments reported in this paper the only instance of lack of appetite was in the second week of the first and in the last week of the last experiment where copulation did not occur. Jägerroos lays stress on the fact that a "period of retention is always followed by a reaction," and Bar gives expression to the same thought in his "phase of saturation" idea. He seems not altogether satisfied with this explanation, however, for he mentions the possibility of proteolytic enzymes (cytolysins) set free by the fetus and acting on the maternal proteins. Inspection of the whole record as it now stands does not seem to favor the "reaction" idea. In eight out of thirteen experiments tabulated above a minus balance occurs in the fourth week of pregnancy.[30] In seven certainly, and probably nine, of the thirteen a negative balance occurs in the fourth week. Two of the dogs in which it does not occur were on very high protein, and should therefore, one would think, show the "reaction" earlier, if that were the true explanation.

It is the writer's belief that the presence of the embryos is in some way responsible for the nitrogen loss. Whether this is due to enzyme action it is at present impossible to say with certainty. Graefenburg[31] has recently found abundant evidence of the effects of proteolytic enzymes produced by the ovum at the time of implantation. In fact, there is no other rational way to account for the growth of the ovum previous to the establishment of the placental circulation save by the production of such enzymes. The placenta itself is now looked upon as an organ which has a digestive function[32] and may be said to serve

[30] It probably would have occurred in another — the III of the present series — making nine out of thirteen.

[31] Graefenburg: Zeitschrift für Geburtshülfe und Gynaekologie, 1909, lxv, p. 1.

the purpose of restricting the action of enzymes to materials furnished directly by the maternal blood instead of permitting them to act indiscriminately. It appears from the investigations of Bonnet [33] on the embryology of the dog that the placenta is fully matured at about the time (thirtieth day) when rapid growth begins; or perhaps we ought now to say that rapid development begins at this time because the placenta has been fully established. It is probable, therefore, that the period of greater nitrogen loss simply marks the culmination of the more or less indiscriminate enzyme action, and that the recovery therefrom marks the complete establishment of the placental functions.[34] The plus balance occurring immediately after conception in so many of the cases may mean only that the quantitative effect of the enzymes is not yet sufficient to counterbalance the natural tendency of the maternal tissues to maintain themselves in equilibrium. The amount of nitrogen lost on this hypothesis might reasonably be expected to bear some direct relation to the number of embryos formed, and this, as we have seen on page 192, is the case.

Space forbids fuller discussion of this point at present, but it should be noted that the biological reason for the production of enzymes by the developing ovum is identical with the reason for the existence of such enzymes in the adult stomach, namely, to enable the organism to build foreign materials into its own type of protoplasm.

If, as is generally supposed, though by no means definitely proved, the enzymes produced by the ovum can act on the maternal proteins and the ovum can establish placental (enzyme) connection with the uterine wall of its own physiological species only, the enzymes so produced become an essential part of the mechanism of heredity. We should then be obliged to say that the reason why dog ova grow into young dogs is partly at least because they can grow at the expense only of old dog proteins. At all events, the existence of a negative

[32] See L. Zuntz: Ergebnisse der Physiologie, 1908, vii, p. 430.

[33] Bonnet: Anatomische Hefte, 1er Abtheilung, 1902, xx, p. 477.

[34] According to Grosser (Entwickelungsgeschichte der Eihaüte und der Placenta, Wien u. Leipzig, 1901, p. 118), two stages of placentation are to be distinguished among the Placentalia including man, — an earlier in which the nutrition is mainly embryotrophic, and a later in which the nutrition is mainly by diffusion between the two circulations. In the woman it is believed that the disappearance of morning sickness is coincident with the complete formation of the placenta. See, *e. g.*, Wood, Washington medical annals, July, 1908.

balance at a certain stage of the gestation in nearly every case of pregnancy in the dog so far studied fits in well with the hypothesis that such enzymes are at work, and that their influence may extend into the whole maternal system, causing the destruction or "mobilization" of more proteins than are needed by the embryos.

Explanation of the plus balance in the latter half of pregnancy is a much simpler matter. This condition has been found in the last three weeks at least of every case yet investigated which has reached term. Bar was the first to show that the retention of nitrogen for this period is parallel with the needs of the embryo, and Hoffström found that the same was true in his single case also for the retention of phosphorus, sulphur, calcium, and magnesium, although a large quantity of each substance was not delivered at birth, but was retained by the mother's own body as a "reserve fund."

It is to be supposed that, as growth proceeds and more and more protein materials are diverted from the maternal system to the fetal

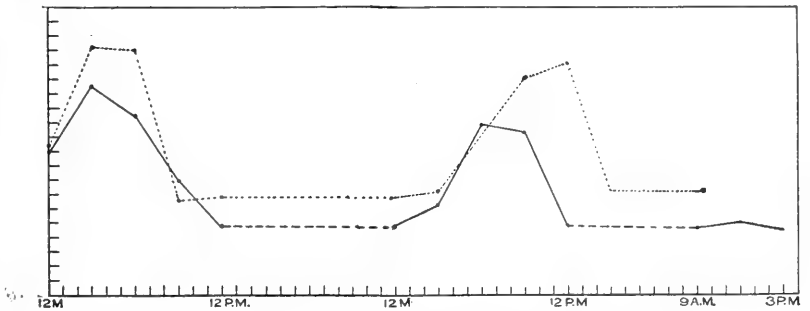

FIGURE 2. — The daily curves of nitrogen excretion in the urine of the Dog A, in the last week of fourth pregnancy (unbroken line) and in sexual rest (dotted line). In each case 8.9 gm. N were fed. The abscissæ indicate tenth grams of nitrogen, and ordinates are hours.

system, the maternal tissues react by holding back the nitrogenous bodies which otherwise would find their exit from the body mainly as urea. The question arises whether the rate of excretion of the total nitrogen, that is, its daily curve, would be affected by the presence of the embryo. To determine this point Dog A in the last week of her fourth pregnancy (Experiment IV), and while on the same diet as had been used up to the sixth week, was catheterized every three hours for twelve hours immediately after feeding on both January 11

and 12, 1910. On January 14 five puppies were born. The experiment was repeated in every particular after the dog had been several days on the diet again on March 24 and 25, two weeks after lactation had ceased. The results are plotted in the two curves represented in Fig. 2.

It is seen that the curve for the pregnant condition runs below that for the non-pregnant condition at a fairly uniform distance. In other words, the amount of nitrogen diverted to the embryos from a given diet is about the same from hour to hour.[35]

The curve is also significant in that it shows that the great bulk of the daily intake of nitrogen is removed by the action of the liver and kidneys within from nine to twelve hours, whether the animal be pregnant or not. This result obviously favors the idea of frequent small meals of protein for the pregnant organism rather than one large meal.

Summary and Conclusions.

1. The nitrogen balance was followed through two complete periods of gestation beginning with copulation, through parts of two others beginning several weeks previous to menstruation, and through one period of menstruation which was not followed by copulation.

2. In the first two experiments the diet contained 70 cal. and 0.65 to 0.75 gm. N per kilogram per day. From the first pregnancy one puppy was born, and the result was a net gain of 8.69 gm. N to the mother's body. From the second four puppies were born, and the result was a net loss of 55.6 gm. N from the mother's body. Up to the fourth week in these two experiments (on different dogs) the amounts of nitrogen lost from the mother's body were proportional to the weights of puppies delivered.

3. The effect of the menstruation is to cause a retention of nitrogen, which may be explained, in part at least, as a compensation for the amount of blood lost.

4. The results of these experiments support the idea that nitrogen loss from the mother's body is characteristic of the first half of normal pregnancy in the dog, particularly of the third and fourth weeks.

[35] See Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, p. 126.

This is probably due to the action of proteolytic enzymes produced by the embryo and not yet limited by the placenta in their action to the maternal blood.

5. Nitrogen retention has been found in these, as in all other experiments, in the last half of the pregnancy. The curve of nitrogen elimination in the urine shows that the retention in the last week of pregnancy is fairly even from hour to hour.

ON THE NERVOUS MECHANISM OF THE RIGHTING MOVEMENTS OF THE STARFISH.

By A. R. MOORE.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

IT was first noted by Romanes [1] that the detached arm of the starfish, when placed upon its aboral side, spontaneously righted itself. Afterward Loeb [2] showed that if the oral nerve ring be cut in two places, all the arms attach to the bottom, so that the animal is not able to right itself. Loeb concludes from this experiment that the righting of the normal starfish is possible because inhibiting impulses set up by the arms which have the securest attachment pass through the oral nerve ring and cause the attaching of the remaining arms to be inhibited.

Opposed to Loeb's analysis is the "trial and error" hypothesis advocated by Jennings and others. According to this hypothesis the starfish possesses a sort of consciousness whereby the animal is enabled to co-ordinate its movements so as to accomplish a desired act; all movements not contributing toward the success of the animal's purpose are sooner or later abandoned as "errors," thus allowing only "successful" trials to persist. In support of such a view Jennings [3] has attempted to show experimentally that the starfish can learn by experience to right itself in a certain way. The following quotation from this writer indicates clearly the point of view taken by the "trial and error" school of naturalists. Speaking of the efforts of an inverted starfish to right itself, he says: "As soon as these one or two arms have been successful the others cease their efforts; the attached arms turn the body over. If all the arms attempted to turn the body over at the

[1] Romanes: Jellyfish, starfish, and sea urchin, New York, 1898, p. 294.

[2] Loeb: Comparative physiology of the brain, New York, 1900, p. 63.

[3] Jennings: University of California publications, Zoölogy, November, 1907, p. 156.

same time, in other words, if there were no way of *recognizing*[4] success in the trial, the animal could not right itself."[5]

Georges Bohn,[6] however, has called attention to the significant fact that old and mature starfish make a great many more "trials and errors" in the act of righting than do the young starfish of the same species; whereas if the "trial and error" hypothesis were true, facility should be the product of experience, and the old starfish should be more expert than their offspring in the common act of turning over. The truth of Bohn's observation is apparent to any one who has worked with starfish of various sizes.

For the purpose of gaining more light on this question I have made observations on a large number of Asterina miniata and Asterias ochracea. I find that there is a common or normal way in which a starfish rights itself.[7] For example, in Fig. 1, any two arms, say *A* and *B*, face each other ventrally and attach; *C* and *E* may have obtained a hold, but soon withdraw their tube feet, rise orally, pulling *D* with them, and thus complete the somersault. During this process *D* may either remain passive or hold to the bottom with its tube feet and so retard the righting. Taking Loeb's view that *C* and *E* have their attaching inhibited by impulses sent from *A* and *B* and move upward at once, it is significant that *D* is affected by such impulses very slightly if at all. Repeated observations of the passiveness of *D* led me to think that the inhibiting impulses arising from *A* and *B* lose so much in effectiveness, as they travel away from the point of origin, that they affect only adjacent arms sufficiently to cause movement. That is to say, an inhibiting impulse from *A* rouses only *E*, and the impulse from *B* causes only *C* to act. If such were the case, we should have additional proof that the hypothesis of Loeb is correct and that the righting of the starfish is accomplished by the simplest sort of mechanism.

This rapid decrease in the effectiveness of a nerve impulse as it travels away from its point of origin is a phenomenon familiar to physiologists in the spreading of impulses, and was described by Pflüger and

[4] The italics are ours.

[5] Jennings: Carnegie Institute publications, 1904, p. 245.

[6] Bohn: Bulletin de l'Institut général psychologique, Paris, 1908, p. 95.

[7] Moore: Biological bulletin, 1910, xix, p. 237.

later by Sherrington [8] with reference to the vertebrates. According to these authors, "the degree of reflex spinal intimacy between afferent and efferent spinal roots varies directly as their segmental proximity." To illustrate, Lee,[9] speaking of the shark, says: "The power of the fins to make compensatory movements [due to the stimulation of the eighth cranial nerve] diminishes in an antero-posterior direction, the most delicately responsive [fins] being the two pectorals and the anterior dorsal. Even these do not in these respects equal the eyeballs."

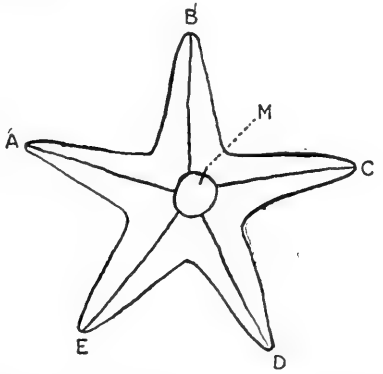

FIGURE 1.—Showing oral nervous system of the starfish.

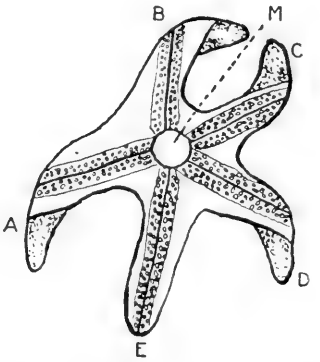

FIGURE 2.—Showing attempt at righting after cut is made.

In order to test whether such were also true of the nervous system of the starfish, I cut the oral nerve ring of one of these animals between the arms *B* and *C* at *M* (Fig. 1). In the case of a starfish so treated, if *A* and *B* face each other ventrally and attach, *E* is inhibited, but *C* either remains passive or attaches and holds to the bottom until pulled loose by *E* and *D*. In addition to *A* and *B* attaching coordinately, *C* and *D* may also attach and face each other ventrally. In this case the starfish remains for some time in the position shown in Fig. 2, until finally *E* pulls either *A* or *D* loose and thus decides the course of the righting. The time required for the righting to take place in this way is two or three times as great as that needed before the cut was made. Of course if *B* and *C* should attach and inhibit *A*

[8] SHERRINGTON: The integrative action of the nervous system, New York, 1906, p. 158.

[9] LEE: Journal of physiology, 1894, xv, p. 321.

and *D*, then the righting would be accomplished as in the normal starfish. Likewise, if *A* and *E* or *E* and *D* attach co-ordinately, their connection with the adjacent arms is unimpaired and the righting is performed in the normal way. But when the nerve ring is cut at *M*, if *A* and *B* attach co-ordinately, *C* is inhibited very slightly or not at all and does not rise orally to assist in the righting as in the case of the uninjured animal; on the contrary, *C* acts as an independent arm and renders no assistance by co-ordinated movement.

Numbers of control experiments have been performed in which the cut between the arms was made from the outside so as not to injure the oral nerve ring. In these cases the co-ordination of the arms was not interfered with in the least, and the righting was accomplished in normal time. This shows that the interference in co-ordination caused by making one cut in the oral nerve ring is not due to general injury to the animal.

The experiment was recently given to a class of eighteen students in nerve physiology, to perform. The material consisted of small specimens of Asterias ochracea. Although the students had no idea of the result expected, their observations entirely harmonized with those which I had previously made.

In making the one cut in the nerve ring of the starfish, none of the arms of the animal are isolated nervously, all are connected; but instead of the old circular connection there now obtains a linear system comparable to the nerve cord of worms and crustaceæ. If conscious judgment ever were present in the starfish, it scarcely could have been destroyed by our operation, because each part of the animal still has perfect nervous connection with every other part. The fact that the arm *C* does not co-ordinate with the active arms proves that direct nervous connection across the cut is necessary for co-ordination; that *C* can only be caused to move "intelligently" by an impulse from an adjacent arm. This is different from saying that the co-ordinated movements of the starfish are due to judgment exercised by a "psychoid" entity. Hence it is evident that there is no nervous centre in the starfish, for any one of the five arms may give rise to impulses which strongly affect only adjacent arms. These impulses rapidly diminish in strength as they travel away from their point of origin, and their effect is usually imperceptible in the movements of other than adjacent arms. The simple nervous mechanism herein described is suffi-

cient to account for the co-ordinated righting movements of the starfish. The hypothesis which seeks to account for the co-ordinated movements of the starfish by postulating judgment, decision, entelechy, etc. for that animal is unnecessary, and since each arm is capable of initiating impulses which bring about co-ordination, the hypothesis of a psychoid unity or centre for the starfish is untenable.

In conclusion, I wish to thank Professor Maxwell and Dr. Burnett for valuable criticisms and suggestions.

THE EFFECT OF LESIONS OF THE DORSAL NERVE ROOTS ON THE REFLEX EXCITABILITY OF THE SPINAL CORD.*

By CLYDE BROOKS.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Introduction.

PROFESSOR CARLSON, after some preliminary experiments in which he observed, especially in the dog, temporary depression of the crossed reflexes following transection of the dorsal nerve roots, asked me to determine whether the changes in the reflex excitability of the cord following lesions of the dorsal nerve roots were related in any way to those following the transection of the cord itself.

After a somewhat prolonged investigation extending over most of the species of animals commonly employed in laboratory experiments and also including some that are not ordinarily used, the results indicate that there is a parallelism between the effect upon reflex excitability following transection of the dorsal roots and that following transection of the cord itself. In all those animals which show depression of the reflex excitability of the spinal cord following transection of the cord, the transection of the dorsal roots is followed by a depression comparable in amount and duration. Further, some animals which are usually said to show no spinal shock do show it in a slight degree when more delicate methods are employed for its detection; and they also show a parallelism between the amount of depression caused by section of the cord and that caused by section of the dorsal roots.

These results are of interest because they bear upon the mechanism of spinal shock; for if section of the dorsal roots alone may cause

* A preliminary report was published in Science,[1] and a brief report was also made before the American Physiological Society at the Baltimore meeting in December, 1908.

phenomena simulating those of spinal shock, doubt is cast upon the hypotheses which assume that spinal shock is due only to the interruption of the reflex arcs themselves; section of the dorsal roots does not transect the reflex arcs of the crossed reflexes, and yet apparently produces shock.

Statement of Present Opinions.

It is very well established that transection of the dorsal roots in the frog,[2] dog,[3] or monkey [4] is followed by a decrease in reflex irritability of the spinal cord and loss of tonus of the limbs. But depression does not invariably follow section of sensory roots. For example, Merzbacher [5] has shown that there is good tonus and movement of the tail of the dog after section of the sensory roots of the cauda equina; and Trendelenberg [6] has found that there is a slight increase in tonus and reflex irritability of the pigeon's wing after section of its sensory nerve roots.

At a very early period spinal shock was known to occur in adult animals [7] and to be absent in very young animals.[8] At present, while there is very good agreement as to the occurrence of spinal shock, there is a good deal of difference of opinion as to the mechanism of it. The Goltz view [9] seems to be largely superseded by the view that spinal shock is the result of disruption of nervous connections between the distal segment of the cord and the higher parts of the central nervous system.[10] As to the functions of these connections, nothing is directly known. Some [11] are inclined to what has been called the "anatomical view," [12] which supposes that normally the reflexes travel with less resistance through the upper part of the cord and central nervous system, and that transection of the cord breaks the normal reflex paths; while others [13] take the "functional view," [12] which supposes that the severance of connections of the distal segment of the cord from its higher relations either leaves it bereft of influences which normally tend to maintain the normal state of tone or reflex irritability, or else that it initiates processes in the lower segments which cause depression of its tone and irritability.

More detailed explanations have been suggested for the functional view. Moore and Oertel [12] regard spinal shock as the result of the removal of higher regulatory influences which are normally exercised

upon the cord by the higher parts of the central nervous system. Von Monakow [14] has suggested that a "Diaschizis" takes place between his "Schaltzellen" and blocks the impulses. To Sherrington [15] spinal shock suggests a loosening of the nexus between links of the mechanism composing an arc; a defect in transmission at the synapse. According to Munk's conception [16] the irritability of the motor cells is constantly kept up to the normal height by two influences: one, the "centrogenous," is from the central nervous system; the other, the "neurogenous," is from the periphery *via* the sensory roots. According to this, transection of the cord cuts off the centrogenous influence, which results in loss of reflex irritability, or spinal shock. Recovery occurs by the gradual restoration of reflex irritability through the medium of the neurogenous influence.

Babák [17] has made a systematic investigation of spinal shock in the frog and has considered the problem from the view-point of ontogeny and phylogeny. He found spinal shock absent in the tadpole and developing gradually during and after metamorphosis until in the adult frog it reaches the maximum. He also found that there is more shock when the transection is made in the upper part of the cord, especially at the level of the first or second vertebra, and less when made in the lower part, especially the level of the fourth to the sixth vertebra. These results are very difficult to harmonize with the anatomical view. Pike [18] also has made studies from this view-point, and has questioned Babák's conclusions. Babák, however, considers that Pike's criticism is due to misunderstanding his position.[19]

Experimental Methods.

In all the species examined, the same general plan of experimentation was followed. The animals were fixed or suspended in some suitable way while the reflexes were elicited by mechanical, thermal, or electrical stimulation. In most of the animals fine wire electrodes were fastened to the skin of one limb and connection made with an induction machine so that electrical stimuli could be applied to the skin. After obtaining the normal reflex reactions the cord was transected high up and the reflexes measured from time to time to note whether there was any change in excitability. After recovery from the effects of transection of the cord the dorsal roots of the limb oppo-

site to the one to which the wire electrodes were attached were transected. If the electrodes were attached to the right hind leg, the dorsal roots were transected on the left side of the lumbar enlargement of the cord. After section of the dorsal roots the reflex irritability was noted from time to time until recovery had occurred. Modifications were made in order to adapt the plan to certain species. In the frog and the turtle, which were the species most used, the graphic method was also employed, and the stimuli were thrown in by an automatic key connected with a metronome. The following are the various species employed: dog, cat, rabbit, guinea pig, chicken, pigeon, alligator, snapping turtle, terrapin, large bullfrog, small leopard frog, and necturus. Some of these (cat and dog) were studied in the very young as well as in the adult animal. Usually ten or twelve animals of each species were used, but the number of snapping turtles was about sixty, of frogs about thirty, and of pigeons about twenty.

Experimental Results.

The dog. — As mentioned in the introduction, it was Professor Carlson's results especially on the dog that led to this research. Other experiments have confirmed the first results showing that section of the dorsal roots in the dog is followed by temporary depression of the crossed reflexes, lasting for an hour or more. This depression is comparable to that caused by transection of the spinal cord itself.

The dogs were anæsthetized with ether and the spinal cord exposed and transected in the lower cervical or the upper dorsal region. The dorsal roots were exposed on one side of the lumbar enlargements of the cord. The reflexes were tested at intervals before and after the operation. After recovery from the preliminary operation the wound was reopened and several dorsal roots cut, and the cross reflexes were tested before and after the operation.

The following abbreviated protocol shows the result of such an operation on an adult dog:

September 18, 1908. — 11.00 A. M. Small female dog in good flesh and vigor. Reflexes normal.

11.10 A. M. Anæsthetized with ether. Exposed the last thoracic and first three lumbar dorsal nerve roots on the right side. Closed the wound.

11.25 A. M. Transected the cord in the upper dorsal region. Removed the anæsthetic and dressed the wounds.

11.35 A. M. No reflexes obtained by pinching or striking the hind legs. Legs hang limp and feel soft and relaxed.

1.10 P. M. Cross reflexes on pinching toes or tail are good. Same side reflexes also good. Placed dog in hospital.

September 19, 1908. — 10.45 A. M. Placed dog on its abdomen with its hind legs hanging over the edge of the table. Removed dressing. Wound in good condition. Opened wound. Exposed four dorsal roots.

11.20 A. M. Reflexes good when tested by pinching or striking hind legs or tail.

11.30 A. M. Cut four dorsal roots on right side (last thoracic and first three lumbar).

11.40 A. M. No crossed reflexes; only local contraction of muscle caused by direct stimulation with electricity or sharp blow. Muscles appear to have lost tone.

12.15 P. M. Apparently a little more tone in muscles now. The left hind leg contracts on striking or pinching it hard.

12.40 P. M. There is some slight movement in the right leg on striking or pinching the left sharply.

1.15 P. M. Good cross reflex (movement of right leg) on pinching and on striking the left leg.

1.35 P. M. Reflexes still improving. Tonus returning.

In the experiments on the dog the depression of the cross reflexes was much the same whether caused by section of the cord itself or by section of the dorsal roots. The results of similar experiments upon the young puppy are given later.

The cat. — The methods used in the experiments upon the cat were similar to those used upon the dog. The cats were etherized, the spinal canal was opened, and the cord exposed. The cord was then transected in the thoracic region and the wound closed. The reflexes were observed from time to time. After recovery on the following day, or later, the wound was reopened and the several dorsal roots were transected, and the effect upon reflex excitability noted by pinching or striking the hind legs and tail, and also by stimulation with the induced interrupted electrical current. The following notes from one of the protocols shows the chief feature of the results:

September 13, 1908. — Young adult female cat.

2.20 P. M. Reflexes normal. Etherized.

2.25 P. M. Opened spinal canal. Exposed dorsal roots in lumbar region.

2.27 P. M. Transected cord at the level of the last cervical or the first thoracic vertebra.

2.30 P. M. Closed wound. No reflexes by pinching or striking. Hind legs limp.

2.35 P. M. No reflexes. Legs limp.

3.05 P. M. Electrical stimulation of hind legs causes no movements.

3.10 P. M. Slight crossed reflex on electrical stimulation of the left hind foot.

3.15 P. M. Crossed and homolateral reflexes good. Tonus in legs improved. Stimulation of one hind leg causes toes of other hind leg to clench.

7.00 P. M. Good reflexes by pinching, striking, or stimulating with electricity. Legs in very poor tonus. Put animal in hospital in good condition.

September 14. — 8.00 A. M. Cat in good condition; mews and purrs. Pinching its tail causes lively kicking in both hind legs. Picking up one leg causes kicking. Pinching toes causes several successive kicks in that leg and one or two of the opposite hind leg. Crossed and homolateral reflexes more readily elicited than normally.

September 15. 8.00 A. M. Condition of cat remains good.

September 16. — 2.30 P. M. Condition of cat good.

2.35 P. M. Removed dressing from lumbar wound. Wound clean. Reopened wound and exposed dorsal roots.

3.15 P. M. Cut five dorsal roots on the right side at level of the lumbar enlargement.

3.20 P. M. No crossed reflexes; cat lies still, breathes regularly. No crossed reflexes from pinching or striking the left hind leg.

4.05 P. M. Reflexes have returned. Pinching tail causes kicking of both legs. Pinching left leg causes right to kick.

5.00 P. M. Crossed and same side reflexes are stronger.

6.00 P. M. Crossed and same side reflexes good.

From these experiments we find that in cats there is temporary depression of the crossed reflexes, either by transection of the cord or by transection of the dorsal roots, but the depression is not so long continued as in the dog.

The results of observations on the young kitten are given later.

The frog. — A large number of experiments have been made upon the frog, partly because it has long been the object upon which such research has been made, and also because the frog shows more shock than most of the other cold-blooded animals. In these experiments very large Indiana bullfrogs were most frequently employed, but the ordinary leopard frog was also used a good deal. The experiments were conducted on the same plan as those described above for the dog and the cat. The reflexes were tested from time to time during the experiment by pinching with forceps or striking with some light instrument. Then transection of the cord was made. After recovery from this operation the dorsal roots of one hind leg were transected. It was found that transection of the cord in this species resulted in depression of the reflexes lasting some minutes, also that section of the dorsal roots from one hind leg was followed by a similar temporary depression of reflex irritability. These results are in accord with those of most of the writers mentioned above.

The following brief protocol shows the effect upon the cross reflexes of section of the dorsal roots of the large Indiana bullfrog:

July 28, 1909. — Large specimen in excellent condition. Reflexes normal.

3.00 P. M. Opened spinal column. Exposed dorsal roots of left hind leg. Reflexes still good.

3.07 P.M. Cut four dorsal roots on left side of lumbar enlargement. Left leg hangs limp.

3.08 P. M. No cross reflexes.

3.13 P. M. Cross reflexes very slight.

3.19 P. M. Good cross reflexes.

3.27 P. M. Cross reflexes good.

This experiment shows depression of the cross reflexes lasting for almost twelve minutes before complete recovery following transection of the dorsal roots. Transection of the cord itself in other specimens gave similar results.

The alligator. — A number of observations were made upon alligators which were about 75 cm. long. They were suspended in a sling and their reflexes tested by pinching or striking, and also by applying fine wire electrodes to the skin of the thigh and stimulating with interrupted induced current.

In normal intact alligators it was difficult to distinguish the exact

strength of stimulus just sufficient to give a distinct cross reflex, because the animal apparently inhibited all responses until a certain strength of stimulus was reached, when it quickly began to flounder and struggle vigorously. But after transection of the cord the cross reflexes were more readily obtained. It was further observed that these animals were more susceptible to hemorrhage or asphyxia than were turtles or frogs. In some of the experiments when hemorrhage was profuse, in the first ten or fifteen minutes there was a heightening of reflexes, probably due to anæmia or asphyxia, followed by a gradual persistent depression which ended in complete disappearance of reflexes in about seventy to eighty minutes.

The following extracts from a protocol show the slight depression caused by section of the cord or dorsal roots:

April 14, 1909. — Young alligator 70 cm. long. Lively and vigorous.

9.00 A. M. Applied the electrodes to the skin of the posterior part of the thigh of the left hind leg.

9.31 A. M. Coil at 16.5 cm. No movement.
Coil at 14.5 cm. Flexion of same leg (left).
Coil at 8.0 cm. Vigorous general movements.

9.50 A. M. Transected cord just below line drawn across the back at the anterior border of scapula.
Coil at 12.9 cm. gave reflex on same leg.
Coil at 8.0 cm. gave reflex on same leg.
Coil at 7.0 cm. gave general movements.

9.55 A. M. Retransected cord at 2 cm. below first transection.
Coil at 12.4 cm. gave reflex same side.
Coil at 8.3 cm. gave cross reflex to right leg.
Coil at 6.8 cm. gave general movements.

After recovery, section of the dorsal roots was followed by a corresponding temporary depression of the cross reflexes.

The chicken and the pigeon. — These two species may well be classed together, as they gave very similar results. Experiments upon these have been made by the simple method of pinching and striking the toes or the abdomen and observing the resulting movements; then transection of the cord under light ether anæsthesia, or under local anæsthesia, or in some cases (the chicken) the bird was quickly decapitated without any anæsthesia and artificial respiration employed.

The result of such experiment has been that there is no apparent depression of reflexes when tested by the method, whether from transection of the cord or by section of the dorsal roots. In fact, there often seemed in the pigeon to be increase in irritability.

Necturus. — The experiments upon the necturus were made by observing the reflexes obtained by pinching or striking or stimulating by interrupted induction shocks the limbs, the body, or the tail of the animal, and then making high section of the cord. The observations by these methods showed no change of reflex irritability so long as the general condition of the animal remained good.

The kitten and the puppy. — As noted by many other observers in very young animals, no depression of reflex irritability occurs upon transection of the cord or dorsal roots, and indeed when ordinarily tested this appears to be the case; but when measured stimuli are used even in the very young puppy or kitten there is observed some distinct though very slight temporary depression of the reflexes.

In these experiments the electrodes were applied to the skin of the ball of the foot of one hind leg. After measuring the stimulus just necessary to cause a distinctly perceptible reflex on the same side and also that just sufficient to produce a reflex to the opposite hind leg, transection of the cord was performed. After allowing time for recovery the dorsal nerve roots were exposed and transected on the side opposite the leg with the wire electrodes applied to the feet. As the kitten and the puppy gave very similar results, they may be considered together.

The following abbreviated protocol shows the results of such experiments upon a kitten:

March 26, 1909. — Young maltese kitten about nine days old.

2.05 P. M. Intact animal; gave cross reflexes with secondary coil at 11.65 cm.; at 10 cm., strong crossed reflexes.

2.20 P. M. Etherized kitten and exposed cord. After allowing thirty minutes for recovery from the anæsthetic the reflexes were again tested.

2.55 P. M. Coil at 11.5 cm. gave very slight cross reflex.

Coil at 11.0 cm. gave stronger cross reflex.

3.05 P. M. Transection of the cord in the upper thoracic region. Kitten kicks vigorously with both hind legs for a few seconds, then legs hang limply down.

3.07 P. M. Coil at 9.5 cm. gave slight cross reflex to tail.

Coil at 9.0 cm. gave distinct cross reflex to right hind leg.

3.23 P. M. Coil at 10.0 cm. Cross reflex.

3.48 P. M. The dorsal roots on the right side of the lumbar enlargement cut. As soon as possible after this the reflexes were tested as before.

3.50 P. M. Coil at 9.0 cm. Distinct though very slight cross reflex.

Coil at 8.0 cm. Strong cross reflex.

4.10 P. M. Not quite complete recovery.

The rabbit and the guinea pig. — Since the results obtained on rabbits and guinea pigs are very similar, they may be reported together. The cord was either transected under local anæsthesia and afterwards the dorsal roots sectioned, or else the animals were decerebrated under ether and transection of the cord and of the dorsal roots made after recovery from the first operation. The reflexes in these animals are only slightly and temporarily depressed by section of the cord or dorsal roots. It was only by using delicately graduated strengths of stimuli that the depression was noticeable.

May 15, 1909. — 3.00 P. M. Guinea pig of large size and in good condition.

3.25 P. M. Coil at 10.8 cm. Cross reflexes.

3.38 P. M. Transected cord. Ethyl chloride anæsthesia. Apparently good tonus in legs immediately after cutting cord.

3.39 P. M. Coil at 10.1 cm. Homolateral reflex.

3.45 P. M. Coil at 9.5 cm. Crossed reflex.

After recovery three dorsal roots on the right lumbar enlargement were transected. This was followed by a similar temporary depression of cross reflex irritability.

Experiments on the rabbit gave very similar results. There appeared to be very slight loss of tonus as judged by the position and resistance of the limbs in handling. There was a slight but distinct temporary depression of irritability as judged by the strength of interrupted induction current necessary to cause a cross reflex.

The turtle. — It is upon the turtle that the greatest number of our experiments have been performed. More than sixty large lake snapping turtles and a half dozen terrapin have been used. In the snapping turtle, as in the kitten and puppy, the earlier experiments which were made by pinching or striking the legs and observing the resulting reflex movements, no depression was discernible after tran-

section of the cord or section of the dorsal roots. But later, by using delicately graduated electrical stimuli, a distinct though slight fall in cross reflex irritability was observed. These experiments were made at room temperature. The stimuli were from an induction machine with about fifteen to thirty interruptions per second. The stimuli were made by a key operated by hand and were one second in duration, as measured by a second pendulum and a stop watch. The following abstract of one of the protocols illustrates the results:

March 17, 1909. — Large lively snapping turtle. Room temperature, 27° C.

8.39 A. M. Intact animal.
Coil at 9.0 cm. Homolateral reflex.
Coil at 8.6 cm. Cross reflex.
8.42 A. M. Transected cord just below the medulla oblongata.
8.44 A. M. Coil at 8.0 cm. No response.
8.45 A. M. Coil at 7.0 cm. Cross reflex.
8.48 A. M. Coil at 8.5 cm. Homolateral reflex.
9.16 A. M. Coil at 9.0 cm. Homolateral reflex.
Coil at 7.0 cm. Good cross reflex and general movements.
Placed in ice box, leaving electrodes attached to legs.

March 18. — 10.00 A. M. Coil at 8.6 cm. Homolateral reflex.
Coil at 8.0 cm. Crossed reflex.
10.17 A. M. Cut seven dorsal roots to right side of lumbar enlargement.
10.20 A. M. Coil at 7.0 cm. No reflex movements.
10.23 A. M. Coil at 1.0 cm. No reflex movements.
10.26 A. M. Coil at 0.0 cm. Homolateral reflex.
10.41 A. M. Coil at 5.0 cm. Homolateral reflex.
10.45 A. M. Coil at 8.5 cm. Homolateral reflex.
Coil at 8.2 cm. Crossed reflex.

A series of experiments was performed upon the turtle based upon the above method, but registering the reflex movements graphically, and also using the key connected with a metronome in order to obtain more exact duration of the interrupted electrical current.

In beginning the experiment the normal turtle was placed upon the stand and rigidly clamped in position. Each hind leg was attached to its writing lever and its electrodes. The levers were adjusted to the smoked drum so that the right leg traced about 3 cm. from the top of the drum while the left leg traced about 3 cm. below that. Flexion of

the hind limbs caused a downward stroke of the writing points. When both hind legs were completely relaxed and quiescent, a signal magnet which indicates the period of stimulation was adjusted so that it was on a level with the base line of the right leg, and a Jaquet instrument marking time in seconds was adjusted so that it traced the base line for the left leg. By this arrangement there were recorded on the drum the homolateral and cross reflex movements and tonus changes of the normal intact turtle. The tracings showing the effects of decapitation and of section of the dorsal roots were recorded and compared with the normal. After decapitation a syringe bulb with tracheal cannula and T-piece was connected with the trachea of the turtle and artificial respiration was given. The spinal cord was cut across with a narrow sharp chisel so that very little blood was lost. Later, after the effects of transection of the cord were recorded on the drum, the dorsal roots of the right hind leg were transected and the effect upon the reflex irritability of the cord recorded on the drum. The results of this method agree with those obtained where the stimulation was regulated by hand and the resulting reflex movement judged by the eye.

These tracings also show the changes in tonus of the muscles of the hind limbs that accompany the changes in irritability.

Summary.

The above results as a whole show a parallelism between the changes in the reflex irritability of the cord following transection of the spinal cord itself, and those following transection of the sensory nerve roots of the cord. The dog, cat, and frog show a marked depression following either of these operations. The alligator shows some depression. The rabbit, guinea pig, pigeon, chicken, or the turtle, shows no depression when tested roughly by observing the reactions following pinching, or striking, or mechanical, electrical, or thermal stimuli. Neither do very young kittens or puppies show any shock when tested by these methods. But when the turtle, kitten, and puppy are tested with more delicate methods, using carefully graduated stimuli and avoiding all sources of error possible, they do show some slight but distinct depression following the transection of the spinal cord or section of the dorsal roots. Very probably other species would show similar results.

From the experiments so far performed the changes in tonus that accompany the above findings are not clear. At present there is some evidence that a change in tonus always accompanies the decrease in irritability of the spinal cord, and that this change is usually a decrease; but in some instances it is an increase of tonus.

These results tend to strengthen the view of Von Monakow, Sherrington, Moore and Oertel, Babák, Munk, and others, who hold that spinal shock is due to the functional separation of the cord from the higher structures; and to cast doubt upon the theory that spinal shock is due solely to the anatomical severance of the reflex arcs directly involved.

This influence of the higher parts of the central nervous system and sensory roots upon the spinal cord at present appears to be most satisfactorily designated as regulatory, that is, a sort of balancing of excitatory and inhibitory influences. According to this view, in the intact animal the cord is normally in a state of relative tonic equilibrium; when the cord is transected or when a sufficient number of dorsal roots are cut, this state of relatively stable equilibrium is temporarily disturbed, resulting in more or less depression of reflex irritability of the spinal cord, that is, spinal shock.

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[17] BABÁK: Archiv für die gesammte Physiologie, 1902, xciii, p. 134; *Ibid.*, 1905, cix, p. 78; Zentralblatt für Physiologie, 1907, xxi, p. 9; *Ibid.*, 1907, xxi, p. 513.

[18] PIKE: This journal, 1909, xxiv, p. 124.

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A QUANTITATIVE STUDY OF FARADIC STIMULATION. — V. THE INFLUENCE OF TISSUE RESISTANCE AND OF KATHODE SURFACE ON STIMULATING EFFECTIVENESS.

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IN the beginning of this investigation [1] the resistance of the secondary circuit was set down as one of the variable factors to be considered in determining the stimulating values of induced currents. At that time, however, the tentative assumption was made that break induction shocks are not modified in efficiency by alterations in secondary resistance. This assumption [2] was based upon certain experiments of Helmholtz,[3] and has been shown by the work of Hoorweg [4] and Gildemeister,[5] as well as by the experiments reported in this paper, to be erroneous. For the earlier parts of the investigation it was immaterial whether or not the secondary resistance affects the values of stimuli; therefore this phase of the problem was put over till the other phases had been considered.

Inasmuch as the research of which this paper is a part is primarily an attempt to increase the practical usefulness of faradic stimuli, and concerns itself only incidentally with the theoretical considerations which arise in connection with their study, the questions to be asked with regard to the influence of the secondary resistance are: (1) In what direction does this influence manifest itself, and is it measurable? and (2) Is it so extensive that it must always or often be taken into account? This latter question is one of great practical

[1] Martin: This journal, 1908, xxii, p. 72.

[2] See Martin: *Loc. cit.*, p. 118.

[3] Helmholtz: Poggendorf's Annalen der Physik und Chemie, 1851, lxxxiii, p. 536.

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bearing, for it must be admitted at once that if the influence of the secondary resistance has to be taken into account in every quantitative use of faradic stimuli the difficulty of such use becomes very great, and will often be found prohibitive.

The subject matter of this paper falls naturally into two divisions: the first dealing with the measurement of the influence of the secondary resistance, and the second with the question of how important it is to take this influence into account, and in what classes of work, if any, it may be disregarded.

The Measurement of the Influence of Secondary Resistance.

The relation of tissue resistance to secondary resistance as a whole. — The secondary circuit usually has a comparatively high resistance. Most inductoria used in physiological laboratories have secondary coils with resistances mounting into hundreds of ohms, and the resistances of the tissues undergoing stimulation are usually high likewise. In numerous determinations of the resistance of stimulated tissues I have met with only one or two under 1000 ohms and have found many exceeding 50,000 ohms.

Since it has been shown conclusively that the stimuli imparted by faradic currents as well as those of galvanic origin arise from the kathode,[6] and since the resistance of the physiological kathodes must be small in comparison with that of the whole mass of tissue traversed by the current, we are justified in considering tissue resistance as external to the actual seat of stimulation, and need make no distinction between this and the other resistances that may be included in the secondary circuit.

The method of experimentation. — In studying the influence of secondary resistance experimentally the usual procedure has been to introduce known, non-inductive resistances into the secondary circuit and to observe the effect of their introduction upon the stimulating value of the shocks sent through the circuit. As a check upon this method some experiments were performed in which different amounts of tissue were included between the stimulating electrodes,

[6] Chauveau: Journal de la physiologie, 1859, ii, pp. 490, 553. See also Biedermann: Elektrophysiologie, Jena, 1895, ii, p. 622.

and thus the resistance of the tissue itself was varied. This latter method is of course less certain than the former, since the inclusion of more or less tissue in the circuit may mean a variation in the number and irritability of the physiological kathodes involved.

Tissue resistances were determined by means of an ordinary wheatstone bridge according to the Kohlrausch method, using an alternating current to avoid polarization, and a telephone in place of the galvanometer. The average of three readings was always taken. This procedure, in the hands of one experienced in its use, gives results accurate within 4 or 5 per cent, a degree of accuracy sufficient for the purposes of this inquiry.

The measure of stimulating effectiveness was the same as in the earlier parts of this research, namely, the stimulus required to produce a minimal contraction in a frog's leg muscle, stimulated either directly or indirectly. Break shocks were used throughout this part of the work and the expression for the value of the stimulus is Z, determined from the formula,[7]

$$Z = \frac{M}{L} \times I. \quad (1)$$

The effect upon the stimulus of varying the secondary resistance. — The effect upon the value of Z of varying the secondary resistance is shown in two representative experiments cited in Table I. As appears from this table, stronger stimuli are required to produce a given physiological effect when the secondary resistance is high than when it is low. That there is a definite mathematical relationship between the effectiveness of the stimulus and the secondary resistance is shown by plotting these values as a curve. Such a curve for the first experiment of Table I is given in Fig. 1. It is virtually a straight line having the general equation

$$Z = \frac{\beta R + A}{A}, \quad (2)$$

in which Z is the intensity of the shock required at resistance R to produce the desired effect, and β and A are constants. This formula has been found to hold in every experiment, numbering more than fifty, in which it has been applied. The value of the constant β in

[7] Martin: This journal, 1909, xxiv, p. 271.

any given experiment can be determined geometrically by producing the curve to where it cuts the ordinate for zero resistance. According to Fig. 1, the value of β for the experiment of Table I from which that curve is derived is 3. Since this represents the value of Z, whose effect at zero resistance would equal that of the various other values of Z at their respective resistances, it affords a measure of the irritability of the physiological kathode where the stimulus actually arose, assuming that the resistance of such kathode is negligibly small. We have, therefore, in β an expression for the value of any stimulus as it affects the seat of actual stimulation, namely, the physiological kathode, irrespective of the resistance of the secondary circuit.

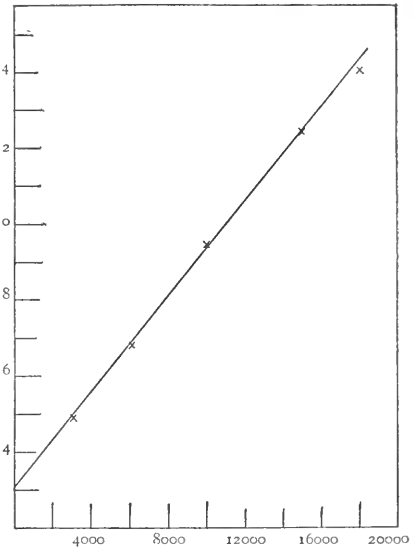

FIGURE 1. — Showing that the curve of increasing stimulus against increasing resistance is a straight line. Ordinates represent resistances in ohms; abscissæ represent values of Z.

TABLE I.

THE INFLUENCE OF SECONDARY RESISTANCE UPON THE STIMULATING VALUES OF INDUCED CURRENTS.

Experiment of Dec. 15, 1909. RESISTANCE OF SECONDARY COIL = 1400 OHMS; OF TISSUE = 1700 OHMS. TISSUE = FROG'S GASTROCNEMIUS, UNCURARIZED.					
Resistance in secondary circuit	3100	6100	10100	15100	18100
Value of Z	4.96	6.81	9.45	12.45	14.1
Experiment of March 1, 1910. RESISTANCE OF SECONDARY COIL = 1400 OHMS; OF TISSUE = 16600. TISSUE = FROG'S SARTORIUS, UNCURARIZED.					
Resistance in secondary circuit	18000	28000	48000	68000	
Value of Z	3.97	5.24	6.8	9	

By a slight transposition of equation (2) the equation for β becomes:

$$\beta = \frac{ZA}{R + A}, \quad (3)$$

and it is clear that if the value of Z for any secondary resistance is known the actual or "specific" stimulus can be calculated from equation (3), provided only the value of the other constant, A, is known. For measuring stimuli with reference to the resistance through which they are applied, therefore, there must be added to the determinations previously required not only the secondary resistance, but a constant A.

Kathode surface an important factor. — That the value of A depends upon the *surface offered* by the physiological kathode or kathodes is strongly indicated by the results of a series of thirteen experiments upon frog's leg muscles, gastrocnemius, triceps femoris and sartorius, in which the surface of the physical kathode was accurately determined. The method was as follows: The kathode was a cylindrical platinum wire of known diameter. This was thrust a measured distance into the muscle tissue. Thus the kathode surface could be calculated. In the thirteen experiments under discussion there was a direct proportionality between the kathode surfaces and the values of the constant, A, as determined from the plotted curves of the experiments. This proportionality is brought out in Table II. I do not wish to urge that these experiments show anything more than a dependence of the constant upon the kathode surface. They cannot be looked upon as establishing a simple method of determining its value. In fact in seven other similar experiments there was no marked proportionality between kathode surface and the value of A. Nor was I able in numerous experiments in which nerves were stimulated instead of muscles to get evidence of any simple relation between kathode surface and the value of A. Failure to find such proportionality regularly does not, however, invalidate the idea that there is a definite relationship of some sort, when we consider the many factors which go to determine the actual physiological kathode, of which the surface of the physical kathode is but one; and when we recall that this idea is merely the application in special form of a fact

long recognized, namely, the influence of current density on stimulation value.[8]

TABLE II.

INDICATING A DEPENDENCE OF THE CONSTANT, A, UPON KATHODE SURFACE.

Kathode surface.	Value of A.	$\frac{A}{\text{Kath. surf.}}$	Muscle stimulated.
sq. mm. 5.6	4500	800	gastrocnemius
11.2	9000	800	"
16.9	13500	800	"
13.2	10500	800	"
7.5	6000	800	"
6.0	4800	800	"
6.0	4800	800	"
7.2	5700	790	triceps femoris
4.6	3700	800	" "
8.6	6900	800	gastrocnemius
5.2	4200	810	"
1.2	1000	830	sartorius
6.0	4800	800	gastrocnemius

I know of no reliable method of determining the value of the constant, A, other than that used in this work, namely, to establish experimentally at least two values of Z for different secondary resistances, and from these values compute the value of A. This can be done by means of the equation

$$A = \frac{Z_R R' - Z_{R'} R}{Z_{R'} - Z_R}, \quad (4)$$

in which Z_R and $Z_{R'}$ are the stimuli required with resistances R and R' respectively to produce the minimal contractions used as the index.

[8] BIEDERMANN: *Loc. cit.*, i, p. 185.

The factor of secondary resistance is thus, as we see, indissolubly connected with another, depending on the kathode surface and requiring the determination of a constant more difficult to obtain than is the secondary resistance itself.

Before imposing this additional burden upon physiological experimentation we may well inquire how great errors are likely to arise in comparing faradic stimuli if these two factors are completely disregarded.

How Extensive is the Influence of Secondary Resistance and Kathode Surface?

We must recognize at the outset of this part of our inquiry that if comparisons are attempted between stimuli used under conditions of widely varying secondary resistance and divergent kathode surface, disregard of these two factors is sure to lead to erroneous conclusions; but in a majority, probably, of physiological experiments the stimuli to be compared are produced under conditions which tend to be closely similar. It is with regard to such cases as these that we may properly inquire whether the factors under consideration need be taken into account.

Successive stimulation of the same tissue. — Probably the experiments in which accurate comparisons of stimuli are most needed are those in which a given tissue is to be stimulated successively. But in experiments of this class neither the tissue resistance nor the electrode surfaces undergo noteworthy variation during the course of the experiment and so do not enter as modifying factors.

Stimulation of corresponding tissues in different animals. — Next in importance are cases in which it is desired to impart comparable stimuli to corresponding tissues through a series of experiments. Cases of this sort arise very frequently in the course of physiological research, and I have therefore given them special consideration.

Mr. E. L. Porter has been carrying on in this laboratory an investigation which involves, among other things, determining in a series of cats the threshold stimulus for producing extension of the wrist, the stimulus being applied to the deep branch of the radial nerve below the elbow; and reflex flexion of the hind leg through stimulation of the musculo-cutaneous branch of the peroneus. Here

was presented a typical example of the class of experiments described in the paragraph heading, and I therefore secured Mr. Porter's co-operation in utilizing it in the study of my problem. At my request he determined in several cases the threshold stimulus when the tissue only was in the secondary circuit, and immediately afterward, the threshold when an additional resistance of 10,000 ohms had been introduced. I was thus able in these cases to compute the value of the constant, A, and from it, to obtain the solution of the equation for "specific" irritability, $\beta = \frac{AZ}{R + A}$. In the experiments of this series, ten in all, the secondary resistances ranged from 2800 ohms to 6000 ohms, averaging 3900 ohms. The values of A ranged from 4300 to 14,000, averaging 7800. The statistics for this series are given in Table III.

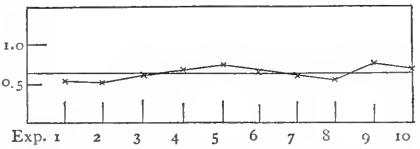

FIGURE 2. — Illustrating the relatively slight departures of individual ratios of β to Z from the average. Ordinates represent successive experiments; abscissæ represent ratios of β to Z. The horizontal line is drawn at the level of the average ratio.

Inspection of the table reveals a definite tendency of β to vary as does Z. The closeness of this tendency is brought out more strikingly, however, in Fig. 2, where the ratios of B to Z in successive experiments are plotted. The horizontal line represents the average ratio of β to Z as determined in these experiments; the variations from this line of the different actual ratios are, as is seen, relatively inconsiderable, the greatest being 18.5 per cent, the average of all slightly under 11 per cent.

Assuming the data cited in Table III to be fairly representative of the relations between β and Z that are likely to occur in experiments of the sort under consideration, to what extent are we justified in such experiments in making use of the values of Z for expressing quantitative relationships?

The figures show clearly, I think, that all except the finest relationships are revealed with sufficient exactness by the values of Z. While it cannot always be known certainly, in cases in which several nearly equal values of Z are under comparison, which will give smaller and which larger values of β; yet, if the experiments are carefully performed, one can be practically certain whenever the values of Z

differ by more than 15 or 18 per cent that the larger Z means also a larger β. In other words, the values of Z are accurate within about 15 to 18 per cent. With this degree of accuracy assured, probably the demands of most researches of this class are fully met, and all

TABLE III.

Illustrating the Tendency of β and Z to vary Similarly in Direction and Extent, Z represents the Stimulus producing just perceptible Wrist Extension in Cat. Stimulus applied to Deep Branch of Radial Nerve.

Date 1910.	Secondary resistance.	Value of A.	Value of Z.	Value of β.	Ratio $\frac{\beta}{Z}$.
Aug. 8 . . .	6000	7600	1.16	0.65	.54
July 28 . . .	4400	5000	1.33	0.71	.53
Aug. 5 . . .	4800	8000	1.60	1.0	.62
Aug. 3 . . .	3400	7800	1.80	1.25	.70
Aug. 9 . . .	3000	9800	2.3	1.76	.77
Aug. 2 . . .	4600	9600	2.52	1.7	.68
Aug. 17 . . .	2800	4600	10.6	6.6	.62
As above except that Stimulus was applied to Musculo-Cutaneous Branch of Peroneus, and Reflex Flexion of Hind Leg was Movement Evoked.					
July 18 . . .	3000	4300	1.70	1.0	.59
July 22 . . .	4000	14000	2.75	2.1	.76
July 20 . . .	3000	7000	10.3	7.2	.70
Average					.65

such may safely disregard both the secondary resistance and the kathode surfaces.

Differing from the series of experiments quoted above in that they offer wider variations in both secondary resistance and electrode surface, and therefore greater likelihood of error if these factors be disregarded, is a series of observations on frog's gastrocnemius muscle, carried out by myself. The method of stimulation was that described

in an earlier paper of the series,[9] the electrodes being platinum needles thrust directly into the muscle substance.

TABLE IV.

ILLUSTRATING TENDENCY OF Z AND β TO VARY TOGETHER. FROG'S GASTROCNEMIUS STIMULATED DIRECTLY.

Date.	Secondary resistance.	Value of A.	Value of Z.	Value of β.	Ratio $\frac{\beta}{Z}$.
Feb. 24, 1910 . . .	5400	6900	2.55	1.5	.59
Oct. 28, 1909 . . .	6500	6000	2.60	1.2	.46
Dec. 15, 1909 . . .	3100	5000	4.96	3.0	.60
Jan. 19, 1910 . . .	8400	4800	5.10	1.85	.36
Feb. 17, 1910 . . .	6800	7500	5.15	2.7	.52
Feb. 24, 1910 . . .	6400	4200	5.20	2.2	.42
Feb. 18, 1910 . . .	5000	4800	5.3	2.5	.47
Jan. 31, 1910 . . .	11400	13500	5.4	3.0	.56
Mar. 7, 1910 . . .	5400	4800	6.1	2.9	.48
Feb. 18, 1910 . . .	5500	5500	6.1	3.2	.52
Nov. 18, 1909 . . .	7700	4200	6.4	2.15	.34
Feb. 10, 1910 . . .	13000	6000	7.0	2.3	.33
Jan. 31, 1910 . . .	10400	9000	8.0	3.6	.45
Dec. 13, 1909 . . .	5000	5000	8.3	4.0	.48
Jan. 31, 1910 . . .	6200	10500	9.2	6.0	.65
Feb. 18, 1910 . . .	5000	4800	9.5	4.5	.47
Feb. 17, 1910 . . .	6000	10500	10.3	6.7	.65
Nov. 4, 1909 . . .	3200	2600	17.3	8.0	.46
Average					.49

In the series of eighteen experiments cited in Table IV the secondary resistances ranged from 3100 to 13,000, and the values of A from 2600 to 13,500. Yet, in spite of these wide ranges in the values

[9] MARTIN: This journal, 1908, xxii, p. 117.

of the factors determining the relation of Z to β, this latter relation varies to a surprisingly moderate degree. The average ratio of β to Z is .49. The widest departures from this are ratios of .33 and .65, amounting to 33 per cent in each case, while the average variation is only 15 per cent. If the experiments of Table IV represent fairly the variations in secondary resistance and kathode surface likely to be met with in experiments on frog's gastrocnemii, we can safely conclude that the values of Z, obtained in any such experiment, represent the true relative values of the stimuli used within less than one third.

In a series of ten experiments on frog's gastrocnemii stimulated through the sciatic, with resistances ranging from 6300 to 38,000 ohms, and values of A from 6000 to 23,000, the ratio of β to Z averaged .48, and the widest variation was a ratio of .28, amounting to 42 per cent, the average variation being 20 per cent. In the experiments cited in the two series above no attempt was made to keep conditions of tissue resistance and kathode surface approximately uniform. On the contrary, these conditions were purposely made to vary widely from one experiment to another. I feel, therefore, that they cover the range of variation likely to occur in ordinary experimentation.

Discussion.

The data thus far cited seem to me to show that in the hands of a careful experimenter, who will take pains to keep his conditions of stimulation as uniform as possible, quantitative results of great value may be obtained without the labor involved in taking account of secondary resistance and kathode surface. By the use of the method outlined in previous papers of this series the strengths of stimuli employed in any given case may be expressed in terms of stimulation units, and if the conditions of experimentation, such as nature of electrodes used, distance between them, and method of applying them, are carefully described, other experimenters can duplicate the stimuli very closely. Certainly this method allows comparisons of much greater accuracy than can be made by the methods of describing stimuli in vogue at the present time.

While it is true that in many investigations, in which accurate knowledge of the strengths of stimuli used is of paramount importance, careful account will have to be taken of the two factors, sec-

ondary resistance and kathode surface, yet in a far greater number of researches in which approximate knowledge of the stimuli used is all that is desired, these factors may be wholly disregarded.

Use of high additional secondary resistance. — In connection with the discussion of the influence of secondary resistance and kathode surface it is important to emphasize the fact that kathode surface is fully as influential in modifying the strength of stimulus as is secondary resistance. That this fact has not been appreciated hitherto is shown by the frequent use of a device supposed to overcome any inequality in stimulation strength due to differences in secondary resistance, namely, the introduction of a very high additional resistance into the secondary circuit, thereby making fluctuations in tissue resistance relatively negligible. That this device is perfectly adequate in experiments in which a single tissue of varying resistance is under examination is of course obvious; there being under such circumstances no variations in kathode surface. But in experiments in which different tissues are being compared the introduction of high additional resistance into the secondary circuit is more apt to be misleading than otherwise because of the cumulative effect of variations in kathode surface. The point can best be illustrated by a concrete example:

Experiment of March 7, 1910. — Frog's gastrocnemius muscle stimulated directly. In the first test the kathode was in contact with the surface of the muscle, but did not penetrate it. When the tissue only was in the secondary circuit, the total secondary resistance was 17,000 ohms. A minimal contraction was secured with a value of Z equal to 6.6. When 70,000 ohms' additional secondary resistance was introduced, the value of Z was 16.8. By calculation the value of A was found to be 24,000 and of β to be 4. In the second test the kathode was thrust directly through the muscle tissue; the secondary resistance was 5400 ohms; the value of Z was 6.1. When 70,000 ohms' additional resistance was introduced, the value of Z was 40.5. The calculated value of A was 4800, and of β 2.9. In this case the values of Z as determined with the tissue only in the secondary circuit represent much more nearly the true relationships between the stimuli than do the values as determined with a large additional resistance in the circuit. In reality the stimulus applied in the first test was stronger than in the second, whereas, if reliance were placed upon the results given when the high additional resistance was in circuit, it would appear that the second

stimulus was more than twice as strong as the first. The subjoined tabulation will serve to emphasize the error:

	Z (tissue only).	Z (70,000 ohms added).	β.
First	6.6	16.8	4
Second	6.1	40.5	2.9
Ratio of 1st to 2d	1.08	0.41	1.38

Summary.

1. This paper deals with two factors upon which the effectiveness of any faradic stimulus depends: they are the resistance of the secondary circuit and the surface presented by the physiological kathode. These are so closely interconnected that account cannot be taken of either independently of the other.

2. If it be assumed that the resistance of the physiological kathode, which is the point actually stimulated, is negligibly small, the value of the stimulus applied to this point is expressed by the equation $\beta = \frac{AZ}{R + A}$, in which β is the actual or "specific" stimulus, Z the stimulus as determined regardless of the resistance of the secondary circuit, R this resistance, and A a constant depending upon the kathode surface.

3. To determine the value of A, corresponding values of Z at different secondary resistances must be found and A computed from the formula

$$A = \frac{Z_R R' - Z_{R'} R}{Z_{R'} - Z_R},$$

in which Z_R and $Z_{R'}$ are the values of Z for the resistances R and R' respectively.

4. By a study of series of experiments it is shown that the influence of the two factors under discussion upon stimulating effectiveness is in many cases too slight to require attention. This is true: (*a*) In experiments in which neither secondary resistance nor kathode surface varies during the course of the experiment. (*b*) In those in which certain nerves or muscles are to be stimulated in essentially similar fashion in a succession of animals; in which case it is possible, by care in maintaining uniform conditions, to reduce to a minimum

the variations in secondary resistance and in kathode surface. Experiment shows that with due care the probable error need not exceed 18 per cent in these cases. (*c*) In experiments in which it is not so important to know the actual value of the stimulus used as to describe it so that other investigators can duplicate it. If the conditions of stimulation are carefully described and the value of Z given, it is possible in practically every such case to duplicate the stimulus within one third or less by following closely the conditions given and employing the same value of Z.

5. It is necessary to take into account the factors of secondary resistance and kathode surface in experiments whose prime object is the comparison of stimuli applied to different tissues; and in all experiments requiring a higher degree of accuracy than is indicated in the paragraph above, in which either factor changes during the course of the experiment.

6. On account of the close interdependence between secondary resistance and kathode surface the introduction of a high additional resistance into the secondary circuit for the purpose of rendering negligible fluctuations in tissue resistance is permissible only in cases where the kathode remains unaltered throughout; the method is likely to give wholly misleading results if used in experiments which involve changes in the position of the kathode, or shifting of the stimulus from one tissue to another.

NOTE. After this paper had been sent to press I discovered an error of calculation in the determination of the mutual induction of the standard coil used in calibrating my inductoria (see this journal, 1908, xxii, p. 121), which has led me to use throughout the work a scale smaller than the true one. This error in no wise affects the validity of the quantitative method developed in this series of papers. On account of it, however, all figures for *strength of stimulus* reported by me are too small. The correct values can be obtained in every case by multiplying my figures by 2.4.

ON THE DYNAMICS OF CELL DIVISION. — II. CHANGES IN PERMEABILITY OF DEVELOPING EGGS TO ELECTROLYTES.

By J. F. McCLENDON.

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THE process of cell division may be divided into two distinct phenomena, the division of the nucleus and of the cytoplasm. Although these processes are closely interrelated, they can occur separately. Karyokineses may occur without subsequent cytoplasmic division, and cytoplasmic division may occur without the presence of a nucleus or chromatin in any form.[1] The division of the cytoplasm in plant cells is accomplished by the formation of a division wall, but in most animal cells by simple constriction.

The constriction of the cell seems to be a special case of these protoplasmic movements that were shown by Quincke to resemble movements accompanying surface tension changes.[2] If the constriction of the cytoplasm were due to surface tension changes, we should expect a band of greater surface tension to include the cleavage furrow. In this case there would be a flowing of the superficial cytoplasm from the poles of the cell toward the cleavage furrow, and of the deeper protoplasm in the opposite direction.

By a study of fixed material Nussbaum [3] showed movements of the pigment granules in cells of frog's embryos from the interior to the surface and along the surface to the position of the future cleavage furrow. On constriction of the cell the granules were massed in the form of a plate in the cleavage plane. These movements indicate the surface tension changes described above.

[1] McClendon: Archiv für Entwicklungsmechanik, 1908, xxvi, p. 662.

[2] Buetschli: Archiv für Entwicklungsmechanik, 1900, x, p. 52.

[3] Nussbaum: Anatomische Anzeiger, 1893, viii, p. 666.

Conklin [4] found evidence for such movements in the changes in position of certain structures in Crepidula eggs.

The first observation of this process in living cells was made by Erlanger,[5] who saw movements of superficial granules toward the cleavage furrow, and of internal granules toward the poles, of Nematode eggs.

Gardiner [6] observed, in living eggs of Polychœrus caudatus, colored granules move to the surface and then along it to the position in which the cleavage furrow appeared immediately afterward. Fischel's observations are considered below.

The fact that cells usually round up before cleavage, if not previously spherical, indicates a general increase in surface tension, and it is only necessary to assume a greater increase to be localized along the cleavage furrow to account for the constriction.

Robertson [7] floated an olive oil drop on water and laid across it a thread moistened with soap (or soap-forming) solution. After the thread reached the edges of the drop the latter was torn in two. Since soap decreases the surface tension between oil and water, he concluded that cytoplasmic division is due to a decrease in surface tension along the cleavage furrow. As this view has been accepted by Lillie [8] and Loeb,[9] it seems worth whlie to point out Robertson's error.[10] In Robertson's experiment three different surface tension films occur, between air (*A*) and water (*W*), air and oil (*O*), and water and oil (Fig. 1), and an equilibrium is established when the water-air surface tension equals the horizontal components of the air-oil plus the oil-water surface tensions. When the moistened thread is laid across the oil drop, two more films are added, *i. e.*, air-soap solution (*S*) and oil-soap solution (Fig. 2). At opposite edges of the drop where the thread touches the water, the soap would decrease the water-air surface tension, and the undiminished pull on the remainder of the edge of the drop would pull it in two (Fig. 3).

[4] Conklin: Biological lectures at Woods Hole, 1908, p. 69.

[5] Erlanger: Biologische Centralblatt, 1897, xvii, p. 152.

[6] Gardiner: Journal of morphology, 1897, xi, p. 55.

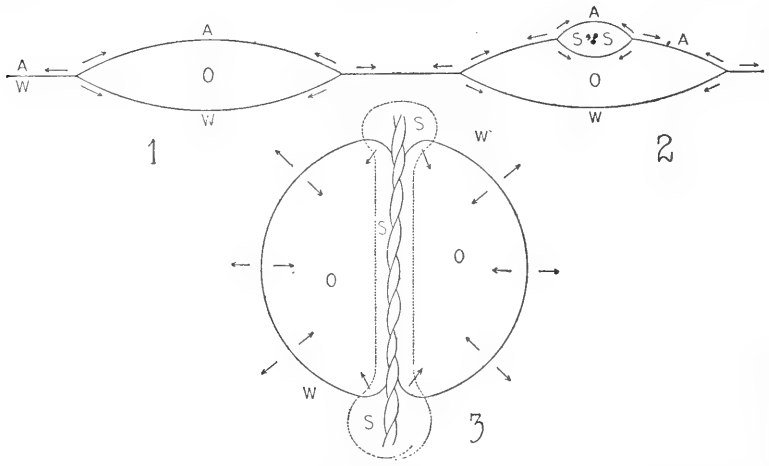
[7] Robertson: Archiv für Entwicklungsmechanik, 1909, xxvii, p. 29.

[8] Lillie: Biological bulletin, 1909, xvii, p. 203, footnote.

[9] Loeb: Chemische Entwicklungserregung des tierischen Eies, p. 5.

[10] This was first pointed out by me before the American Society of Zoölogists, see Science, xxxi, p. 467. It was discussed by A. B. Macallum, Science, 1910, xxxii, pp. 498–500.

I have repeated Robertson's experiment and also modified it by entirely submerging the oil drop. Enough alcohol was added to the water to make the oil sink below the surface, and the soap solution introduced through a capillary pipette, or a piece of solid soap held near the oil drop, or a thread covered with solid soap was wrapped around the oil drop. Very little movement of the oil occurred, but



FIGURES 1 to 3. — *A*, air; *O*, oil; *W*, water; *S*, soap solution. The arrows show the direction of the pull of surface tension. The dotted line in Fig. 3 bounds the soap solution. Further explanation in text.

that which did occur was always a bulging toward the soap, and never a constriction or receding from the soap. Similar experiments were also tried on the under side of oil drops floating on water, and unless the soap reached the water-air film, the oil advanced toward the soap and no constriction occurred. We may conclude, then, that the cleavage furrow is a region of *increased* surface tension, as shown by Bütschli and others, and not of decreased surface tension, as Robertson, Lillie, and Loeb maintain.

One may ask why such movements as seen by Erlanger, Gardiner, and others have not been observed in all dividing cells that have been studied alive. The answer to this may be sought in the structure or consistence of protoplasm. If the cytoplasm present an alveolar structure, the spreading of the surface in regions of reduced surface tension would be almost entirely confined to the individual alveoles, and the general effect would be a slow stretching of the surface in

areas of less surface tension and a slow contraction of the surface in areas of greater surface tension, as shown by Bütschli's microscopic oil foams. The constriction of the cleavage furrow would then take place as though a rubber band around the cell contracted.

In the constriction of the egg of the sea urchin, Arbacia punctulata, usually just such a number of chromatophores are carried into the cleavage furrow that when the two daughter cells are formed the pigment is evenly distributed over all parts of their surfaces. Under certain abnormal conditions in which the cleavage is more violent the pigment is massed in the furrow. Fischel observed a massing of pigment along the lines of the future cleavage furrows in the eggs of Arbacia pustulosa. The reasons we get so little movement of pigment in the egg of Arbacia punctulata probably are the alveolar structure and the presence during cleavage of the "hyaline plasma layer," or "Verbindungsschicht," to which the surface movements are chiefly confined. The hyaline plasma layer is said to be formed by a recession of granules toward the interior of the egg, leaving the superficial layer free from granules and almost invisible. It is formed before the first cleavage and becomes heaped up in the cleavage furrow.[11] Whenever such an outer layer occurs, as in eggs of sea urchins and ctenophores, it becomes heaped up in the cleavage furrow; this indicates increased surface tension in this region (or decreased surface tension at the poles).

In the cutting off of the micromeres in the Arbacia egg the pigment entirely disappears from the micromere pole, indicating spreading movements due to the surface tension being less here than in the region of the future cleavage furrow. Similar movements of granules have been observed in the cutting off of polar bodies in various eggs, and it may be concluded that for the separation of a very small cell from a large mass of protoplasm a very great difference in surface tension between the pole of the small cell and the cleavage furrow is required.

Changes in surface tension may be the result of the presence of certain substances in one of the two fluids in contact, changes in temperature, or a difference in electric potential across the boundary. In numerous instances electric changes have been found to accompany vital movements. Hyde[12] detected electric changes accompanying

[11] Goldschmidt and Popoff: Biologische Centralblatt, 1908, xxviii, p. 210.

[12] Hyde: This journal, 1904, xii, p. 241.

cleavage, and the question whether the constriction of the cytoplasm is due to electric changes seems worth investigation.

If an electric current were passed through a solution containing a living cell, and if the cell surface offered more resistance to the passage of ions than either the medium or the cell interior, a difference of potential would be produced between the inner and outer sides of the cell surface, and would be proportional to the angle that the surface made with the current lines cutting it, *i. e.*, it would be greatest at the point where the surface was at right angles to the current lines and equal to zero at the point where the surface was parallel to the current lines. The surface tension would be reduced at the poles (the points nearest the electrodes), and the equator would lie in a region of relatively greater surface tension. This would result in the protrusion of the polar regions and constriction of the equator, thus producing the form change of the first stage of cleavage.

When a current of a certain density was passed through an unfertilized Arbacia egg, the surface nearest the anode showed spreading movements and bulged out. We might conclude from this that this egg was less permeable to anions than to cations. The confined anions caused a difference of potential between the two sides of the surface nearest the anode, thus decreasing the surface tension, and spreading of the surface and bulging of the egg followed. At the surface nearest the cathode, the anions that could not enter could pass around the egg, and therefore the difference of potential was not so great as at the opposite pole. If the egg were as poorly permeable to cations, we should expect a reduction of surface tension at the pole nearest the cathode.

It seemed to me that an analysis of artificial parthenogenesis might throw light on the question of cell division, and in the summer of 1909 I began an attempt in this direction.

Artificial Parthenogenesis.

It is well known that the eggs of different individuals of the same species vary in response to stimuli. Investigators usually suppose that they have normal material when a large per cent of the eggs develop in a control to which sperm is added. In order to test this method of

controlling experiments and be sure no unknown factor vitiated the results, I made a series of experiments by fertilizing eggs of the same mother with sperm of different fathers, and eggs of different mothers with sperm of the same father, with the following results, giving the percentage of eggs developing:

Fathers.	Mothers.			
	A	B	C	D
1	100	98	93	98
2	100	97	94	96
3	100	98	92	97
4	100	96	90	100

It may be seen by inspecting the table that development depends more on the eggs than on the sperm, so the practice of keeping a fertilized control seems to be a good one. Fertilized and unfertilized controls were kept to all of my experiments, and the experiment thrown out if fertilization did not occur.

The question which concerns us first is, in what ways have cells been caused to divide, and maturation (in most cases), as well as segmentation, is cell division. We may summarize the methods used as follows:

Hypotonic solutions (distilled water, Schücking).

Nearly isotonic solutions made by adding to sea water or to distilled water the following substances:

Acids (Delage, Fischer, Herbst, Lefevre, Loeb, Lyon, Neilson, Schücking, Tennent).

Alkalis (Delage, Loeb, Schücking).

Neutral salts (Delage, Lillie).

Hypertonic solutions:

Acids (Delage, Loeb).

Alkalis (Delage, Loeb).

Neutral salts (Bataillon, Bullot, Delage, Fischer, Hunter, Kostanecke, Loeb, Lyon, Mead, Scott, Treadwell, Wilson).

Non-electrolytes (Delage, Loeb).

Mechanical shock (Delage, Fischer, Mathews, Scott).

Thermal changes (Bataillon, Delage, Greeley, Lillie, Loeb, Schücking).

Electric changes (Delage, Schücking).

KCN or lack of oxygen (Loeb, Lyon, Mathews).

Fat solvents (Loeb, Mathews).

Alkaloids and glucosides (Hertwig, Loeb, Schücking, Wassilieff).

Blood sera (Bataillon, Loeb).

Soap and bile salts were found effective by Loeb when followed by hypertonic solutions.

As all of these agents were not tried and found effective on eggs of the same species, their differences might be thought to correspond to differences in the eggs, and therefore I thought it worth while to try a large number of them on the egg of Arbacia punctulata at Woods Hole, Mass. Segmentation was produced by the following methods: the time indicated is the optimum duration found after a series of experiments, the tables being omitted:

Hypotonic solutions (70 c.c. sea water + 30 c.c. tap water or distilled water, one to one and a half hours).

Nearly isotonic solutions:

Acids (50 c.c. sea water and 3 c.c. 1/10 normal acetic, fifteen to sixty seconds. Or sea water charged with CO_2 in a "sparklet fountain," five to ten minutes).

Alkalis (1.2 c.c. 1/10 normal NH_4OH or NaOH + 50 c.c. sea water, twenty to sixty minutes).

Salts (5/8 normal NaCl, one-half to two hours, this is very slightly hypertonic).

Hypertonic solutions (100 c.c. sea water + 15 c.c. 2½ normal NaCl, one hour. Or sea water boiled down to .76 of its volume, one hour.)

The eggs were also made to segment by placing them in sea water brought from Boca Grande Key, twelve miles west of Key West, Florida, in steamed out, glass-stoppered and paraffine-sealed bottles. The eggs were allowed to lie twenty minutes in Woods Hole sea water, then placed for two and a half hours or four hours in Boca Grande sea water and returned to Woods Hole sea water, or allowed to remain indefinitely in Boca Grande sea water. At the end of nine hours segmentation had occurred in all three lots, about 10 per cent were segmented in that left four hours in Boca Grande sea water.

Woods Hole sea water has a $\Delta = 1.818$, specific gravity = 1.024 (Garrey). Boca Grande sea water has a $\Delta = 2.05$, specific gravity = 1.0248. The alkalescence of the Boca Grande sea water is greater than that of Woods Hole. The hypertonicity and greater alkalinity may have both aided in producing the segmentation. This experiment producing such poor results (10 per cent), is given so much space merely because the effects were produced by natural sea water. However, this does not prove that Arbacia grown at Boca Grande would be naturally parthenogenetic.

Mechanical shock (shaking in vial, by hand, five minutes. Or pouring from one dish to another every ten minutes for three hours. One effect of agitation is the removal of the jelly-like covering from the eggs, after which, perhaps, the mere contact of the egg with another surface will start development. Mathews supposes the effect of shaking is rupture of the nuclear wall, at least in the starfish egg).

Thermal changes (keeping at 32° C., four minutes; keeping at 1° C., one to eleven hours; or at 10° C., one to twenty hours. By the end of twenty hours some were already segmented).

Electric changes (several entire ovaries were placed in longitudinal series in a glass tube, and an alternating current from a small induction coil was passed through it for two hours. To avoid error from polarization at the electrodes, only the eggs from the central ovary were observed).

KCN or lack of oxygen (a stream of hydrogen eight to twenty-two hours, with or without previous boiling of the sea water. Or 1/500 normal KCN, seventeen to thirty-two hours. Or 1/1000 normal KCN thirty-two hours).

Fat solvents (½ saturated solution of ether in sea water, ten minutes).

Certain combinations such as carbonic or fatty acid followed by hypertonic sea water, or tannic acid + an excess of NH_4OH,[13] or tannic

[13] In making his "ammonium tannate" solution, DELAGE considered tannin a hexivalent acid, but I can find no confirmation of this view in chemical handbooks. On adding this solution to sea water, a slight precipitate, probably calcium or magnesium tannate, forms, and the fact that DELAGE obtained as good results by adding the "ammonium tannate" to a sugar solution does not prove that the precipitation of some salt in the sea water solution was not in this case a factor in the production of parthenogenesis.

or acetic acid followed by NH_4OH or NaOH, seemed to produce better results than the single treatments.

The following results were obtained on other species:

First, at Woods Hole, eggs of Cumingea and Mytilus were caused to maturate by treatment with hyperalkaline sea water.

Second, at Tortugas, Fla., where I found the $\Delta = 2.03$, specific gravity = 1.0246, and alkalescence greater than at Woods Hole.

A few of the immature eggs of Ophiocoma rizii maturated when left twelve hours in 50 c.c. sea water + one drop of dilute ammonia, whereas none maturated in the control.

Eggs of Toxopneustes (Lytechinus) variegatus and Tripneustes (Hipponöe) esculentus were made to segment by placing a test tube of sea water containing them for one minute in water at 38°–44° C., then pouring the eggs into a dish of sea water at the normal temperature.

After treatment with sea water carbonated in a "sparklet syphon," followed by hypertonic sea water, development went farther in both species than after any of the single treatments tried. By increasing the duration of the treatment the rate of development was increased (approached or equalled that of fertilized eggs), but the percentage of resulting larvæ decreased, indicating injury to the eggs.

The optimum for Toxopneustes was: carbonated sea water one and one-half to five minutes, followed by 100 c.c. sea water + 16 c.c. 2½ normal NaCl, thirty to forty-five minutes. If eggs had remained a long time in sea water before the beginning of the experiment, a shorter stay in carbonated sea water was required than if they had been just taken from the ovaries. In this connection it is to be remarked that carbonated sea water hastens the solution of the jelly-like coverings, which takes place more slowly in natural sea water.

The optimum for Tripneustes was carbonated sea water ten minutes, followed by hypertonic sea water one hour. If we look for something in common in all of these methods of artificial parthenogenesis, we meet with many difficulties. I concluded that all of the methods of artificial parthenogenesis could not directly initiate any one single chemical reaction in the egg, but must have their first common effect in some physical or physico-chemical change.

The osmotic methods have this in common, that in all there is an *increase* in osmotic pressure. If the eggs are placed in sufficiently

concentrated sea water or other hypertonic solution, some may segment while remaining in the hypertonic solution, but if placed in distilled water (Schücking) or diluted sea water (McClendon) they segment only after removal to natural sea water, which means an increase in osmotic pressure of the medium. I do not, however, conclude from this that in the latter instance it is the return to sea water rather than the sojourn in the hypertonic solution that starts the development of the egg.

Traube [14] showed that "fertilization membrane-forming" substances are effective in greater dilution the more they lower the surface tension of water. If the egg surface contain lipoids, such substances will be adsorbed or absorbed by the lipoids in the ratio that they lower the surface tension of water. But in what way can an absorption or adsorption by the lipoids of the cell, of a host of different substances, cause the development of the egg, and how can we explain those methods in which no lipoid soluble substance is used?

Loeb had shown the similarity between methods of artificial membrane formation and hæmolysis, and many eggs segment after artificial membrane formation. But in my opinion Loeb has not given a satisfactory explanation of the mechanism of hæmolysis. It seems to me that the "membrane theory" of hæmolysis, so admirably presented by Stewart,[15] is a satisfactory explanation.

Lillie [16] advanced the view that the essential element in artificial parthenogenesis is the increase in permeability of the plasma membrane to CO_2, allowing the chief end product of oxidation to escape and the rate of oxidation to increase, the more rapid oxidation causing development. But Lillie has never published any determinations of changes in permeability of the egg to anything except pigment, and there is no certain proof that the escape of pigment is due to increased permeability of the plasma membrane, as the pigment must first be liberated from the chromatophores, in which it is held physically or chemically.

I know of no method of determining changes in permeability of the egg to CO_2, but have thought of five methods for detecting changes in

[14] Traube: Biochemische Zeitschrift, 1909, xvi, p. 182.

[15] Stewart: Journal of pharmacology and experimental therapeutics, 1909, i, p. 49.

[16] Lillie: Biological bulletin, 1909, xvii, p. 188.

the permeability of the egg to electrolytes in general: 1. Electric conductivity of masses of eggs; 2. Electric conductivity of individual eggs as determined by destructive effects of the electric current on the cell; 3. Plasmolysis; 4. Chemical analysis of masses of eggs; 5. Microchemical analysis of single eggs. These will be considered in the order given.

Brown [17] concluded that the membrane of the Fundulus egg is practically impermeable to salts and water during the first eight hours, and becomes most permeable after eighteen to twenty hours. Apparently he refers to the thick membrane which is pushed out after oviposition, but Sollmann [18] observed that the "yolk" swells in distilled water or one-fourth molecular cane sugar, obliterating the perivitelline space, and thus indicating that the plasma membrane is less permeable to salts than is the thick egg membrane or chorion (vitelline membrane of Sollmann).

Biataszewitz [19] found that the absorption of water by the unfertilized frog's egg increased five times for every rise of 10° C., and concluded from this that heat increased the permeability of the plasma membrane to water.

The Electric Conductivity of Masses of Eggs.

This work was done at the Tortugas Laboratory of the Carnegie Institution. The experiments were made on board the yacht "Physalia" anchored off Boca Grande Key, twelve miles west of Key West, Fla. I am indebted to Dr. W. R. Warren of Key West for the use of a centrifuge, as the one provided was left behind. My thanks are also due Dr. Alfred G. Mayer for the unusual facilities at my disposal.

The determinations were made by Kohlrausch's method. A resistance box of 15,000 ohms and a metre sliding resistance were used. A number of difficulties arose in eliminating possible sources of error, and these will be considered in order:

First. The procuring of sufficient quantities of suitable eggs to

[17] Brown: This journal, 1905, xiv, p. 354.

[18] Sollmann: This journal, 1904, xii, p. 112.

[19] Biataszewitz: Bulletin de l'Académie des Sciences de Cracovie, Math. Nat., October, 1908.

make accurate determinations. This was met by going to Boca Grande Key, where the sea urchins, Toxopneustes variegatus and the Tripneustes esculentus, could be picked up by the ton in shallow water. As the ripe eggs of the former species were more abundant, they were used exclusively.

Second. The handling of the eggs with sufficient rapidity to insure their being in normal condition. Washing the eggs repeatedly by allowing them to settle in sea water requires much time, though it will be shown later that for the first washing this is an advantage.[20] But the time required for them to settle into a compact mass for the conductivity determination must be shortened as much as possible, as the continued crowding of the eggs might produce abnormal effects. No suitable conductivity vessel on the market could be placed directly in the centrifuge.

I made a conductivity vessel at the Tortugas Laboratory. It consisted of two glass tubes, the inner one fitting nicely into the outer one. Owing to a very slight curvature of the tubes, a rotation of one in the other would clamp them tightly together. The inner tube was 131 mm. long, 10 mm. inside diameter, and sealed at the lower end. Two small glass tubes were placed longitudinally within the outer tube and sealed into its upper end. One of these projected 25 mm. below the other. Platinum electrodes 6 × 9 mm. were sealed in the lower ends of the two smaller tubes. The electrodes were "platinized" with platinic chloride solution containing a little lead acetate.

The advantages of this conductivity vessel were the comparatively large surface area of the electrodes, 108 sq. mm. each; the distance between them, 25 mm.; the small volume of eggs, less than 3 c.c., required to cover the electrodes, and the short time required for its contents to reach the temperature of the thermostat. The electrodes were plane and vertical, and hence could be pressed down into a mass of eggs with the least possible disturbance of them. The inner tube, containing the eggs, could be placed directly in the centrifuge, and thus the eggs could be washed with sea water or other solutions without removal from the conductivity vessel. By inserting the lower end of the outer tube into a short, closely fitting test tube, the electrode could be protected from drying while the inner tube was in the centrifuge.

[20] The eggs of Toxopneustes are of but very little greater specific gravity than sea water and hence settle much slower than those of Tripneustes or Arbacia.

Third. The temperature of the thermostat, if constant, would on some days be very far from that of the sea surface, which varied greatly (from about 25°–30° C.), and the sudden change of the eggs from one to the other might affect them in some undesirable way, as they could be caused to segment by a change in temperature; also the time required for the eggs to reach this temperature would be greater. This was obviated by making the temperature of the thermostat about one degree higher than that of the sea before each set of determinations and then keeping it constant, within one tenth of a degree. The thermostat held 20 litres of water, was closed at the top and well insulated at the sides, stirred with a paddle attached to a rod going through a small hole in the cover, and regulated by hand with a minute flame beneath.

Fourth. The spaces between the eggs might vary. This was obviated by centrifuging the eggs in the conductivity vessel and marking their upper limit accurately in indelible ink with the finest drawing pen, and before each reading centrifuging them again to the same line.

The egg as it leaves the ovary is surrounded by an invisible gelatinous covering, which I have called the "jelly," since it shows similarity to the jelly-like coat of the frog's egg, a mucin which yields galactosamin (Schulz and Ditthorn). Loeb applies the name chorion to it. This jelly seems to be a mucin which in sea water slowly dissolves. The solution of the jelly is aided by weak or strong alkalis and very weak acids, though it is coagulated by tannin and basic dyes, and seems to contract (coagulate?) on addition of strong mineral acids. Delage says it is lifted from the egg before the formation of the fertilization membrane. It is possible that this appearance was due to contraction caused by the dye used to make it visible.

If eggs bearing this jelly are put in the conductivity vessel and centrifuged, and later mixed with sea water and centrifuged again, much less force is required to precipitate them to the same level, and they will slowly settle below this level by gravity while the eggs are being brought to the temperature of the thermostat. This is due to the washing away of some of the jelly. To prevent this occurrence, the jelly had to be entirely washed off of them before they were first placed in the conductivity vessel, a process accomplished by stirring in large quantities of sea water and repeated centrifuging. The

jelly could be removed completely from Toxopneustes eggs and with more difficulty from Tripneustes eggs. Care was taken that no treatment was used that would cytolyze even a few eggs, as cytolysis causes swelling or disintegration which would affect the volume of the eggs and therefore the spaces between them when they were precipitated to a certain level.

The pushing out of fertilization membranes might affect the shape of the spaces between the eggs and thus change the free paths of the ions in the sea water filling those spaces. This was obviated by washing the eggs so long in sea water that no fertilization membranes were pushed out when they were fertilized or treated with the solutions used. This was tested by microscopic examination of control eggs and of the eggs taken from the conductivity vessel after each experiment. Such eggs develop.

It has been objected that a membrane was formed and not pushed out. I found two methods of detecting membranes that lie so close to the egg as not to be distinguishable with the microscope. If the egg be plasmolyzed with a molecular solution of cane sugar, such membranes are often if not always lifted from the egg. Harvey says the fertilization membrane is relatively impermeable to sugar, and one would suppose that sugar would push the membrane closer to the egg. From many of my experiments it is evident that sugar will go through the fertilization membrane, and Harvey has not stated the degree of impermeability to sugar that he observed. The second and more certain method is as follows: If an electric current of sufficient density be passed through, the membrane will be lifted from the cathode end of the egg.

It may be objected that the sugar solution and electric current caused the membranes to be formed, and that they were lifted from the egg in the usual manner. I can only answer to this that the same sugar solution and electric current were tried on normal unfertilized eggs and no membranes appeared.

Under the same conditions as those of the later conductivity experiments, eggs did not form membranes that could be detected by either of the above methods, and similar eggs developed. Harvey [21] says that after an egg stands in sea water twenty-eight hours and is then fertilized, it becomes surrounded by a thick adhering membrane

[21] Harvey: Journal of experimental zoölogy, 1910, viii, p. 365.

which, on cleavage of the egg, surrounds each blastomere. He calls this a fertilization membrane, and maintains that fertilization membranes are formed on all developing sea urchin eggs. Evidently in the above case and in that of eggs placed in hypertonic or calcium-free sea water he mistook the so-called "hyaline plasma layer" or "Verbindungsschicht" for the fertilization membrane.[22] Harvey states that he saw membranes on Hipponoe eggs on slightly "high focus." A membrane on the surface of a sphere could only be determined positively with so high a power that the optical section was extremely thin in comparison to the diameter of the sphere, and passed exactly through the point of contact, with the sphere, of a tangent drawn from the eye to the sphere. Hence there is only one focus, and "high" or "low" focus means out of focus. One need only try this "high focus" on an air bubble or oil drop to see what appearances of "membranes" are thus obtained.

But it is impossible for me to see how a change in a surface film, of immeasurable thickness, of the egg, thus forming a "membrane" in close contact with the egg, can cause such a change in the conductivity of the inter-egg spaces as to account for the great differences in the conductivity of unfertilized and fertilized eggs which I obtained. I account for the change in conductivity by a change in the surface film of the egg, allowing ions to pass through more easily. Probably such a change would be accompanied by a visible change if microscopic technique were sufficiently developed to detect it, but I would not call this the formation of a new membrane; it is a change in the "*plasma* membrane," a condensed surface tension film or haptogen membrane.

The conductivity of the spermatic fluid and of the acidulated sea water were slightly less than that of natural sea water, so that if they replaced natural sea water between the eggs, they would cause a slight decrease in the conductivity reading, but they were thoroughly washed out with natural sea water before the readings were taken.

Fifth. The electric current passed through the eggs might alter their conductivity. As no measurement of conductivity could be taken before the current began to pass through, I cannot determine this point. But the first reading and later readings taken at short intervals were always the same provided the eggs had been centrifuged down

[22] See Goldschmidt and Popoff, Biologische Centralblatt, 1908, xxviii, p. 210.

so compactly that they did not settle further by gravity, and the content of the conductivity vessel was at the same temperature as the thermostat. By using a special induction coil with a rheostat, an alternating current of such high frequency and such low amperage was obtained that when passed through my finger from electrodes wet with sea water, I could not feel it, yet this was the current used in the experiments, and variations in it could easily be detected with the telephone used in the experiments.

Sixth. The increased elimination of carbon dioxide by fertilized eggs might cause an increase in conductivity of the sea water between the eggs. To test this, the conductivity of a sample of sea water was determined. It was then charged with CO_2 in a "sparklet syphon," and no increase in conductivity could be detected with the electrodes used in the experiments with eggs. Perhaps with electrodes specially adapted to good conductors like sea water, a change could be detected, but it would evidently be extremely small and incapable of accounting for the large differences observed in the experiments with eggs.

The Conductivity Determinations.

The conductivity of one sample of sea water at 30° C. was found to be .061, while that of unfertilized eggs washed in the same water and precipitated by gravity was .04655 at the same temperature. The conductivity of the same water at 32° C. was .0624 and of the same eggs at 32° C., .0469. At 26° the conductivity of another sample of sea water was .05535, of spermatic fluid direct from testes, .0439, and of unfertilized eggs precipitated with the centrifuge, .002404. In this case the conductivity of the eggs was about one twentieth that of the sea water, although they still contained some of the latter between them. The conductivity of the eggs would have fallen still lower if all of the sea water had been pressed out from between them.

First lot of experiments. — In all of these preliminary experiments the thermostat was kept at 32° C., which was at times very near, but at other times very much above, that of the sea. The eggs were precipitated by gravity. The supernatant sea water in the conductivity vessel was pipetted off, and the vessel shaken, then readings taken, until successive identical results showed that the eggs were of the temperature of the thermostat, after which less than a

drop of sperma was added, the vessel shaken again, and a second series of readings taken. The observed conductivities are given in the following table:

1.	Unfertilized eggs	.05980
	Fertilized eggs	.16950
2.	Unfertilized eggs	.04480
	Fertilized eggs	.04835
3.	Unfertilized eggs	.04690
	Momentarily heated	.05600 [23]

The above figures show a great increase in conductivity on fertilization, or momentary heating to a point that will cause segmentation.

Second lot of experiments. — In this and all subsequent lots of experiments the thermostat was brought before each set of readings to about sea temperature, the eggs were precipitated in the conductivity vessel to such a degree that they were not further precipitated by gravity.

1.	Unfertilized eggs at 27.5° C.	.01524
	Fertilized eggs " "	.01627
2.	Unfertilized eggs " 28° C.	.01076
	Fertilized eggs " "	.01266

Third lot of experiments. — As it was feared that the admixture of but a fraction of a drop of spermatic fluid might raise the conductivity independent of fertilization, and as by this method only a small per cent of the eggs were fertilized, in the third and fourth lots of experiments the eggs were centrifuged in the conductivity vessel and their upper level marked accurately, and the conductivity determined, then they were mixed with sea water containing sperm or acetic acid in the conductivity vessel, and washed by repeated precipitations and precipitated down to the same level, before determining the conductivity of the developing eggs. By this method almost 100 per cent of the eggs could be caused to begin development.

1.	Unfertilized at 26° C.	.002404
	Fertilized " "	.004320

[23] The vessel was set a few moments in water at 45°, then returned to the thermostat; this was shown by control to cause segmentation.

2.	Unfertilized at 25.75° C.	.004445
	Fertilized " "	.006340
3.	Unfertilized at 25° C.	.006523
	Fertilized " "	.009544
4.	Unfertilized at 26.25° C.	.004900
	Fertilized " "	.006390
5.	Unfertilized at 28° C.	.008230
	Fertilized " "	.009220
6.	Unfertilized at 28.17° C.	.006876
	Fertilized " "	.007298
7.	Unfertilized at 26° C.	.005620
	After .006 normal acetic acid in sea water 1½ min. 26° C.	.006000

Fourth lot of experiments, in which all precautions were taken. — In these experiments the eggs were washed so long in sea water that no fertilization membranes could be caused to push out.

1.	Unfertilized at 29.5° C.	.01182
	Fertilized " "	.01537
2.	Unfertilized at 30° C.	.01153
	After .006 normal acetic acid in sea water 1½ min. 30° C.	.0127
3.	Unfertilized at 30° C.	.00877
	After acid sea water 1½ min 30° C.	.00965
4.	Unfertilized at 29.5° C.	.005135
	After acid sea water 1½ min. 29.5° C.	.00839

From the above experiments I have concluded that there is an increase in electric conductivity of the sea urchin's egg at the beginning of development. The question now arises whether the resistance to the movement of ions through the mass of eggs is the impermeability of the plasma membrane or the presence of fat globules or proteid granules within the egg, or the combination of the egg electrolytes with colloids, forming poorly dissociated or poorly diffusible compounds. I found by centrifuging them that there was as great a volume of fat globules and proteid granules in the sea urchin's egg immediately after, as there was immediately before, fertilization.

On the Internal Conductivity of the Cell.

The majority of my experiments on this subject were made at Cornell Medical College.

Höber [24] has devised a method by which the electric conductivity of the cell interior may be measured without breaking the cell wall. The determinations cannot be made with great accuracy, but the results on blood corpuscles clearly demonstrate that the conductivity of the interior is many times greater than that of the corpuscle as a whole, indicating that the greatest resistance to the current lies in the plasma membrane.

Stewart [25] made certain determinations which I take to indicate that hen's egg yolk is a very much poorer conductor than is a solution of its salts made up to the same volume.

The yolk of the hen's egg before it leaves the ovary forms the bulk of a single cell. The yolk of an egg is often considered as "dead" material, but in conductivity experiments we cannot separate "living" and "dead" portions of the cell. There is a small amount of white yolk, but the major volume is yellow yolk.

The yellow yolk under the microscope presents a fluid matrix containing large globules of another fluid of almost the same specific gravity, viscosity, and refracting index as the matrix, the boundary between the two fluids being the seat of little surface tension. Both matrix and globules contain numerous fine granules. On the addition of alcohol, under the microscope, a substance (or substances) in both fluids disintegrates, setting free lipoids which appear as droplets, which are blackened by osmic acid and colored by Sudan III. If one of the large globules is watched closely as the alcohol is applied, fine, lipoid droplets appear and grow and fuse to form larger drops, and some of them may then migrate toward the periphery and fuse to form a lipoid envelope surrounding the globule. The lipoid droplets appearing in the matrix grow and fuse to form larger ones.

The yellow yolk darkens after the addition of osmic acid, the globules becoming darker than the matrix, but if alcohol and then osmic be applied, the lipoid droplets thus formed, quickly become an in-

[24] Höber: Archiv für das gesammte Physiologie, 1910, cxxxiii, p. 237.

[25] Stewart: Journal of experimental medicine, 1902, vi, p. 257.

tense black. Only the lipoid droplets are colored by Sudan III in 80 per cent alcohol.

The white yolk resembles the yellow yolk that has been treated with alcohol in that it contains lipoid droplets. But as there is very little white yolk in the hen's egg before incubation, there are very few lipoid droplets to impede the electric current. The lipoids in the yellow yolk are probably bound up with proteids, forming combinations which are disintegrated by alcohol, as indicated by the above observations.

In order to determine to what extent the granules impede the electric current, I precipitated them with the centrifuge. The large globules cannot be separated from the matrix by this means. With small quantities I was able to obtain the fluid entirely free from granules. It forms 11/17 of the total volume, dissolves in dilute alkalis, and with slight milkiness in dilute acids, and when shaken with water the insoluble portion forms an emulsion or a coagulum resembling yeast plants.

With large enough quantities to fill the smallest suitable conductivity vessel at hand, the precipitation was so slow that I feared decomposition might commence before a granule-free fluid was obtained, so I contented myself with the comparison of a portion containing a very small per cent of granules with a portion containing a very large per cent of granules.

At 25° C. the conductivity of the granule-poor layer was .00302 and that of the granule-rich layer .00278, showing that the granules impede the current to a great extent.

Since dilution with water breaks up many ion-colloid compounds, I used this method to determine whether the electrolytes in the yolk were bound up with colloids. The conductivity determinations at 25° C. are given in the table below:

Portion.	(Undiluted) Vol. = 1.	(+ 1 vol. H_2O) Vol. = 2.	(+ 3 vols. H_2O) Vol. = 4.	(+ 7 vols. H_2O) Vol. = 8.
Granule-poor	.00302	.00268	.00162	.00096
Granule-rich	.00278	.00278	.00200	.00125

The above table shows that whereas the granule-poor layer decreases in conductivity on dilution with distilled water, at first slowly and later slightly more rapidly (which may be partially accounted for by the more rapid increase in ionization of inorganic salts at the

beginning than at the end of the series) the conductivity of the granule-rich layer is not reduced at all by a dilution with one volume of H_2O. It may be said that this is due to the separation of the granules, thus widening the conducting paths, and I have demonstrated that such might occur in an emulsion of oil in soap solution, as shown by the following table of conductivities at 25° C.:

Material.	(Undiluted) Vol. = 1.	(+ 1 vol. H_2O) Vol. = 2.	(+ 3 vols. H_2O) Vol. = 4.
Soap solution	.002490	.001460	.000903
Emulsion of oil, containing 17 per cent soap solution	.000434	.000434	.000335

But how are we to explain the fact that on dilution with one or more volumes of water the conductivity of the granule-rich portion of yolk is greater than that of the granule-poor layer, although the former contains less of the fluid portion of the yolk? Evidently (since there are no inorganic crystals in the yolk) some of the electrolytes must have been bound up in the granules (either by adsorption or chemical combination) and liberated on dilution. Since the fluid portion of the yolk does not entirely dissolve in water, the undissolved portion may impede the current, but this would occur in the granule-rich as well as in the granule-poor portion.

I doubt that Höber's method of measuring the internal electric conductivity of cells is sensitive enough to determine whether the increase in conductivity of the egg is due to liberation of electrolytes in the interior or to increased permeability of the plasma membrane of the egg, but it shows, by exclusion, in case of the cells on which it was used, that by far the greatest resistance to the current lies in the plasma membrane.

Swelling (first stage of cytolysis) of sea urchin eggs causes a decrease in the conductivity of the mass of eggs, as shown by the following determinations of the conductivity:

1.	Unfertilized eggs of Toxopneustes at 27.25° C.	.01354
	After addition of nicotine at 27.25° C.	.01318
2.	Unfertilized eggs at 32° C.	.04850
	After momentary elevation to about 50° C. at 32°	.04780
3.	Unfertilized eggs at 27.5° C.	.01645
	After momentary elevation to about 50° C. at 27.5°	.01626
4.	Unfertilized eggs of Tripneustes at 32° C.	.04730
	After shaking with fraction of a drop of chloroform at 32° C.	.02286

Microscopic examination showed that the addition of nicotine or chloroform, or momentary elevation of temperature in the above experiments, caused the eggs to swell. This could only take place by the absorption of one or more constituents of the sea water between the eggs. If the salts of the sea water did not go into the eggs, the abstraction of H_2O from the sea water would increase the concentration of salts in the sea water remaining between the eggs, and might cause a liberation of electrolytes (by dilution) within the eggs, in the latter case causing increased conductivity of the egg interior without a corresponding decrease in conductivity of the inter-egg spaces. If the membrane became freely permeable to salts, the swelling of the eggs might increase, but should not diminish, the conductivity of the mass. The fact that the conductivity decreased can only be explained by assuming that the salts of the sea water entered the eggs and were adsorbed to or combined with colloids, or that the membrane was very poorly permeable to salts (though perhaps more permeable than the normal egg) and the narrowing of the inter-egg spaces caused the decrease in conductivity. In fact, I think this can be taken as an indication that the egg is a poor conductor not so much because of the low concentration of free electrolytes within it, but chiefly because the electrolytes cannot easily pass the plasma membrane.

As no dilution of the contents (swelling) of the sea urchin's egg occurs at the beginning of development, it is improbable that a liberation of the electrolytes within it, sufficient to account for the increased conductivity, occurs. The only alternative is that the increase in conductivity is due to an increase in permeability of the plasma membrane to electrolytes.

The Electric Conductivity of Individual Eggs.

It is well known that cells may be killed or injured by the passage of electric currents through the media containing them. The current might affect them by raising the temperature, by the passage of ions into or out of the cells, by the accumulation of ions of one sign that are stopped by parts of the cell.

In which of these ways does the current affect the cell most destructively? The heating effect may be practically eliminated. If electrolytes are transported into or out of the cell by the current, they

could also diffuse in the absence of a current, but the accumulation of ions of one sign would not occur by *free* diffusion. Therefore I have regarded the accumulation of ions impeded by the cell structures as explanation of the destructive effects, and the destructive effects as an indicator of the resistance to the passage of ions.

The experiments were made at the United States Bureau of Fisheries at Woods Hole, Mass. The 110-volt direct current from the light circuit was used. Cylindrical non-polarizable electrodes of copper in one-half molecular copper sulphate were plugged at their free ends with absorbent cotton and connected to rubber tubes of 4 mm. internal diameter and about one foot each in length, filled with sea water. The free ends of the rubber tubes were plugged with absorbent cotton, which was allowed to protrude sufficiently to conduct the current to the sea water containing the eggs under a cover glass on a slide on the microscope stage. The current was reduced by passage through a 16-candle power light and further regulated by turning the screw of a pinch-cock which was clamped on one of the rubber tubes. The copper sulphate diffusing into the sea water in the rubber tubes reacted with the calcium carbonate, copper hydrate and calcium sulphate being precipitated and carbonic acid being liberated. The copper sulphate solution and sea water were renewed before each experiment. Usually a piece of ash-free filter paper was cut the size of the cover glass and a hole cut in its middle. This filter paper ring was placed on the slide, and sea water containing eggs placed in the hole in the ring, so that when the cover glass was placed on the preparation, the eggs were contained in a cell which was freely permeable to ions at the sides. At other times the eggs were mixed with sea water containing enough cotton fibres to support the cover glass. Eggs of Arbacia punctulata were used.

When the current is passed through the egg, the latter is affected at that surface nearest the anode, as observed by Brown, who placed the eggs in a molecular solution of urea. Changes in surface tension are indicated by bulging or amœboid movements. The pigment suddenly leaves each of the chromatophores in turn and diffuses into the cytoplasm in this region of the egg, which is turned a red or orange hue (it is a deeper red if the chromatophores have been stained with neutral red), showing that the reaction is not alkaline.[26] The anodal

[26] The pigment extracted from the eggs is red or orange in acid according to dilution; it is violet or green and precipitates in alkali. If the eggs, or especially

end of the cell absorbs water and swells, often a blister is formed and masses of granular cytoplasm pass into the blister fluid and dissolve. Gradually these changes extend from the anode end to the cathode end of the egg, the egg swells enormously and may burst.

Very probably this disintegration commencing at the anode end of the egg is due to the accumulation of anions which cannot pass the plasma membrane. If the plasma membrane is poorly permeable to anions in one direction, it is probably so in the other, and it may be asked why they do not accumulate outside the cell at its cathode end. The anions which are unable to enter the egg at its cathode end are free to move around the egg and hence do not accumulate to form as great a concentration as at the anode end.

Since no destruction of the egg of Arbacia, beginning at the cathode end, was observed, we may conclude that the plasma membrane is more permeable to cations than to anions. This is not true of all eggs, as I observed the eggs of Hydractinea begin to disintegrate at the cathode as soon as at the anode end. However, it is true of a number of living cells.[27]

If fertilized and unfertilized Arbacia eggs are placed in an isotonic sugar solution containing little sea water, through which a current of gradually increasing density is passed, the unfertilized eggs begin to disintegrate, at their anode ends, sooner than the fertilized eggs do. We may interpret this as indicating that the fertilized eggs are more permeable to anions, which therefore accumulate in them to a less extent, or the fertilized eggs are more permeable to electrolytes, which therefore have passed out into the sugar solution to a greater extent, and therefore the current passes through them less, than in case of the unfertilized eggs.

I did not obtain the same results on eggs in sea water, but the uncertainty of the material toward the end of the season prevented the determination of the mode of action of the sugar solution. Possibly the heating effect of the current in sea water increased the permeability of the unfertilized eggs. Sea water is so much better a conductor than the eggs that only a small per cent of the current passes through the latter, and in order to produce visible effects on the eggs an enor-

the perivisceral fluid cells containing much pigment, are killed, the nuclei and some other parts absorb the pigment and turn brownish purple.

[27] See VERWORN'S Physiology.

mous current must be passed through the sea water. It is known that sugar solutions produce abnormal conditions in eggs, but these experiments were made quickly after placing the eggs in the sugar solutions. The nucleus does not begin to disintegrate as soon as the cytoplasm; this is in harmony with McCallum's view that the nucleus contains no free salts. The nucleus as a whole or the contained nucleoproteids migrate toward the anode.

Plasmolysis with Non-Electrolytes.

Osterhout has obtained shrinkage of marine cells in distilled water, and thinks the action of sugar similar; *i. e.*, first the membrane is made permeable and then the salts diffuse out and the cell contracts by some non-osmotic force. But in the only animal cell in which he has obtained this result there is first a swelling, with formation of blisters, and later shrinkage, with the nucleus becoming homogeneous and distinct, which, I think, denotes death and perhaps coagulation. Since the Arbacia egg in an isotonic sugar solution does not swell first and then shrink, I think this objection may not apply to my experiments.

The following tables show that fertilized (Arbacia) eggs shrink more rapidly than unfertilized eggs in molecular sugar solutions, which are calculated to have only slightly greater osmotic pressure than the sea water at Woods Hole, where the experiments were made. It appears that the plasma membranes of the fertilized eggs are more permeable, allowing the salts to diffuse out of the eggs more rapidly, thus lowering the internal osmotic pressure to a greater extent than is the case with unfertilized eggs. Sollmann [28] observed Arbacia eggs contract in hypertonic, and swell in hypotonic, salt solutions.

In normal sea water fertilized are not smaller than unfertilized eggs.[29] Before the first cleavage the hyaline plasma layer forms, thus taking material away from the more opaque portion of the egg, and it might be supposed that the failure to include this layer in taking the measurements caused the appearance of shrinkage, but such would be the case also in the control in normal sea water, and furthermore the measurements were taken before the hyaline layer was formed or had reached visible thickness.

[28] Sollmann: This journal, 1904, xii, p. 111.
[29] McClendon: Science, 1910, xxxii, p. 318.

Fertilized and unfertilized eggs in a molecular solution of dextrose were placed under the same cover glass, which was supported to prevent compression of the eggs, and sealed to prevent evaporation. The fertilized were distinguished from the unfertilized eggs by the presence of the fertilization membrane. The eggs were observed in the order in which they appeared in the field as the slide was moved so as to observe the whole area under the cover glass once and once only. The diameter of each egg in turn was drawn with the camera lucida, and the drawings were measured later with a rule. In case an egg was irregular, approximately its mean diameter was drawn.

The results of two series of measurements are recorded on page 266. In the first column of figures the diameter of the egg in the unit used for all the measurements is represented. In the second and third columns of figures the frequencies of the occurrence of fertilized and unfertilized eggs of the diameters given in the same horizontal line are represented. The fourth and fifth columns of figures represent a second series of measurements in the same manner.

The table shows that there is considerable variation in the size of the eggs, but that the mean (and also the mode if the curve were plotted) of the diameters of the fertilized eggs is less than the mean of the unfertilized eggs. I did not succeed in making measurements fast enough to determine the rate of plasmolysis.

Chemical Analysis of Masses of Cells.

Fertilized and unfertilized eggs may be placed in solutions differing from sea water, and the passage of substances into or out of them detected by analysis of masses of the eggs. There are three sources of error: 1. The presence of the jelly-like coverings on the eggs; 2. The fluid in spaces between the eggs; and, 3. The large surface for adsorption.

I tried some preliminary experiments on yeast cells at a time when suitable eggs could not be obtained. I found yeast and dextrose placed in .3 molecular $MgCl_2$ eliminated CO_2 more rapidly than in .5 molecular NaCl or .325 molecular $CaCl_2$, all of which are calculated to have approximately the same osmotic pressure. Also the CO_2 elimination was more rapid in the magnesium solution than in a solution of the same concentration of magnesium chloride with either of the

Diameter.	Frequency.			
	Fertilized.	Unfertilized.	Fertilized.	Unfertilized.
110	2	..	..	..
111	0	..	..	..
112	2	..	..	..
113	1	..	1	..
114	2	..	1	..
115	2	..	1	..
116	3	1	3	..
117	5	1	3	..
118	3	3	4	1
119	7	2	4	0
120	10	3	3	2
121	9	6	4	0
122	10	5	5	2
123	7	6	6	3
124	6	6	9	3
125	4	7	6	2
126	4	5	6	2
127	1	8	3	3
128	3	7	4	3
129	0	6	1	2
130	2	7	0	1
131	0	3	0	2
132	0	3	1	5
133	1	3	..	7
134	..	2	..	4
135	..	1	..	3
136	..	1	..	3
137	..	1	..	4
138	..	0	..	2
139	..	0	..	2
140	..	0	..	2
141	..	0	..	1
142	..	0	..	..
143	..	0	..	..
144	..	1	..	..
Mean diameter	121	126	122	131

other salts in addition, or in a solution containing NaCl and $CaCl_2$ in the same concentration as in the respective pure solutions, or in a solution containing all three salts, or in tap or distilled water.

The magnesium must have entered the cell or altered the permeability of the plasma membrane to CO_2, sugar, alcohol, the enzyme, or some other substance. In order to determine whether the magnesium entered the cells, I took two blocks of compressed yeast of the same volumes and weights and mixed one with H_2O and the other with a molecular solution of $MgCl_2$ for five hours, then washed each by rapid precipitation in renewed H_2O several times with the centrifuge. The two lots were ashed and weighed with the results: control, ash = .0466 gm.; ash from Mg culture = .048 gm. Evidently the magnesium did not enter the yeast to any great extent and probably acted on the surface, increasing the permeability to some other substance.

Lyon and Shackell have analyzed fertilized and unfertilized eggs placed in salt solutions, and obtained some results indicating that the salts enter and leave the fertilized more easily than the unfertilized eggs. They found an exception in the case of iodine. Iodine (in potassium iodide solution) is absorbed by the unfertilized more quickly than by the fertilized eggs.[30]

I had intended to work along this line, but was forced to postpone it until another season.

Microchemical Analysis of Individual Eggs.

Lyon and Shackell[30] and Harvey have concluded that certain dyes enter fertilized more easily than unfertilized eggs. Loeb supposes that the dye is chemically combined in the fertilized egg and merely in solution in the unfertilized egg. Unfortunately these dyes belong to the class of substances which Overton found to most easily penetrate plant cells, so that a demonstration that they more easily enter the fertilized than the unfertilized egg does not necessarily indicate that the same is true for electrolytes in general.

Harvey[31] found that eggs became more permeable to NaOH after being fertilized or treated with cytolytic agents.

[30] Lyon and Shackell: Science, 1910, xxxii, p. 250.

[31] Harvey: Science, 1910, xxxii, p. 565.

The Migration of the Chromatophores.

The chromatophores of the egg of Arbacia contain a red substance which I found to have an absorption spectrum similar to McMunn's echinochrome, at least in certain solvents. I have crystallized two derivatives of the Arbacia pigment and perhaps the pigment itself, and a chemical study of it is being attempted.

These chromatophores or pigment plastids show similarities to the chloroplasts of some green plants. Similar plastids occur in the perivisceral fluid cells of Arbacia, where they are so closely packed together in the cytoplasm as to be separately distinguishable only on careful observation. In some of the cells the plastids contain pigment and in others they are colorless.

McMunn, finding that the spectrum of echinochrome in certain solvents was changed by strong reducing agents, concluded that it was respiratory in function. Griffiths[32] briefly states that on boiling with mineral acids echinochrome is transformed into hæmochromogen, hæmatoporphyrin, and sulphuric acid, indicating a relation to hæmoglobin.

I separated the cells from about 50 c.c. of the perivisceral fluid of Arbacia and mixed them with sea water to form 50 c.c. This suspension of cells, and 50 c.c. of sea water as a control, were exhausted under an air pump for six hours, during the last half hour at practically water vapor tension. While in the vacuum, the cells must have exerted a reducing action on the pigment if it can be reduced. Each was then shaken with air for thirty minutes in closed apparatus. The suspension of cells had absorbed 1.25 c.c. of air and the control only 0.8 c.c., at atmospheric pressure. The volume of oxygen used in oxidations in the cells during the shaking was probably partly replaced by CO_2 given off by them, but the difference of about half a cubic centimetre does not demonstrate conclusively that the pigment combined with oxygen. Under somewhat similar conditions dogfish blood absorbed many times as much air as the perivesceral fluid of Arbacia.

The migration of the chromatophores in the egg is evidently *not always* in the direction of greater oxygen concentration, but whether it is *ever* a chemotropism toward oxygen I was unable to determine.

[32] Griffiths: Comptes rendus, 1892, cxiii, p. 419.

In 1908 I observed movements of the chromatophores in the eggs of Arbacia punctulata. As Roux had caused a whitening of the cathodal pole of the frog's egg by passing an electric current through it, I tried in 1909 and again in 1910 to move the chromatophores of the Arbacia egg with the electric current. I observed that in the unfertilized egg the chromatophores are distributed throughout the cytoplasm, but after the egg is fertilized or stimulated artificially the chromatophores migrate to the surface.[33]

Harvey[34] says that the pigment comes to the surface within ten minutes after fertilization, but I found that this process sometimes required half an hour, by which time the cleavage spindle had formed. At each cleavage chromatophores sink into the cleavage furrows of the blastomeres. Just before the micromeres are formed the chromatophores move along the surface of the blastomeres, away from the micromere pole of the egg, so that after the resulting cleavage the micromeres are practically free from pigment. Under abnormal conditions there is a great massing of pigment in the cleavage furrow or other regions of the surface or in the interior of the egg. The sinking of pigment into the cleavage furrows and its retreat from the micromere pole are probably due to surface tension changes as discussed above, and perhaps the abnormal massing of pigment at one portion of the surface is due to a local increase in surface tension.

"Membrane-forming" and parthenogenetic agents, even in concentrations too low to produce membranes or segmentation, cause the pigment to come to the surface. If a few normal unfertilized eggs are kept in a relatively large amount of sea water protected from evaporation, and oxygen is very abundant, it appears that there is more pigment at the surface after twelve or more hours than at the beginning of the experiment, but disintegration commences before all the pigment has reached the surface. In an oxygen vacuum this did not seem to occur. The pigment may all come to the surface in a stream of washed hydrogen, but this may be caused by some impurity.

Fischel,[35] observing similar movements of pigment in the egg of Arbacia pustulosa, concluded that the pigment was repelled by the asters according to the forces described by Rhumbler as moving

[33] McClendon: Science, 1909, xxx, p. 454.

[34] Harvey: Journal of experimental zoölogy, 1910, viii, p. 355.

[35] Fischel: Archiv für Entwicklungsmechanik, 1906, xxii, pp. 526–541.

granules toward or away from asters in the cytoplasm.[36] Bütschli and Rhumbler have shown how the contraction of an area in a foam structure causes aster-like radiations around it, and Rhumbler has shown that such radiations to a limited extent may occur around a rigid sphere inserted into a foam or alveolar structure. Rhumbler assumes that the concentration of the alveolar wall substance would increase its surface tension, and that this increase toward the centre of the aster would reduce the thickness of those alveolar walls perpendicular to the astral rays, both of which assumptions have no facts of which I am aware to support them,. On them rests Rhumbler's explanation of the movement of granules away from asters.

However, if those bodies which seem to be repelled by asters (chromatophores of Arbacia eggs, yolk platelets of frog's eggs) lie within or are larger than the largest alveoles, as I have observed to be the case, aster formation might explain their repulsion. Rhumbler's theoretical asters were made of a central body and of alveoles of a uniform size. If the alveoles were of different sizes, the largest ones would seek the periphery of the aster.

I sectioned eggs that had been so treated artificially that all of the pigment came to the surface but no segmentation occurred, and found no asters, though perhaps asters had formed and disappeared.

After the passage of an electric current of a certain density and duration through unfertilized eggs, some of them have their pigment more abundant toward their cathodal surfaces. If the current exceed a certain density, one by one the chromatophores toward the anodal surface of the egg lose their pigment suddenly. When the current was slowly and carefully increased just to the density required to change the distribution of the pigment, no loss of pigment by the chromatophores toward the anode could be observed, but it is mechanically impossible to watch every chromatophore in the anodal region of one egg. I found it possible to observe a single chromatophore for a long time, and attempted, by noting its distance from the anodal surface of the egg, to record its movements. Each time this observation was attempted the chromatophore appeared to move, but its movement was not constant in direction, and a considerable migration in any one direction was not observed, except rarely in case the chro-

[36] Rhumbler: Archiv für Entwicklungsmechanik, 1896, iii, p. 527; 1899, ix, pp. 32 and 63.

matophore was very near the surface. In this exceptional case the chromatophore moved along the surface, toward the cathode, which movement was probably due to surface tension changes. In the egg just taken from the ovary the chromatophores are slightly more numerous near the surface than in the interior, and when the current is passed, this difference is increased. The passage of the current causes the anodal surface of the egg to spread (the increased difference of potential between the two sides of the surface reducing the surface tension), sometimes carrying the more superficial chromatophores along the surface toward the cathode. This is not a cataphoresis of the chromatophores, since they do not go in the direction of the current, but is due to surface tension changes, and is therefore a secondary effect of the current.

Fearing that the high viscosity of the cytoplasm might interfere with the movement of the chromatophores by electric convection, I centrifuged both fertilized and unfertilized eggs until the pigment was massed at one pole of each, and passed the current through solutions containing them. No orientation of the eggs to the potential gradient occurred. I then tried to move the perivisceral fluid cells, which are practically masses of chromatophores, by means of the electric current, but my apparatus did not exclude all sources of error, and this experiment was reserved for another season. The pigment may be caused to leave the chromatophores in these cells by the electric current or by chemicals, to which agents these cells are much less sensitive than are the eggs.

We have, then, no evidence that the chromatophores are electrically charged.

Harvey[37] attempted to explain my observation that the chromatophores come to the surface at the beginning of the development of the egg, by assuming that there is a positive charge over the surface of the egg until the commencement of development, when the surface becoming permeable to anions causes a potential gradient between the surface and centre of the egg. He further assumed that the chromatophores are charged negatively and migrate in the potential gradient.

His evidence for the existence of the positive charge over the surface of the unfertilized egg of Arbacia punctulata is the fact that it is not

[37] Harvey: Science, 1909, xxx, p. 694.

always spherical when it leaves the ovary. His evidence for the loss of the charge is the fact that this egg rounds up more rapidly when it is fertilized than when it is left in sea water without sperm. His evidence for the negative charge on the chromatophores is the fact that they come to the surface after development commences.

My observations indicate that the plasma membrane of the unfertilized egg is less permeable to anions than to cations, which would cause the appearance of the positive charge over the surface provided some electrolyte whose undissociated molecules could not easily pass the membrane was more concentrated in the egg than in the sea water, or was produced with sufficient rapidity within the egg. Carbon dioxide might be this substance. However, my observations seem to indicate that the permeability of the egg is increased suddenly (in less than five minutes) on fertilization, in which case the positive charge over the surface would be lost suddenly, and if the ions within the egg were free to move, the potential gradient would be of momentary duration, whereas the chromatophores require from ten to thirty minutes to come to the surface. Before Harvey made this hypothesis I had attempted, as described above, to move the chromatophores by inducing a potential gradient, in order to determine whether they were electrically charged. Harvey has yet to prove that they are charged, and furthermore that they are negative, and that the potential gradient is of sufficient intensity and duration to move them to the surface. I do not wish to be considered an opponent of his hypothesis, but am merely searching for facts. Garbowski observed chromatophores move toward the centrosomes.

On the Contents of the "Perivitelline" Space.

The assumption has been made by several observers that there exists a colloid between the fertilization membrane and the egg. Here the question arises, what is meant by the surface of the egg? The "hyaline plasma layer," or "Verbindungsschicht," which forms before the first cleavage, is considered by some as part of the egg and by others as a "membrane" outside of the egg. In this section I will not include the hyaline layer in speaking of the egg, as under these experimental conditions the surface of the hyaline layer (if such had formed)

could not usually be distinguished, *i. e.*, the presence of this layer could not be ascertained.

When an electric current is passed through the egg of Arbacia punctulata having a "pushed-out" fertilization membrane, the latter is bulged out toward the cathode, and the egg moved in the opposite direction and pressed against the anodal portion of this membrane. When the current ceases, the egg returns to the centre of the "perivitelline space." I first thought that this was caused by anodal electric convection of the egg, due to confined anions, but sometimes the fertilization membrane bursts at its anodal pole and the egg passes out, and should on this hypothesis continue its migration toward the anode. But as soon as the egg is free from the fertilization membrane it stops its migration, even though floating in a fluid of equal specific gravity. Perhaps an invisible colloid having a positive charge fills the perivitelline space, and its migration toward the cathode pushes the egg in the opposite direction.

Loeb postulated a colloid in the perivitelline space as exerting an osmotic pressure which pushed out the fertilization membrane. This may be true, but the membrane must harden in the expanded condition, for if it is burst by passage of the electric current or other means it does not collapse, but remains spherical unless distorted by violence.

When the electric current causes bulging of the fertilization membrane, the perivitelline space exhibits fine striations radially to the egg or parallel to the current lines. Schücking, Goldschmidt and Popoff, Herbst, and others have described striations or fibres in the perivitelline space, or around the fertilized egg, including the spaces between the early blastomeres, usually under abnormal conditions. These striations are probably due to tension of the colloid filling the perivitelline space (including the hyaline plasma layer or "Verbindungsschicht").

The Action of Parthenogenetic Agents on the Plasma Membrane.

Salts, acids, alkalis, shaking and thermal or electric changes might alter the aggregation state of the colloids of the plasma membrane. Fat solvents, alkaloids, glucosides, blood sera, soap and bile salts

might alter the aggregation state of the colloids, especially lipoids of the plasma membrane.

Lillie [38] found that pure solutions of sodium salts were effective as parthenogenetic agents in the following series arranged according to the anions: $Cl < Br < NO_3 < CNS < I$. This order of anions is reversed in the precipitation of lecithin, and a somewhat similar reversed order of anions occurs in the salting-out of proteids.[39] Hence it appears probable that these salts act by virtue of their dissolving power on the colloids of the plasma membrane.

Alkalis may act not only on the membrane, but by slow diffusion into the egg favor oxidations, as an alkaline reaction favors oxidation in general, and Loeb has shown that an alkaline reaction of the medium is necessary for the normal oxidations in the sea urchin's egg.

KCN or an oxygen vacuum may act by suppressing oxidation until enough of the confined CO_2 can escape to raise the alkalinity within the egg to such a point that when oxygen is readmitted oxidation may proceed with sufficient rapidity to allow the development of the egg.

It seems probable that the undissociated molecules of carbonic acid or CO_2 can diffuse out of the egg at all times. How then could the resistance of the plasma membrane to one or both or its ions so reduce oxidation within the egg as to prevent its development? Perhaps the per cent of CO_2 within the unfertilized egg is sufficient to lower the alkalinity to such a degree that oxidation cannot proceed with sufficient rapidity to allow development.

Loeb [40] finds that an oxygen vacuum or KCN reduces the toxicity of certain poisons to unfertilized and to a greater extent to fertilized eggs (poisons that affect fertilized in less concentration than unfertilized eggs). He concludes that this cannot be explained on the permeability hypothesis (as the mere absence of oxygen would probably not affect the permeability?). It may be that these poisons are toxic because they increase the permeability of both fertilized and unfertilized eggs, but since the fertilized eggs are more permeable before the action of the poison, the additional increase in permeability is fatal because oxidation is abnormally increased. Hence KCN or an oxygen vacuum would be antitoxic.

[38] Lillie: This journal, 1910, xxvi, p. 106.

[39] Hoeber: Zeitschrift für Allgemeine Physiologie, 1910, x, B, p. 178.

[40] Loeb: Biochemische Zeitschrift, 1910, xxvi, p. 288.

Loeb [41] finds that after membrane formation a saccharose solution of much lower osmotic pressure will cause the egg to develop than a pure NaCl solution, which, he says, proves that the membrane is permeable to salts or ions, for the explanation requires that the egg salts diffuse into the sugar in the former case or the NaCl diffuses into the egg in the latter. This shows a certain degree of permeability of the egg to electrolytes after "membrane formation," but proves nothing as regards the normal unfertilized egg. It is not necessary to postulate absolute impermeability, even of the unfertilized egg, to electrolytes, in order to account for development by increased permeability, and I have shown that an increase in permeability follows the action of membrane-forming substances. Furthermore it has not been demonstrated to my satisfaction that the action of the hypertonic solution after membrane formation is purely osmotic.

Lyon has shown that the CO_2 and catalase elimination by the sea urchin's egg increases after fertilization, and Warburg, Mathews, and Loeb and Wasteneys have shown that the oxygen absorption increases. These changes might be due to an increase in permeability.

It is not supposed that an increase in permeability will cause any cell to divide; growth is prerequisite to division. However, permeability might influence growth. Growth is supposed to cause division only when it affects the volume of the cytoplasm more than that of the nucleus. The ratio of the cytoplasm to the nucleus in the egg may be considered sufficient for a number of successive divisions, or the "true" cytoplasm may grow at the expense of the yolk after each division.

[41] Loeb: University of California publications, Physiology, 1908, iii, No. 11, p. 81.

THE RELATION OF AFFERENT IMPULSES TO THE VASOMOTOR CENTRES.

By W. T. PORTER

(With the Collaboration of R. RICHARDSON and F. H. PRATT).[1]

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

I.

THE successful observations in physiology — the observations that largely influence opinion — are commonly attended by parasitic hypotheses. Useful as these may be, they have in themselves no demonstrated truth, but derive their apparent credibility from their associations. Bathed in the radiance of the Great Fact, they seem to shine with their own light, and, as years pass, their doubtful origin is almost or quite forgotten.

It is known that the stimulation of the central end of many nerves causes reflexly a rise or fall in the blood pressure, and that the central mechanism for this purpose lies in the bulb near the calamus scriptorius, since the destruction of this region puts an end to vasomotor reflexes. It is known, further, that the moderate constriction or tonus of the blood vessels is maintained by impulses that stream continuously from this same region, for the blood vessels dilate when the nerves connecting them with the bulb are severed or when the vasomotor region is destroyed. This reflex and this tonus are fundamental truths in the physiology of the circulation.

[1] The stimulations of the sciatic nerve the data of which are used in this paper and the original stimulations of the depressor nerve were made in 1908 (W. T. Porter and R. Richardson: This journal, 1909, xxiii, p. xxxiv; W. T. Porter and F. H. Pratt: *Ibid.*, p. xxxv). These studies were repeated by the writer some months later, and the depressor curves used here are from this second investigation. The writer desires to thank Mr. Richardson and Dr. Pratt, and to express the hope that they will not be held responsible for the views set forth in the present communication, for which he alone is answerable.

With these fundamental truths are associated several hypotheses. Thus the bulbar cells are believed to transmute by an unknown alchemy the afferent into efferent impulses. These same cells are believed to produce the vasomotor tonus as a product of cell life. Not a few physiologists contend that the tonus itself is a reflex, incited by an unceasing flow of impulses from the periphery, but governed by the personal activity of the bulbar centre. Under all these ideas lies the primary hypothesis that both tonus and reflex are controlled by the same masterful syndicate. The importance of this hypothesis is obvious. For if the same apparatus control both tonus and reflexes, the measurement of the vasomotor reflexes will reveal the condition of the apparatus for the maintenance of arterial tone. In other words, the mechanism for arterial tone cannot be impaired so long as the vasomotor reflexes are normal.[2] The present communication brings forward two methods by which this far-reaching assumption may be tested.

II.

The first method to be presented in this communication rests upon interesting propositions. To begin with, it has long been known that muscle will not change its form unless stimulated with a certain intensity and speed. When this threshold stimulus is reached, the muscle will shorten, and, as the successive stimuli increase in force, the shortening is greater and greater until the muscle cannot shorten more, however intense the stimulus. When the stimuli to the gastrocnemius are set down on an abscissa and the changes in the length of the muscle are taken as ordinates, a characteristic curve is produced [3] (Fig. 1, *G*).

The tonus contractions of the heart muscle rise in a similar characteristic curve as the stimulus increases from a minimal to a maximal value (Fig. 1, *H*, and Fig. 2).

Since the heart is a modified blood vessel, it might be expected that

[2] It need hardly be pointed out that a weak or failing reflex does not necessarily indicate depression in the bulbar cells; the difficulty may lie in the afferent path or in the efferent path between the bulb and the blood vessel.

[3] TIGERSTEDT, R., and A. WILLHARD: Mittheilungen vom physiologischen Laboratorium des Carolinischen medico-chirurgischen Instituts in Stockholm, 1883, i, Heft 3, pp. 1–20, Plate I, Fig. 1.

the muscles of the blood vessels would also reply in this characteristic form, and the present investigation will show that this is indeed the

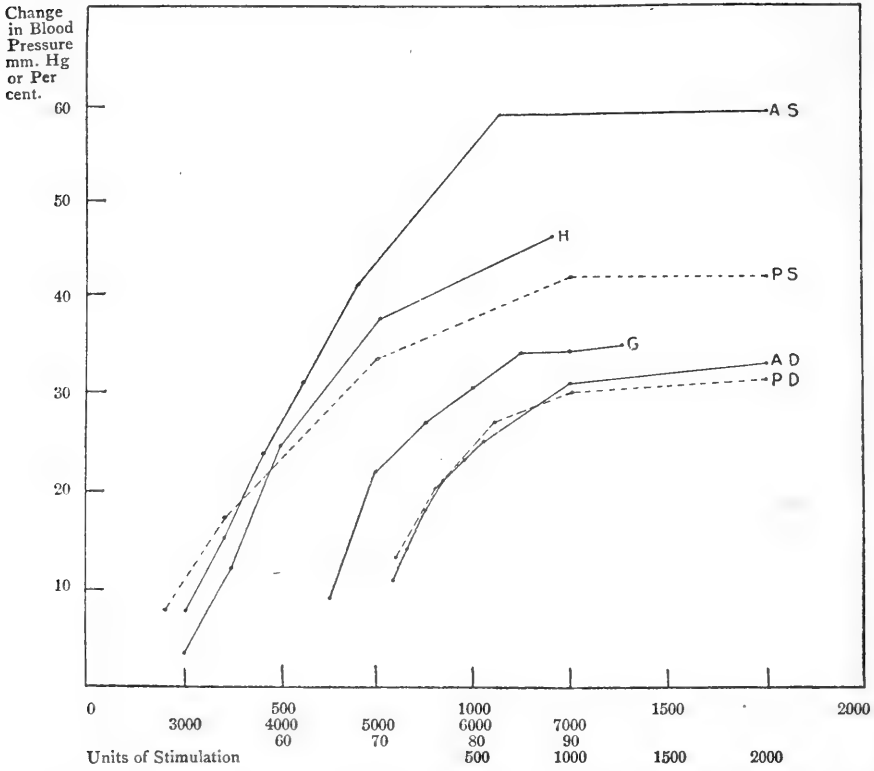


FIGURE 1. — Stimuli progressively increasing from threshold to maximal value cause progressively increasing changes in the skeletal muscles, the tonus substance of the heart, and the muscles of the blood vessels, and these changes are expressed quantitatively in curves of identical form. *A S*, the absolute rise, and *P S*, the percentile rise, in blood pressure following the stimulation of the central end of the sciatic nerve in the cat with Kronecker units from 250 to 2000. *A D*, the absolute, and *P D*, the percentile, fall in blood pressure following the stimulation of the depressor nerve in the rabbit (units 500 to 2000). *H*, the rise in the tonus contractions of the tortoise auricle stimulated directly with 3000 to 7000 units. *G*, Tigerstedt's curve of the contractions of the frog's gastrocnemius stimulated indirectly through its motor nerve (rheochord 60 to 100).

case[4] (Fig. 1). The natural load of the muscles of the blood vessels is the blood pressure: when these muscles contract, the blood pressure

[4] The fact that a strong stimulus produces a greater reflex rise of blood pressure than a weak stimulus was first noted by DITTMAR: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1870, pp. 25 *et seq.*

rises; and when they relax, the blood pressure falls. The extent of the rise or fall in blood pressure is at least an approximate index of the extent of their contraction and relaxation.

The measurement of the contractions between the threshold and the maximal stimulus is naturally much simpler in the cardiac and skeletal muscle than in the muscle of the blood vessels. In the heart the stimulus is applied directly to the muscle itself; in the contractions expressed in Fig. 1, *G*, the gastrocnemius muscle was stimulated through its nerve; but in the vasomotor reflex the stimulus to reach the blood vessels must pass not only through nerve fibres but through many nerve cells. It has just been shown that the form of the minimal maximal curve is not distorted by the intervention of nerve fibres. These act like the indifferent conductors that they are. But what of the nerve cells, which, in the physiological tradition, have so long had a prescriptive right to molest the passing impulse? And, especially, what of the bulbar cells, whose autocratic control of the vasomotor reflexes was at first a wild surmise, then a dignified hypothesis, and, latterly, a dogma?

An answer to this question is not altogether hopeless, as the following reflections will show.

Stimuli desired to be of uniform strength applied to nerves containing vasomotor fibres by no means always cause uniform changes in blood pressure. The application of the stimulus and the measurement of the

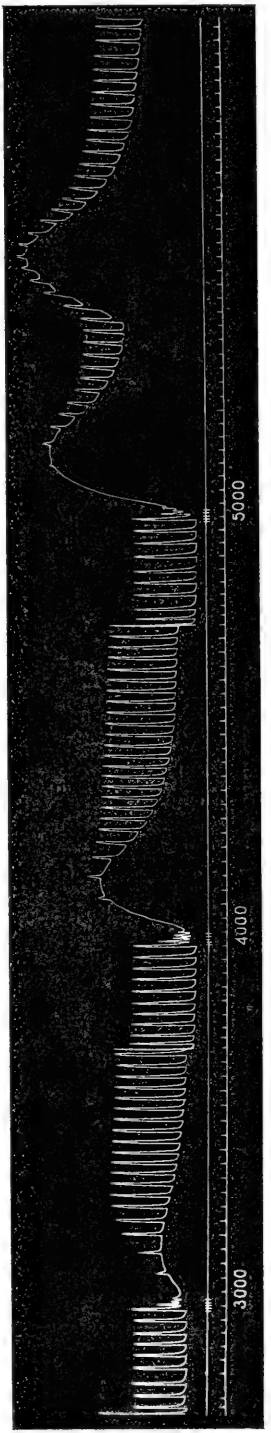


FIGURE 2. — Tonus curves from the auricle of the tortoise. The contraction is proportional to the intensity of the stimulus. The stimuli were the groups of break currents indicated on the middle line. For the first stimulus the secondary coil was at 3000 of the Kronecker scale, for the second at 4000, and for the third at 5000. The fourth tonus curve was spontaneous. The lowest line records every fifth second. (This journal, 1905, xv, p. 8.)

resulting changes in blood pressure are subject to a host of small errors inseparable from any similar operation. But these errors are "accidental."

Statisticians are familiar with the doctrine of "accidental" and "constant" errors. A marksman fires at a distant target on a calm day. Small deviations over which he has no control and which are therefore accidental cause the bullets to strike above, below, to the left, and to the right of the exact centre. If the number of shots be large, the target could be painted out and the centre would still be evident from the position of the bullet marks. They would be found almost or quite symmetrically on all sides of the centre. Those to the right would balance those to the left, and their distribution, if plotted on coördinates, would produce a symmetrical curve, the well-known curve of error.

Far different would be the result if an unsuspected wind blew across the line of fire. Then a *constant* error would operate. The flying bullets would be carried down the wind toward one side of the target. The curve of their distribution would be distorted.

A mathematical curve declares its origin; it does more than picture its constituent data — it is those data self-revealed. Hence, if a curve built up from individual observations of the same entity is symmetrical, the errors involved are accidental; in other words, their distribution is such that the errors on one side of the true value balance those on the other.[5] But if the curve is distorted or "skewed," as statisticians say, a constant error is at work.

Accidental errors in measurement would not therefore affect the form of the minimal maximal curve as an expression of the reflex contractions of the blood vessels, provided only that the number of observations be large enough to give the law of compensation free play.[6] If, on the other hand, the bulbar cells have power to change the vasomotor impulses, the exercise of this prerogative will operate as a "constant" error. In short, if the vasomotor cells are conductors merely, the form of the minimal maximal curve of blood pressures

[5] The number of observations must be large enough to make compensation possible.

[6] Evidently the number of individual observations necessary for satisfactory compensation must be far greater in the case of the complicated vasomotor procedure than with the simpler muscle and nerve preparation.

obtained by stimulating the sciatic and depressor nerves will closely correspond with the curve obtained from a muscle outside the body, but if the vasomotor cells are the masters of the reflex, the curve will not so correspond, but will show a disturbing force, just as the curves at the rifle range showed that the wind blew on one day and not on the next.

I propose therefore to measure the average change in blood pressure on stimulation of the sciatic and depressor nerves by induction currents increasing from the threshold to the maximal value, and with these fixed points to determine the form of the curve that should express the entire range between the minimal and maximal reflex. But first, a few words must be given to the operative procedure.

The vasomotor reflex from stimulation of the sciatic nerve was measured in the cat; for the depressor reflex, rabbits were used.

The cats were etherized, tracheotomized, and cannulas were placed in the carotid artery and in the external jugular vein. The artery was connected to a mercury manometer which recorded the blood pressure upon a kymograph. Curare was injected into the vein. The drug was dissolved in warm normal saline solution and the injection was made very slowly. Artificial respiration was now begun. The sciatic nerve was prepared. It was ligated and severed between knee and pelvis. The peripheral portion was used in testing the effect of the curare. The central portion was freed for a distance of about an inch and three quarters. For stimulation the nerve was lifted into the air and the electrodes were applied at least half an inch from the ligature. The ligature was kept as dry as possible, and during stimulation it was not allowed to touch the surrounding tissues. Between stimulations the nerve was replaced, covered with the neighboring tissues, and the outer wound closed with a pad of cotton wet in normal saline solution. The cotton did not touch the nerve. A very small amount of bleeding was allowed at the bottom of the wound, so that the nerve between stimulations might be bathed in an isotonic solution. The greatest pains were taken not to stretch the nerve, either during its preparation or afterward.

In the depressor observations curare was not employed; the cannula in the jugular vein was omitted and artificial respiration was also avoided.

Extreme care must be given to the ether. The least inattention

will impair the reflex. A few whiffs should be administered and the ether should then be removed. Stimulation should not be made during or very near the periods of ether inhalation.

A standard Kronecker inductorium was employed. It was graduated in Kronecker units. The primary coil was supplied from a Daniell cell of 1.1 voltage. The large smooth zinc was cleaned and amalgamated after each experiment. Except during the experiment itself the porous cup was kept constantly immersed in 20 per cent sulphuric acid.

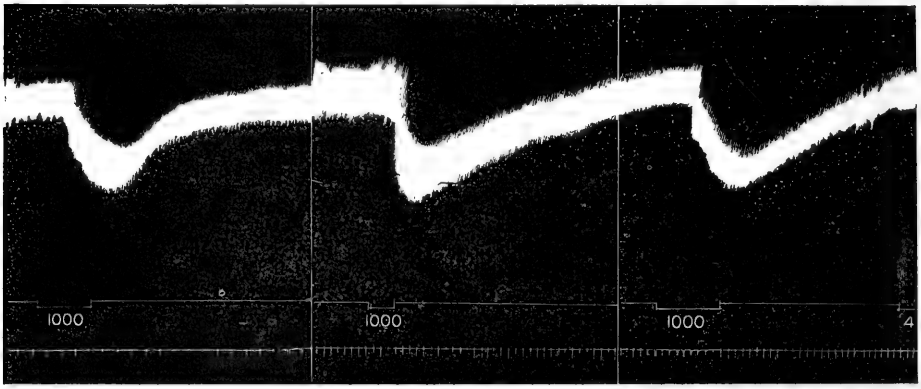


FIGURE 3. — Original size. The fall in the carotid blood pressure of a rabbit on stimulation of the depressor nerve with 1000 units at 10 A. M., 2 P. M., and 5.40 P. M. The blood pressure fell 35, 41, and 42 mm. Hg respectively. To show that the method can be safely employed for quantitative measurements. February 9, 1909.

A quantitative study of the vasomotor reflexes would have little value if the circulation and the reflex nerve apparatus were impaired by the operative procedures or the length of the experiment. It is essential that the physiological *status* be preserved. Fig. 3 is evidence that this condition was satisfied in the present experiments. In the rabbit from which the curves in Fig. 3 were taken, the first reflex was recorded about ten o'clock in the morning. Observations were repeated at frequent intervals for eight hours, but at six in the evening the depressor reflex was still unimpaired and the observations could probably have been continued some hours longer.

Since the correct form of the curve obtained depends on the accurate

placing of the points which it connects, it is desirable to inquire whether the number of observations fixing each point was large enough to permit accidental errors to be compensated. It may at once be said that the number of observations in this investigation, though fairly large for the usual research, is regrettably small for statistical purposes. Indeed, one reason for publishing these results at this time is to urge the collection of such data by many investigators. Nevertheless, the present results appear to be substantially accurate. The following illustration will be of interest. In the experiments of January 21, January 30, February 2, and February 9, the depressor nerve was stimulated eighty times with induction currents of a strength between 400 and 799 Kronecker units. The average absolute fall in the blood pressure was 24 mm. Hg, around which central value the individual observations were symmetrically grouped.

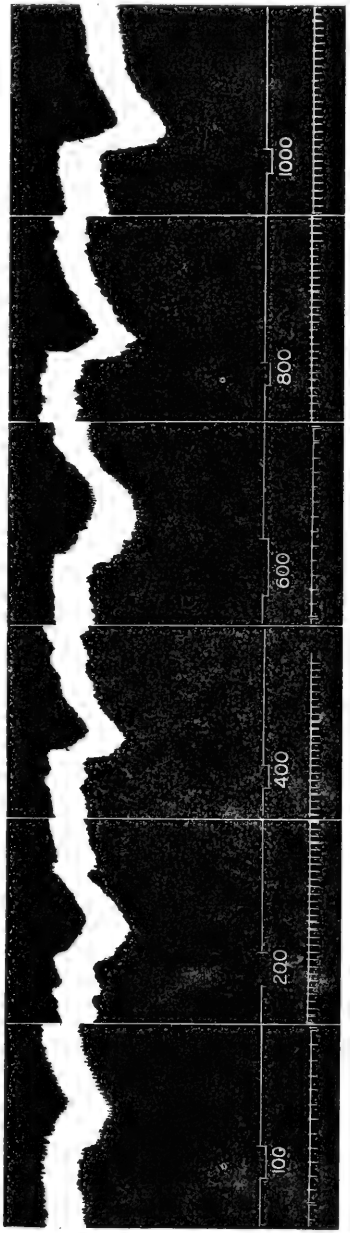


FIGURE 4. — The original size. The stimulation of the depressor nerve with 100, 200, 400, 600, 800, and 1000 units causes the carotid blood pressure of the rabbit to fall, respectively, 11, 17, 20, 25, 28, and 31 mm. Hg as registered by a membrane manometer. Experiment of February 9, 1909.

The blood pressure fell not more than	.		14	mm.	Hg	5	times.
The blood pressure fell between	.	15	and	19	"	"	14	"
The blood pressure fell between	.	20	"	24	"	"	24	"
The blood pressure fell between	.	25	"	29	"	"	20	"
The blood pressure fell between	.	30	"	34	"	"	12	"
The blood pressure fell more than	.	.	.	35	"	"	5	"

It is also desirable to inquire regarding the individual experiments. The averages here offered might be supposed a mere statistical accident. It is not to be denied that the average, or arithmetical

TABLE I.

THE PERCENTILE FALL IN BLOOD PRESSURE ON STIMULATION OF THE DEPRESSOR NERVE WITH INDUCTION CURRENTS INCREASING FROM THE THRESHOLD TO THE MAXIMAL VALUE.

Kronecker units.	Jan. 21.	Jan. 30.	Feb. 2.	Feb. 9.	Number of observations.	Average.
	per cent.	per cent.	per cent.	per cent.		per cent.
50	...	...	...	...	...	...
100	15.3	8	14.0	15.3	37	13
200	...	15	...	19.7		
300	24.9	...	22.3	...	69	20
400	...	16	...	22.1		
500	27.0	...	28.9	...		
600	...	21	...	27.5	58	27
700	34.0	...	25.4	...		
800	...	24	27.4	29.7		
900	...	...	...	...	61	30
1000	...	29	33.0	39.6		
2000	...	29	32.0	...	6	31

mean, gives no information as to the character and distribution of the individual data. The average height of buildings is very likely the same in Berlin and New York. In New York this average conceals the presence, and in Berlin it conceals the absence, of the tallest buildings in the world. Examples of the relation of individual ob-

servations to the averages in this investigation are shown in Table I, in which are presented the depressor reflexes in four animals. As might be expected, not all these rabbits give exactly the same data, but the divergence strengthens rather than weakens the use made of them in this research.

FIGURE 5. — The original size. The stimulation of the sciatic nerve with 250, 500, and 1000 units causes the carotid blood pressure of the cat to rise, respectively, 12, 26 and 54 mm. Hg. Experiments of March, 1908.

With these facts in mind the reader is invited to return to Fig. 1, in which are placed the absolute and percentile changes in blood pressure following the stimulation of the sciatic and depressor nerves. Examples of the observations upon which these curves of Fig. 1 were constructed are given in Figs. 4 and 5. It is clear that the minimal maximal curve is the same in the tonus muscle of the heart, the gastrocnemius stimulated through the sciatic nerve, and in the reflex constriction and dilatation of the blood vessels. If the central nervous system autocratically alters the impulse, the resulting contractions in muscles under its control should differ from the contractions obtained from muscles not under its control.

III.

The relation of afferent impulses to the vasomotor cells is certainly a subject of great difficulty. However clear the results of the method just presented may appear, the prudent investigator will wish to support them by a second method; and he will be glad if he can make this second method totally unlike the first. This welcome contrast has been secured in the procedure now to be described.

The problem is whether the vasomotor cells arbitrarily control the vasomotor impulse. If they do so act upon the impulse, their action should differ as their irritability differs. An impulse arriving in cells that are in a state of augmented irritability should emerge with a force greater than that imparted to the same impulse by cells in a state

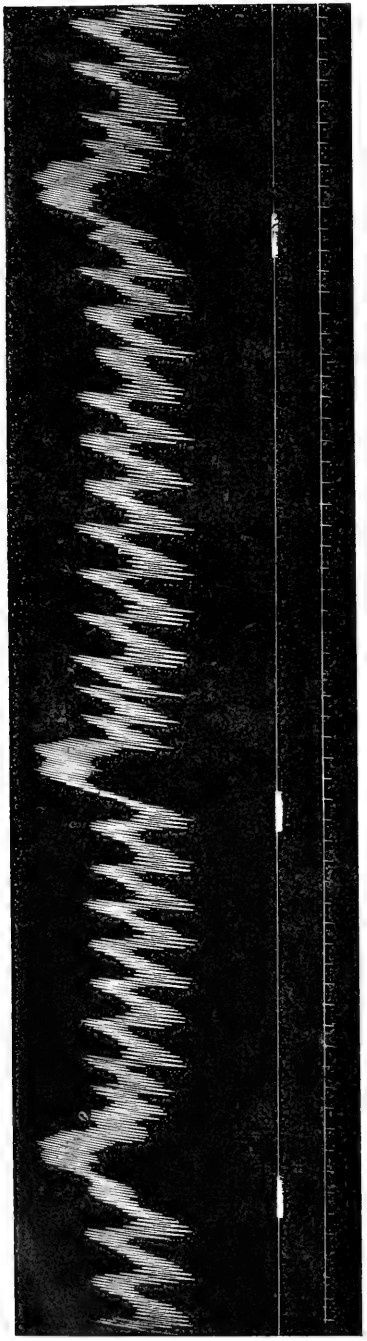


FIGURE 6. — Original size. The carotid blood pressure, recorded by a membrane manometer. A submaximal stimulus applied to the sciatic nerve at the crest of the Traube-Hering wave causes no greater rise than one applied in the trough or half-way down the slope of the wave. As in the other figures, the lowest tracing records every fifth second and the signal magnet marks the stimulus and also writes the atmospheric pressure. From an experiment upon a dog, December 23, 1909.

of lowered irritability. But to make this method practicable, it is necessary to secure wide variations in irritability. The necessary condition is obtained in the Traube-Hering phenomenon. In Figs. 6, and 7 are shown Traube-Hering waves of about twelve seconds' duration and an amplitude of about 40 mm. Hg. They were observed in dogs that had been given 5 c. c. of a 3 per cent solution of morphine and in whom a small quantity of curare had been injected into the crural vein. Artificial respiration was of course employed.

The central end of the sciatic nerve was stimulated with submaximal induction currents of uniform intensity in the trough and on the crest of the waves and at various points between. If the vasomotor cells control the reflex, the rise in blood pressure should have been greater when the stimulus passed through the cells at the height of the wave. As Figs. 6 and 7 testify, this was not the case.

IV.

When two wholly different trains of thought bear the investigator to the same point, the fact of arrival is not to be disputed, at least not by the investigator himself. When the scientist espouses a method, it should be for better or worse. Nevertheless, the writer reserves his

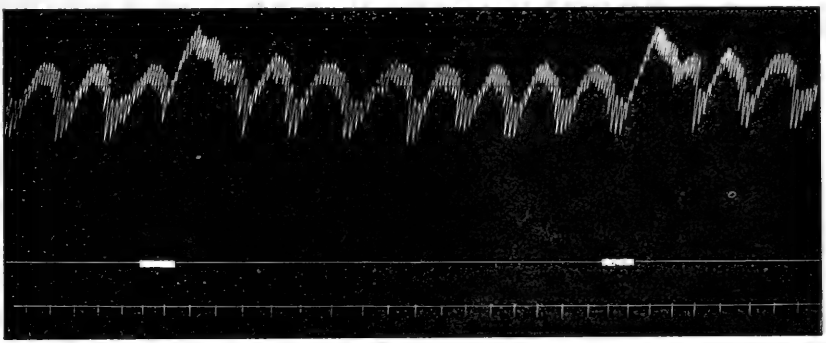


FIGURE 7. — Original size. A submaximal stimulus applied to the sciatic nerve on the downward slope of the Traube-Hering wave causes no greater rise in carotid blood pressure than one applied in the trough of the wave. The difference between the blood pressure at trough and crest is about 40 mm. Hg. There are about five waves per minute. From an experiment upon a dog, December 9, 1909.

judgment. In the present instance the problems involved are very complicated, and they are moreover of grave importance in practical medicine. The hypothesis to which this investigation directs attention has long ruled a state of thought and as a reigning theory is *ex officio* entitled to allegiance until a successor is enthroned. I would not hastily cast aside so old and so important a tool as the control of the vasomotor reflexes by the bulbar cells. It is enough if this investigation shall unmistakably mark the speculative character of that dictum and the need of further research to justify its present acceptance.[7]

[7] It is perhaps advisable to point out that the two methods here described may be usefully applied to other problems in physiology.

THE PHYSIOLOGY OF CELL DIVISION. — III. THE ACTION OF CALCIUM SALTS IN PREVENTING THE INITIATION OF CELL DIVISION IN UNFERTILIZED EGGS THROUGH ISOTONIC SOLUTIONS OF SODIUM SALTS.

By R. S. LILLIE.

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

IN a recent paper[1] I described the results of experiments in which the unfertilized eggs of Asterias forbesii and Arbacia punctulata were exposed for brief periods to pure isotonic solutions of neutral salts of alkali metals and were then returned to sea water. Salts of strong acids with monovalent anions were used, so chosen with the aim of avoiding the possible influence of variations in hydrolytic dissociation, valence of the anion, or calcium-precipitating power, while giving a series whose successive members should exhibit increasing action in promoting colloidal dispersion, namely, chloride, bromide, chlorate,[2] nitrate, iodide, sulphocyanate. It was found that membrane formation and parthenogenetic development could be induced in both eggs by this treatment, but that the two species showed a decided difference in readiness of response: thus with Asterias the more weakly acting salts, chloride and bromide, produced a large proportion of membranes and cleavages, while with Arbacia these salts were almost ineffective, and only those with strongly acting anions, iodide and sulphocyanate and to a less degree nitrate, showed well-marked action. I have since found that chlorate resembles chloride and bromide, though somewhat more active than either, producing membranes readily in starfish eggs, but in only a small proportion of sea urchin eggs.

I suggested that this contrast in responsiveness to the above treatment is to be correlated with the more general physiological con-

[1] Lillie: This journal, 1910, xxvi, p. 106.

[2] Used for the first time last summer (1910).

trast shown by these eggs; parthenogenetic development is much more readily induced in Asterias than in Arbacia, and the former eggs are more easily injured, and hence less uniform in their behavior, and die sooner after maturation unless fertilized. On the view that the critical change in fertilization, as well as in many forms of toxic action and in natural death, is an increase in the permeability of the plasma membrane — only temporary and reversible in the former case — the contrast indicates the existence of a thinner or less resistant plasma membrane in the starfish than in the sea urchin egg. With the latter eggs, having a relatively resistant membrane, only the more active salts are effective in producing the initial rapid increase of permeability with consequent membrane formation followed by cleavage; and the comparative efficacy of the salts in the above series shows an unmistakable parallelism with their comparative power (a function of the nature of the anion) of increasing colloidal dispersion. In Asterias, however, with an easily modified plasma membrane, all of the salts, including chloride, have well-marked and rapid action.

The composition and physical consistency of the plasma membrane are thus to be regarded as important factors in determining what response a particular species of egg will make to treatment by a given parthenogenetic method. It is to be expected that any egg exhibiting unusual powers of resistance to unfavorable conditions, or living relatively long in the unfertilized condition between its maturation and its natural death — peculiarities indicating a plasma membrane of more than average impermeability — will require relatively vigorous treatment to call forth the desired response. This may well be the reason why methods that are perfectly effective with sea urchin eggs produce no impression on (*e. g.*) frogs' eggs. On such an hypothesis, at least, the choice of an appropriate method for a given egg ceases to be a purely empirical matter. Such considerations may suggest appropriate forms of treatment for eggs that have hitherto proved refractory.

The experiments about to be described were performed at Woods Hole during the past summer and form a continuation of those described in the preceding paper. The relation of the increase in permeability induced by the pure salt solution to the initiation of cell division has been again the chief question under consideration. I have therefore compared the action of the pure salt solution with

that of the same solution *plus* a small quantity of calcium or magnesium chloride. The addition of this substance prevents or retards the increase in permeability resulting from the action of the pure salt solution; this, there is good reason to believe, is the basis of the well-known "antitoxic" action of salts.[3] If the initiation of cleavage is due to a similar increase in permeability, the addition of any salt with typical antitoxic action should prevent this result. This has been found to be the case. Experiments were conducted with both Asterias and Arbacia. In the present paper the conditions in Asterias will be more particularly considered. The experiments with Arbacia will be described in more detail in a later paper.

I shall first consider the nature of the changes produced by prolonged exposure to the pure salt solution. Unfertilized mature eggs of Asterias washed in sea water and then transferred to a pure isotonic solution of one of the above salts of (*e. g.*) sodium undergo a progressive series of changes of a very definite kind. Within a few minutes after transfer to the pure salt solution (*e. g.*, 55 m. NaBr) the eggs show a decided tendency to form stringy masses or to agglutinate; the protoplasm appears under the microscope at first unaltered, that is, clear and translucent, but gradually becomes coarser and darker, and within a certain time, varying with the salt, temperature, and condition of the egg, it acquires an opaque and coagulated appearance. The time required for complete coagulation is variable; but typically at normal summer temperature all the eggs in a pure .55 m. NaCl solution are completely opaque and dead within three hours; the onset of death coagulation is more rapid in .55 m. NaBr and $NaClO_3$ solutions and most rapid in .55 NaI and NaCNS. If left still longer in the solutions, the coagulated eggs swell — most slowly in .55 m. NaCl and most rapidly in .55 m. NaCNS and .55 m. NaI — and eventually disintegrate. The above-described changes are of the nature of cytolysis; the coagulation which precedes the swelling is, however, highly distinctive. It invariably takes place both when the eggs are killed by poisons (as saponin) and when they die naturally, as unfertilized mature starfish eggs left in sea water usually do within eighteen hours after removal from the animal. Loeb has shown that

[3] *Cf.* my experiments on Arenicola larvæ, this journal, 1909, xxiv, pp. 14, 459; *cf.* especially pp. 24, 25. Other kinds of antitoxic action have probably an essentially similar basis.

in the latter case the change is independent of bacterial action or other external injury. It is, in brief, a natural process in which oxidations are fundamentally concerned, since eggs kept in sea water freed from oxygen or containing cyanide may live some days without dying or coagulating.[4] One effect of normal or parthenogenetic fertilization is thus to prolong the life of the egg. The above results show that the coagulative change is greatly expedited by the action of pure salt solutions. To what extent oxidative processes are concerned in this salt action is not yet known; the addition of a little calcium or magnesium chloride to the pure solution checks both the agglutinative and coagulative action of the pure solution, so that the essential change appears to consist simply in a marked increase of permeability. In Arbacia eggs, which contain a red pigment, the increase of permeability in the pure solution is rendered more directly evident by the outward diffusion of this substance. A coagulative change also occurs in these eggs under the above conditions, but more slowly;[5] both changes are checked by the addition of a little calcium.

It should be noted that this change, coagulation at death or under the influence of toxic or other unfavorable action, is not peculiar to egg cells, but is shown by all forms of protoplasm; it is, in fact, a general characteristic of living cells.[6] It is associated with an in-

[4] J. Loeb: Archiv für die gesammte Physiologie, 1902, xciii, p. 59.

[5] Loeb (Biochemische Zeitschrift, 1906, ii, p. 81) finds that in Strongylocentrotus, a very resistant egg as shown by its living unfertilized for forty-eight hours in pure isotonic NaCl solution, the toxicity of pure or slightly alkaline salt solutions is greatly diminished by removal of oxygen or addition of cyanide. He concludes that increased or misdirected oxidations are a main source of the toxic action of salt solutions on these eggs. The conditions in Asterias suggest rather, in my opinion, that oxidations are in themselves a direct cause of rapid increase in the permeability of the plasma membranes, and that the injury is to be ascribed primarily to this latter change. Of course, if the rate of oxidations is dependent on the permeability of the plasma membrane, as I have maintained elsewhere, increase in permeability may in itself result indirectly in an injurious increase of misdirected oxidations; but the latter change would be a secondary one, like the increased oxidations in the early stages of death rigor. There is undoubtedly an intimate connection between the rate of oxidations and the condition of the plasma membrane (*cf.* Warburg: Zeitschrift für physiologische Chemie, 1910, lxvi, p. 305).

[6] *Cf.* my paper, this journal, 1908, xxii, p. 75, especially pp. 81–82. I have discussed briefly the essential nature of the connection between increase of surface permeability and death coagulation in this paper.

crease of surface permeability; the diffusion of substances into and out of cells that have undergone death coagulation occurs with great readiness; pigment-containing cells, like sea urchin eggs, lose pigment, and in all cases the protoplasm becomes readily penetrable to dyes and to other substances formerly unable to pass the surface film (thus plasmolysis is no longer possible). The increase of permeability is undoubtedly the primary change, and coagulation of the protoplasmic colloids is one of its secondary consequences. The association of protoplasmic coagulation with the death process is thus simply another indication that the latter is associated with an increase of surface permeability sufficient to destroy the normal or physiological semi-permeability. Since such a change destroys the normal structure of the cell by altering the physiological state of aggregation of its colloids, and profoundly alters its chemical constitution by allowing free diffusion of crystalloidal compounds through the plasma membrane, the properties of the cell as a living system are soon irrecoverably lost.

To explain this connection between increase of surface permeability and coagulation of the protoplasmic colloids, I advanced some time ago the following hypothesis, which it seems desirable to restate here in somewhat amplified form. The clear translucent appearance of living protoplasm indicates an extremely fine dispersion of the cytoplasmic colloids. The electrical charge of the colloidal particles is undoubtedly negative; and the fineness of the subdivision indicates the existence of a considerable potential difference across the surface of the particles, corresponding to the low surface tension which such fine subdivision implies. It is assumed that this condition is promoted by the electrical polarization of the plasma membrane which allows certain cations (supposedly H-ions) to pass, but not anions. The influence of the cations on the colloids within the membrane is thus reduced, while that of the anions remains unaltered. Now, if the potential difference across the interface of each colloid particle with the medium be determined (1) by the specific ion-liberating properties of the colloid and (2) by the difference between the respective adsorptions [7] (or other form of combination) of anions and cations in

[7] For evidence that the hypothetical ion-protein compounds of Loeb and Pauli are of the nature of adsorption complexes, *cf.* Pauli: Beiträge zur chemischen Physiologie und Pathologie, 1908, xi, p. 415.

the medium, it is clear that conditions tending to diminish adsorption of cations will increase the negative charge on the colloid. These conditions exist in the cell because of the ability of certain cations to penetrate the plasma membrane, producing the characteristic polarized condition of the plasma membrane, whose outer surface appears to be about one tenth of a volt more positive than its inner. The consequence of this will be that the portion of the adsorption potential [8] at the surface of the colloidal particles due to adsorption of cations by the particles will be correspondingly decreased. The negative charge on the colloid will thus be increased by the physiological polarization of the plasma membrane, and a low surface tension (possibly negative in value) of the colloid particles, with consequent fine subdivision, will result. This is the condition in the resting cell during life. Decrease in the polarization of the plasma membrane following increased permeability will increase the adsorption of cations by the colloidal particles, decrease their negative charge, and hence increase their surface tension.[9] Coagulation will result if this increase in surface tension is sufficient and lasts sufficiently long.

The immediate action of the pure salt solution and presumably of other membrane-forming agencies on unfertilized eggs is thus to produce an increase in the permeability of the plasma membrane. Such a change in a system like the living cell involves far-reaching consequences. Increased rapidity of interchange across the plasma membrane will follow, and probably an increased rate of oxidations. It is known that cells when dying evolve carbon dioxide in increased quantity: thus the carbon dioxide output of muscle increases markedly at the onset of rigor; the increase in oxidation following fertilization [10] is thus, in all probability, to be referred, at least partly, to the increase in permeability. On the present view these and other changes in the egg system, conditional on temporarily increased permeability, initiate, in some manner as yet mainly obscure, the complex series of

[8] The potential due to unequal adsorption of anions and cations at the surface. *Cf.* Michaëlis: Dynamik der Oberflächen, Dresden, 1909.

[9] Stimulation of a contractile tissue probably involves this kind of change, the surface tension of the contractile elements undergoing increase with increase in the permeability of the plasma membrane.

[10] Lyon: Science, 1904, N. S. xix, p. 350; Warburg: Zeitschrift für physiologische Chemie, 1908, lvii, p. 1.

chemical and physical changes of which cleavage and development are the normal expression.

It should be emphasized that any marked increase of permeability under physiological conditions can be only temporary and not too prolonged, otherwise injurious alteration of cell organization must follow. It has, in fact, usually been found to be essential that the eggs should be exposed to the membrane-forming conditions for only a brief period, a few minutes at most, and should then be returned to normal sea water. In the case of sea urchin eggs, subsequent treatment with hypertonic sea water, cold, or lack of oxygen greatly increases the proportion of developing eggs; and we may infer from this, in conformity with the above hypothesis, that the essential effect of such after-treatment is *to restore the normal permeability*. The whole procedure would then produce, first, a well-marked and rapid increase of permeability; this condition would then be partly reversed by transfer to sea water and completely by further treatment with hypertonic sea water or cyanide. Presumably a rhythm of increased followed by decreased permeability is thus started; such an alternate increase and decrease of permeability is, on the present view, an essential condition of cell division.[11]

If an initial rapid increase in permeability is the first critical change in the initiation of cleavage, it is clear that any conditions that check or inhibit this process should also prevent fertilization, whether normal or artificial. The addition of small quantities of calcium chloride to pure sodium salt solutions has a marked action in preventing the increase of permeability normally induced by the pure solu-

[11] It seems probable from LOEB'S researches with ELDER (Chemische Entwicklungserregung, p. 249) that in normal fertilization the superficial action of the spermatozoon, which apparently increases permeability, must be followed by a second action, exercised after the sperm has penetrated the egg, and which restores the latter to a state favorable for normal development. I make the suggestion, in conformity with the above, that this second action consists essentially in restoring the normal permeability. The spermatozoon thus first by its contact increases, and then by its penetration decreases, the permeability of the egg. This is probably not by any means the whole matter; but the continued life of the fertilized egg demonstrates that its permeability is normal, while the fact that most eggs die soon unless penetration occurs indicates that abnormal permeability persists under these conditions. This hypothesis should be susceptible of experimental confirmation or disproof.

tion. I have found that it also checks or prevents membrane formation and initiation of cell division by the above salts.[12]

The addition of small quantities of calcium and potassium salts to pure solutions of sodium salts has long been known, since the researches of Sidney Ringer, to diminish the toxicity of such solutions. The researches of J. Loeb and his pupils have since shown that a large proportion of plurivalent cations have this "antitoxic" power — so called by Loeb to draw attention to the resemblance to the toxin-antitoxin relation. Now the toxic action of pure solution of various sodium and potassium salts on sea urchin eggs runs parallel with their laking or permeability-increasing action, and is probably directly dependent on the latter, increase of permeability beyond a critical degree being in itself destructive to the cell.[13] This increase in permeability, as shown in sea urchin eggs by the diffusion of the characteristic red pigment into the solution, may be checked or prevented by the addition of salts of calcium or other appropriate metal to the solution. The following experiment will illustrate. Equal quantities of Arbacia eggs were placed in a series of twelve test tubes to the depth of *ca.* one-half inch in each; the sea water was removed as far as possible and the following solutions were added: (A) pure .55 m. NaCl, NaBr, $NaClO_3$, $NaNO_3$, NaI, NaCNS, and (B) the same series *plus* 1 c.c. $m/2$ $CaCl_2$ to each 20 of solution. Within five minutes the eggs in the pure solutions .55 m. NaI and .55 m. NaCNS showed exit of pigment; .55 m. $NaNO_3$ showed perceptible extraction within ten minutes, and the others not till considerably later. But all the calcium-containing solutions remained perfectly colorless for many hours; next day, after eighteen hours, all were colorless except those of iodide and sulphocyanate, the most toxic salts. The eggs were then transferred to sea water and sperm added; of those left in the pure solutions none developed, while those from the calcium-containing solutions gave larvæ in all cases — though few from the iodide solution — except the sulphocyanate. Antitoxic action and prevention

[12] Newman, working with Mathews, found calcium salts particularly effective in preventing the normal fertilization of Fundulus eggs by spermatozoa. The effect was reversible. This action is similar to the above; the calcium salt prevents the initial increase of permeability which the sperm or the salt would otherwise produce. *Cf.* Newman: Biological bulletin, 1905, ix, p. 378.

[13] R. Lillie: This journal, 1910, xxvi, pp. 116 *et seq.*

of increase of permeability thus run parallel; the antitoxic action is less complete with the more toxic salts iodide and sulphocyanate than with the others. In all cases, however, the increase of permeability, with the associated toxic action, is greatly checked.[14]

Calcium similarly antagonizes the membrane-producing and cleavage-initiating action of the pure solution. In the following series of experiments the eggs were allowed to mature and were then exposed for brief periods — five and ten minutes — (*a*) to the pure solution and (*b*) to the same solution *plus* a small quantity of *m*/2 $CaCl_2$; they were then returned to sea water. The result has always been that eggs thus exposed temporarily to the pure solution showed a large proportion of membranes and form changes and developed in a considerable proportion of cases to blastulæ; while those similarly exposed to the calcium-containing solutions remained — with a few exceptions, especially with iodide and sulphocyanate solutions — essentially unaltered. The presence of calcium, in other words, prevents the initiation of cell division through the action of the salt. In each series of experiments some eggs were allowed to remain in the pure solution and in the calcium-containing solution; the former always underwent rapid agglutination followed by coagulation, while

[14] The action of calcium salts in checking hæmolysis by various toxic substances (saponin, digitalin, quillain) is an instance of the same kind (*cf.* J. B. MacCallum: University of California publications, 1905, ii, pp. 87, 93). So long as a cell retains the normal impermeability of its plasma membrane to diffusible substances, the latter, if toxic, can have little injurious action. It seems, however, to be a general characteristic of toxic substances, of whatever nature, to increase the permeability of cells. Their action is thus twofold: first, they break down the barrier normally existing between the cell and its environment, a change which, besides destroying the osmotic equilibrium, permits abnormal loss of diffusible constituents from the protoplasm and entrance of abnormal substances from outside; and, second, they may then enter the cell and exert a destructive action on the protoplasm itself. The presence of any substance which opposes this increase of permeability has thus necessarily an antitoxic action. In former papers I attempted thus to explain the antitoxic action of salts; *cf.* This journal, 1909, xxiv, pp. 14, 459; such an explanation, if valid for salts, is almost undoubtedly valid for antitoxic action in the more general sense. *Antitoxins would thus protect cells against the permeability-increasing action of toxins.* This seems unquestionably true in the case of hæmolysins and anti-hæmolysins. If agglutination is also a consequence of increased permeability, as I have elsewhere maintained (*Loc. cit.*, p. 21), the anti-bodies act by preventing increase of permeability also in this case.

the latter remained normal in behavior and in appearance, with clear protoplasm and capable of fertilization for several hours, showing, in fact, little difference from eggs kept in normal sea water. Eventually such eggs die and coagulate, as do also eggs in normal sea water, but the contrast to those left in the pure solution is always marked, especially with the less toxic salts, chloride, bromide, nitrate, and chlorate; in the case of iodide and sulphocyanate the toxic action is less completely checked by the calcium.

The series on page 299 is typical.

A marked contrast is thus shown between the effects of the pure and of the calcium-containing solutions, the latter having little or no action in increasing the proportion of parthenogenetically developing eggs. It should be added that the eggs used in this series were not entirely normal and showed rather more than the average tendency to spontaneous membrane formation and change of form; this tendency was undoubtedly spontaneous, since (1) the animals were all thoroughly washed in fresh water before being opened, and (2) what cleavage appeared was much slower and less regular than in the fertilized control, and no eggs developed to the blastula stage. It should also be noted that the calcium-containing solutions are not absolutely indifferent in their action on starfish eggs; they usually show some slight action in increasing the proportion of spontaneously cleaving eggs; this is especially likely to be the case with the more strongly acting salts sulphocyanate and iodide; in the above series the calcium-containing nitrate solution showed noticeably greater action of this kind than the other calcium-containing solutions. In view of the susceptibility of starfish eggs to the action of membrane-forming agencies this is not surprising, since a solution of sodium chloride containing calcium is far from being precisely equivalent to sea water.

[15] This tendency to spontaneous membrane formation and form change is very frequently seen in starfish eggs that have lain some hours in sea water. It is not to be regarded as evidence of the accidental presence of spermatozoa, since the succeeding cleavage is imperfect and development rarely goes far, though occasionally blastulæ are formed. The starfish egg is on the verge of parthenogenesis, and cases of natural parthenogenesis are known (Greef and others). Eggs exhibiting such spontaneous development are always few and often absent. It is evident, however, that in parthenogenetic experiments with starfish comparison of the experimentally treated eggs with untreated controls is especially necessary. Such spontaneous development is never seen in sea urchin eggs.

TABLE I.

June 18, 1910. — The starfish used were washed in tap-water to destroy any adhering spermatozoa, and the eggs from several animals were used. Most of these eggs underwent apparently normal maturation. A few after some hours showed membranes and irregular form change.[15] The eggs were exposed to the following solutions for five and ten minutes in each case with the following results:

Solution.		Time of exposure and result.
A. .55 m. NaCl.	1. 5 m.	Good proportion of membranes. A few larvæ.
	2. 10 m.	Considerable number of larvæ (< A1).
A *ca.* .55 m. NaCl (250 c.c.). + $m/2$ $CaCl_2$ (12.5 c.c.).	3. 5 m.	Little change in three and one-half hours. Next day a few membranes; all dead and coagulated. No larvæ.
	4. 10 m.	Like A_3. (One feeble abnormal larva found.)
B. .55 m. NaBr.	1. 5 m.	Fair proportion of membranes and irregular forms after three and one-half hours. A few larvæ next day.
	2. 10 m.	Like B_1, but larvæ more numerous and more active.
B *ca.* .55 m. NaBr (250 c.c.). + $CaCl_2$ (12.5 c.c. $m/2$).	3. 5 m.	A few membranes, imperfectly separated; practically like unfertilized control. No larvæ.
	4. 10 m.	Similar to B_3, but one larva found.
C. .55 m. $NaClO_3$.	1. 5 m.	After three and one-half hours a somewhat small proportion of membranes and irregular forms. A fair proportion of larvæ next day.
	2. 10 m.	More favorable than C_1; considerable number of larvæ.
C *ca.* .55 m. $NaClO_3$ (250 c.c.). + $CaCl_2$ (12.5 c.c. $m/2$).	3. 5 m.	Little change in three and one-half hours; a *few* membranes and irregular forms. No larvæ next day.
	4. 10 m.	Like C_3. Very few form membranes. No larvæ.
D. .55 m. $NaNO_3$.	1. 5 m.	Membrane formation in a somewhat small proportion. A fair proportion of larvæ, irregular and feeble.
	2. 10 m.	More favorable than D_1; considerable number of larvæ, many active and vigorous.
D *ca.* .55 m. $NaNO_3$ (250 c.c.). + $CaCl_2$ (12.5 c.c. $m/2$).	3. 5 m.	Little change, but a *few* form membranes and cleave; one or two feeble distorted larvæ found.
	4. 10 m.	Practically like D_3; one or two feeble larvæ.

Control of unfertilized eggs. — All are dead and coagulated next day. Almost all of the coagulated eggs are compact and without separated membranes; a few, however, show widely separated membranes; such eggs show more complete disintegration. No larvæ.

Control of sperm-fertilized eggs. — Membrane formation is defective in many eggs and the majority die before reaching a larval stage. Many form larvæ, largely irregular or thick-walled.

Eggs remaining in the pure solutions (A, B, C, D). — After three hours all are coagulated and opaque. A few have membranes, most not. The tendency of the eggs to cohere or agglutinate in the pure solution increases in the order $NaCl < NaBr < NaClO_3 < NaNO_3$ (condition after seven minutes in the solutions). The eggs were returned to sea water after three hours and sperm was added; none developed.

Eggs remaining in the Ca-containing solutions (A *ca, etc.*). — After three hours all have clear normal-looking protoplasm as in the unfertilized control. The tendency to agglutinate is absent, there being only slight coherence as in normal sea water. On transfer to sea water and fertilization with sperm after three hours in the solutions, each lot yields a considerable number of blastulæ.

The further addition of magnesium and potassium salts, as in van't Hoff's solution, is necessary to produce a practically indifferent medium. The contrast between the action of the calcium-containing solution and that of the pure salt is, however, invariably a striking one.

Two exact repetitions of the above series gave the same result. Experiments with sodium and potassium iodides and sodium sulphocyanate showed a less decided difference between the pure and the calcium-containing solutions. The cytolytic and coagulative action of the pure solution is markedly diminished by adding calcium, but the prevention of membrane formation and development is less complete with these salts than with those having less strongly acting anions; with sodium sulphocyanate, in fact, comparatively little difference was found between the pure and the calcium-containing solution.[16]

In one series magnesium chloride, as well as calcium chloride, was used to offset the permeability-increasing action of sodium chloride. Eggs were exposed for five and ten minutes as above to (1) pure .55 m. NaCl, (2) a mixture of 250 c.c. .55 m. NaCl and 15 c.c. *m*/2 $CaCl_2$, and (3) 250 c.c. .55 NaCl *plus* 18 c.c. *m*/2 $MgCl_2$. Magnesium showed the same action as calcium, but was somewhat less effective in preventing cytolysis; in correspondence with this difference more eggs were found to form membranes and to cleave after treatment with the magnesium-containing than with the calcium-containing solution. Only a small proportion of eggs, however, were thus affected as compared with those treated by the pure solution which formed membranes on the majority of eggs. Doubtless salts of other bivalent metals would be found to act similarly; experiments with these have not yet been tried.

Nature of the membrane-forming action. — The separation of a sharply defined thin film or membrane from the surface of the egg immediately after fertilization is highly characteristic of echinoderm eggs but not of eggs in general; it must therefore be regarded as dependent on special conditions peculiar to these and a few other eggs (certain Mollusca, Amphioxus). What appears to be fundamental and universal in the

[16] This is in agreement with the general experience as to the relative difficulty of counteracting the action of these salts by the addition of others. *Cf. e. g.* my results with cilia, this journal, 1906, xvii, pp. 104 *et seq.*

fertilization process is a temporary initial increase in surface permeability, and the separation of the surface film in certain eggs is, I believe, to be regarded as a largely incidental consequence of this primary change. Since the volume of fluid enclosed by the membrane — which at first is indistinguishable from the general cell surface — increases immediately after fertilization, an increase in the effective osmotic pressure of the cell contents against the membrane is indicated. This points to a disturbance of osmotic equilibrium as the essential condition; the observed effect would result from an increase in the permeability of a detachable surface film sufficient to allow ready passage of salts but insufficient for the passage of the more complex diffusible substances and colloids in the surface layers of the protoplasm; under these conditions the outwardly directed osmotic pressure of the latter substances would no longer be equilibrated by the salts of the external medium and the volume of fluid enclosed by the membrane would increase. This is what actually happens.

It is characteristic of echinoderm eggs that the surface of the egg beneath the detached membrane remains sharply defined, while the volume of the egg remains practically unaltered. The space between the egg surface and the separated membrane is occupied by a clear fluid which apparently contains colloidal material derived from the egg.[17] Loeb has suggested that on fertilization a colloidal substance is set free in the superficial layer of protoplasm by a cytolytic action, and absorbs water or swells, pushing a modified portion of the surface film away from the egg surface. I believe that in its main features this explanation is essentially correct, but suggest further that *what conditions this swelling* is simply an increase in the permeability of the surface film, so that the osmotic pressure or "Quellungsdruck"[18]

[17] Herbst: Biologisches Centralblatt, 1893, xiii, p. 14; J. Loeb: Archiv für Entwicklungsmechanik, 1908, xxvi, p. 82.

[18] It is not clear to me that any fundamental distinction has been established between these two phenomena. As I have already urged (This journal, 1907, xx, p. 127; *cf.* pp. 133, 140), the absorption of water by a liquid colloidal system separated from the solvent by a membrane — a process usually ascribed to osmotic pressure — appears to be essentially the same phenomenon as the absorption of water by a solid colloid — in this case called *swelling*. Pauli and Handowsky have maintained that the osmotic pressure of protein solutions is a phenomenon of swelling or hydration different from true osmotic pressure (Biochemische Zeitschrift, 1909, xviii, p. 340); and Handowsky states that there

of this colloid substance (which need not necessarily be set free *at* the time of fertilization) is no longer equilibrated by that of the external salts. The film is then separated from the egg surface as the colloid absorbs water.[19] This view implies the essential identity of the fertilization membrane with the plasma membrane. So far as can be distinguished by observation with the water-immersion lens, the most external layer of the unfertilized egg of Arbacia and the fertilization membrane are identical in thickness and optical properties. They are probably therefore composed of the same material. The separated membrane, however, has different osmotic and probably other properties from the surface film of the egg; thus it is demonstrably freely permeable to salts, though impermeable to colloids like serum albumin or egg albumin[20] and difficultly permeable to sugar;[21] whereas the plasma membrane of the unfertilized egg is practically impermeable to salts. This is shown by the fact that the whole egg including the visible surface film shrinks when placed in hypertonic sea water; if this film — which is afterwards, on the present view, separated as the fertilization membrane — were, like the latter, freely permeable to salts, the egg ought to shrink away from the membrane.[22] The separation of the two cannot, however, be accomplished until after normal or parthenogenetic fertilization; while immediately after this event

is no corresponding depression of the freezing point in such solutions (Kolloid-Zeitschrift, 1910, vii, p. 193). This appears to me thermodynamically impossible; a solution exhibiting a "swelling pressure," as well as one showing "true osmotic pressure," must have a lower vapor tension than the pure solvent in proportion to the height to which the pressure can raise the level of the solution above that of the pure solvent. If its vapor tension is lower, its freezing point, *i. e.*, the temperature at which contiguous solid and liquid phases are in equilibrium, must also be proportionately lower. The explanation of the failure to detect any depression of the freezing point in protein solutions exerting an osmotic or swelling pressure simply lies, in my opinion, in the fact that such determinations become uncertain when the osmotic pressure is very low.

[19] "A small concentration of sugar or protein, even though its osmotic pressure were far less than that of sea water, would be capable of absorbing sea water through a membrane perfectly permeable to sea water." E. N. Harvey, Journal of experimental zoölogy, 1910, viii, p. 363.

[20] Loeb: *Loc. cit.*

[21] Harvey: *Loc. cit.*

[22] Unless, indeed, there were close adhesion between the two — a supposition which seems inconsistent with the ease of separation of the membrane.

the film separates from the egg surface and proves itself, on examination, to be freely permeable to salts but impermeable to colloids. The facts thus seem clearly to indicate that the surface film of unfertilized eggs, like plasma membranes in general, is semi-permeable in relation to the salts of the medium, and loses this semi-permeability temporarily in consequence of fertilization; it is then separated from the surface as a result of absorption of water by the superficial colloid substance while it is yet impermeable to the latter. This altered surface film (which probably represents only the most external layer of the true plasma membrane, since it is unaffected by lipoid solvents) is the fertilization membrane. A new plasma membrane with a surface film similar to the original is normally soon afterward re-formed by the protoplasm (see below, p. 305, footnote).

Further evidence that the egg surface becomes more permeable to dissolved substances immediately after fertilization has during the past summer been brought forward by McClendon,[23] Harvey,[24] Lyon and Shackell[25] and by Loeb[26] himself. These investigators agree in finding that the rate of diffusion of substances into or out of eggs undergoes marked increase shortly after fertilization. In harmony with these observations I may cite another of apparently different nature which I have frequently made with Asterias eggs: when treated with isotonic sodium chloride for five minutes or warmed to 35° for thirty seconds, not all of the mature eggs form membranes, and of those which do only a small proportion undergo favorable development; the remainder, usually after undergoing irregular cleavage or change of form, die and disintegrate. When the eggs are examined after eighteen hours, a striking contrast is invariably shown between the eggs with membranes and those without; the latter, while dead and coagulated, are only slightly swollen and present a compact ap-

[23] McClendon: Science, 1910, xxxii, pp. 122, 317.

[24] Harvey: Science, 1910, xxxii, p. 565.

[25] Lyon and Shackell: Science, 1910, xxxii, p. 249.

[26] J. Loeb: Science, 1910, xxxii, p. 411. Loeb had formerly (*cf.* Biochemische Zeitschrift, 1906, ii, p. 87) considered the possibility that the greater toxicity of pure NaCl solutions on the fertilized as compared with the unfertilized egg was due to the greater ionic permeability of the former; in the paper cited he rejects this explanation in favor of one based on differences in the oxidative metabolism of the two kinds of eggs. There is, however, no conflict between these two possibilities, since changes in permeability and in rate of oxidation appear to go hand in hand.

pearance and definite outline; while the eggs with membranes are always found to have undergone extensive disintegration and are usually converted into masses of loose detritus filling the whole space enclosed by the distended fertilization membranes.[27] It is plain that in these eggs the resistance to swelling or disintegration is slight compared to that of the eggs without membranes. This can only mean that the surfaces of eggs with separated membranes have an unusually high permeability; that an effective surface of separation between egg and medium has in fact practically ceased to exist. While the phenomena of dead eggs can be regarded as throwing only a partial light on the conditions during life, the above contrast is so constant and striking as to leave no doubt of a marked difference in permeability between the two classes of eggs.

Eggs like those just described appear to have undergone the typical coagulative disintegration or cytolysis as a result of the destruction of the semi-permeable properties of their plasma membranes. Loss of semi-permeability, if the present point of view is valid, is a necessary accompaniment or consequence of the change leading to membrane formation; hence such a change, if not reversed, must eventually lead to the dissolution of the egg protoplasm. This, I believe, is why the majority of eggs (particularly sea urchin eggs) subjected to a simple membrane-forming treatment die without development. In order that favorable development shall follow, the initial increase of permeability must be succeeded, after an appropriate interval, by a *decrease*, that is, by a return to or toward the original condition. To produce this effect some further treatment is necessary. This inference is a simple corollary of the membrane theory as applied to the case of the dividing cell. For the concrete evidence that the initial increase of permeability is followed by a decrease, I may point to the observation of Lyon[28] that a temporary slight loss of pigment takes place from Arbacia eggs during the period immediately following fertilization; this outward diffusion of pigment then ceases, indicating decrease of permeability. Harvey also states that in Toxopneustes eggs "between ten and fifteen minutes after fertilization the eggs return to the same condition of permeability, with respect to alkali, as the unfertilized. There appears to be a second increase at the time

[27] R. Lillie: Journal of experimental zoölogy, 1908, v, p. 375; *cf.* p. 387.
[28] Lyon: *Loc. cit.*

of the first cleavage."[29] The evolution of carbon dioxide and the susceptibility to poisons also follows a rhythm which, as I have already pointed out, corresponds to the rhythm of changing permeability required by the present theory. We may therefore infer that in the normally dividing egg or other cell the permeability undergoes a series of alterations which might be represented by a curve which would correspond in its general form to the curve of carbon-dioxide production. Any artificial means of starting cleavage is presumably successful in proportion to the faithfulness with which it produces a curve of permeability change corresponding to the normal.

I have already expressed the opinion that the parthenogenetic methods act in this manner.[30] The treatment, after the preliminary membrane formation, with hypertonic sea water, cold, or cyanide, according to the methods discovered by Loeb, produces, on this hypothesis, a return to the normal semi-permeable condition of the plasma membrane. Exactly how this result is accomplished it is impossible to say at present.[31] In the egg of Strongylocentrotus the hypertonic solution fails to produce its effects in the absence of oxygen; so that the physical action of the solution and some chemical action of the nature of oxidation appear to combine in producing the effect. How far these conditions are general remains to be determined.

[29] HARVEY: *Loc. cit.*

[30] See above, p. 295.

[31] Hypertonic sea water, by decreasing the volume of the egg, *i. e.*, concentrating its constituents, may favor the re-formation of a normal impermeable surface film. Cold retards the chemical processes which further disintegration, and thus gives the egg time to recover, and the same may be true of cyanide. Oxidations are apparently favorable to increase of permeability in the unfertilized egg in sea water; if they are checked the counter-changes are more likely to restore the semi-permeable surface film. That a surface film of the same nature as in the unfertilized egg is actually re-formed after the separation of the fertilization membrane, is shown by the fact that a second membrane, in all respects like the first, may be produced by appropriate treatment (*cf.* HERBST, Biologisches Centralblatt, 1893, xiii, p. 14; TENNENT: Journal of experimental zoölogy, 1906, iii, p. 538; HARVEY, *Ibid.*, 1910, viii, p. 363).

It seems to me highly interesting that hypertonic solutions have been used with success in checking the hæmolytic action of bacterial toxins in the intact organism. This effect is comparable to the above and probably has a similar basis. *Cf.* W. D. SUTHERLAND: Biochemical journal, 1910, v, p. 1. This result suggests that the scientific therapeutics of the future will pay close attention to the conditions by which the permeability of cells may be kept normal, or restored to the normal after alteration.

In the following experiments I have studied the action of cyanide and of hypertonic sea water on eggs previously exposed to the membrane-forming salt solution. These experiments, though incomplete, have shown that after-treatment with hypertonic sea water increases the proportion of eggs developing to a larval stage. The effect, however, in the experiments so far performed has been much less decided with Asterias than with Arbacia. In the latter form eggs treated for five minutes with isotonic sodium or potassium iodide or sulphocyanate solutions and transferred after an interval of ten minutes [32] to hypertonic sea water (250 c.c. sea water *plus* 15 c.c. 2.5 m. NaCl) for half an hour yield a remarkably high proportion of active larvæ (from 50 to 80 per cent), many of which swim at the surface and appear fully normal; the hypertonic sea water produces, in fact, the same result as after membrane formation by fatty acid.[33] Asterias eggs treated with .55 m. NaCl solution for five minutes, followed, after a brief interval, by exposure to hypertonic sea water for thirty minutes, also yield a larger proportion of larvæ than eggs treated with the isotonic salt solution alone; but the increase in my last summer's experiments was comparatively slight, and the great majority of eggs died in an early stage of development. One significant difference was noted between the eggs treated with hypertonic sea water and the others: the degree of disintegration in the dead eggs after eighteen hours was distinctly less in the treated than in the untreated eggs, indicating that the increase in permeability had been checked by the hypertonic sea water — an observation in conformity with the above hypothesis that the hypertonic solution acts by decreasing the permeability. After-treatment with cyanide — a method highly effective with Asterias eggs in which membranes have been formed through brief warming — was found to produce little or no increase in the proportion of developing Asterias eggs and only a slight increase with Arbacia. It would thus appear that the injurious action of the pure salt solution is less

[32] Ten minutes is the most favorable interval between return from the salt solution to sea water and transfer to the hypertonic solution. Eggs transferred to hypertonic sea water at twenty, thirty, and forty minutes after membrane formation show a progressively smaller and smaller proportion of favorable development.

[33] Apparently it makes little difference whether the membrane-forming solution has a general action on the colloids of the membrane, or affects specifically the lipoids.

easily reversed than that of temporary warming; also that after-treatment with cyanide produces a much less close approach to the normal conditions than after-treatment with hypertonic sea water. I hope to continue these experiments next summer.

Summary.

The chief experimental results and conclusions of the foregoing paper may be briefly summarized as follows:

1. The addition of small quantities of calcium chloride to isotonic solutions of sodium salts (1) prevents the rapid increase in permeability produced in the unfertilized eggs of Asterias and Arbacia by the pure solution, (2) produces at the same time a marked decrease in the toxicity of the solution, and (3) prevents the membrane formation and initiation of cell division which are typically induced by the pure solution. The view is thus confirmed that both the toxic action of the pure salt solution and its action in initiating cell division are due primarily to the production of a condition of increased surface permeability.

2. This increased permeability is, however, temporary in normal or in favorable parthenogenetic fertilization. Treatment with hypertonic sea water after the formation of fertilization membranes by salt solutions results in an increase in the proportion of favorably developing eggs, especially in the case of Arbacia, of which the majority of the eggs thus treated may reach active larval stages. Since hypertonic sea water thus prolongs the life of the egg — an effect comparable to that of calcium in the above antitoxic action — and since prolonged life implies a practically normal permeability, the inference is drawn that the essential effect of such after-treatment is to bring the permeability — which has been increased by the initial membrane-forming treatment — again to the normal. An artificially induced increase is thus followed after a favorable interval by an artificially induced decrease of permeability. Without such after-treatment few eggs succeed in developing beyond an irregular early cleavage stage and development is abnormal.

EFFECTS OF PRESSURE ON CONDUCTIVITY IN NERVE AND MUSCLE.

By WALTER J. MEEK and W. E. LEAPER.

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MECHANICAL stimulation of nerve and muscle as measured by the resulting muscular contraction has been thoroughly investigated, but the effect of mechanical factors on conductivity in these tissues has received scant attention. The literature consists of few papers and these are full of conflicting statements. That nerve compression might offer an opportunity for a clearer insight into the phenomenon of conduction seems never to have been generally appreciated. The purpose of the present work has been chiefly to devise an instrument suitable for the application of pressure to muscle and nerve, and to measure the amount of pressure necessary to block conduction in these tissues. In addition the relations existing between pressure, time, and strength of stimuli, velocity of conduction under compression, irritability, and fatigue have also been studied.

The problem as outlined was suggested by Erlanger's experiments on artificial heart block. In his work the auriculo-ventricular bundle was compressed with a special screw clamp. Since the bundle contains both nerve and muscle tissue, it seemed advisable to investigate the effects of compression on these tissues separately.

The first work on nerve compression was done by Fontana as early as 1797. Hermann[1] quotes Fontana as having pointed out that pressure on a nerve interferes with conduction without causing stimulation. Interest in the subject seems next to have been reawakened by the clinical methods of nerve stretching which were in vogue during the middle part of last century. Weir Mitchell[2] determined roughly

[1] Hermann: Handbuch der Physiologie, 1879, ii, p. 95.

[2] Weir Mitchell: Injuries of nerves and their consequences, Philadelphia, 1872.

the amount of pressure necessary to block the nerve impulse. He found that 18 to 20 inches of mercury acting for ten to thirty seconds was sufficient to interrupt conduction. Schiff, Laferon, Heidenhain, Tigerstedt, and Wundt all touched on the problem from time to time, but the first paper dealing definitely with the question was that of Lüderitz.[3] This worker used rabbits and attempted to determine whether sensory or motor fibres were first affected by compression. He found that sensory impulses passed through the compressed area at a time when motor impulses were no longer able to reach the muscle. Zederbaum[4] found that pressure on a frog's sciatic nerve increased its irritability and that sensory nerves were blocked before motor. Efron[5] also found an increase in irritability and conductivity in the compressed area. Calugareanu[6] worked with smaller pressures than the preceding authors. He found no increase in irritability. The threshold value of a stimulus applied above a region of compression was always increased.

Ducceschi[7] in 1901 published an extensive study of the effects of compression on conduction in nerve. He determined the pressure necessary to produce block, and showed that both reflex motor and sensory impulses are interrupted before motor impulses from stimuli applied directly above the point of compression. Ducceschi rarely found an increase in irritability due to pressure. He found that pressure acts differently on impulses produced by different kinds of stimuli. With increasing degrees of compression impulses from chemical stimuli were the first to be blocked, while those from mechanical and electrical stimuli were next affected in the order named. Muscle curves taken while the nerve was under increasing amounts of pressure were found to vary only in height.

Bethe[8] in 1903 verified some of Ducceschi's data. He frequently observed an increase in irritability after pressure was applied. In an attempt to prove that the neurofibrils are the conducting parts of the nerve, Bethe directed his attention toward the histological effects of

[3] Lüderitz: Zeitschrift für klinische Medizin, 1881, ii, p. 97.

[4] Zederbaum: Archiv für Anatomie und Physiologie, 1883, p. 161.

[5] Efron: Archiv für die gesammte Physiologie, 1885, xxxvi, p. 467.

[6] Calugareanu: Journal de physiologie et de pathologie, 1901, pp. 393, 413.

[7] Ducceschi: Archiv für die gesammte Physiologie, 1901, lxxxiii, p. 38.

[8] Bethe: Allgemeine Anatomie und Physiologie des Nervensystems, 1903, p. 248.

compression. Semenoff [9] has also studied compression of nerve, but his problem was to find whether pressure gave the same changes in irritability as those described by Wedensky in the phenomenon of parabiosis.

Many different methods of securing compression were used by the above-mentioned workers. Lüderitz bound the leg of the rabbit with rubber bands and silk ligatures, including both bone and muscle with the nerve. Zederbaum applied weights to a platform resting on a piston which was fitted into a glass cylinder. The foot of the piston was provided with a piece of hard rubber which rested on the nerve. Efron used a lever of the second class with weights in a scale pan hung at the end. Calugareanu employed a device similar to that of Zederbaum, the nerve in his experiments resting in a small groove into which the piston fitted. Ducceschi devised a more refined method in that the area compressed was greatly reduced. Two small holes were drilled through a glass plate about .3 mm. apart, and through these a thread was looped over the nerve. Below the plate the thread was attached to a scale pan. The latter could be lowered gradually by means of a screw. The area of compression was thus equal to the diameter of the thread and the pressure could be applied slowly without injury.

It will be seen that none of the methods just described is physically perfect since each involves deformation of tissue. For the most part they are crushing methods. The actual pressure varies with the size of the nerve and the friction to be overcome, and it is impossible to say just how much pressure has been applied per unit of surface. The point of blocking can never be an exact one as expressed in terms of weight. From a physical point of view the only perfect way to measure pressure is by fluid transmission. The first requisite for our work was to find an apparatus that would transmit fluid pressure to nerve and muscle.

It proved a task of considerable difficulty to devise an instrument that would overcome all objectionable features of manipulation and at the same time give accurate readings. The prosecution of the work was finally made possible by the ingenious apparatus described below, which was designed by Dr. Erlanger. We wish to express our indebtedness to Dr. Erlanger for help throughout the entire work.

[9] Semenoff: Archiv für die gesammte Physiologie, 1903, c, p. 182.

Fig. 1 illustrates the compression apparatus. A large air tank *A* was connected at *H* with a half-inch pipe leading first to a pressure gauge and then to a cylinder *C*. This cylinder, which contained about a pint of heavy machine oil, was connected by means of a valve *V* with a brass tube *R*, which led to a brass T-tube *B*. The horizontal arm of the brass T-tube measured 7 mm. in length and 2.5 mm. in diameter. Through the arm of this T-tube a short piece of a carotid artery from a calf was drawn, reflected over the ends, and tied tightly with a heavy linen thread. The pressure system was thus closed. The nerve or muscle to be studied was then drawn through the lumen of the blood vessel. On letting air into the system from the compressed air tank pressure was transmitted by the oil directly to the blood vessel and thus upon the nerve or muscle drawn through its lumen.

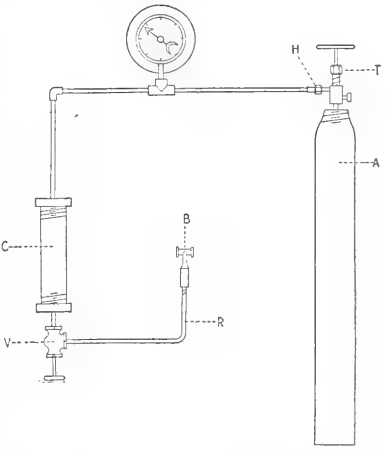

FIGURE 1. — Compression apparatus. Description found in text.

The pressure gauge was tested and found accurate. Its registering capacity was 200 pounds, and each division of the scale represented 5 pounds. By watching the gauge and opening valve *T* of the air tank any desired pressure could be quickly secured. To remove the pressure it was necessary to disconnect at *H*. By closing valve *V* in advance the pressure could be raised in the oil cylinder independent of the T-tube. On opening the valve pressure could be admitted almost instantaneously to the preparation of nerve or muscle in the T-tube.

At first there was some difficulty in getting a suitable kind of blood vessel. All the various vessels from the cat, rabbit, and dog were tried with imperfect success. The carotid artery of the calf finally proved satisfactory. Sections of some length can be found entirely free from branches, and if drawn tightly through the tube this artery will withstand over 100 pounds' pressure. Usually there was a slow leakage of oil through the artery if the pressures were high. Unless the leakage became as much as a drop every three to four seconds the

reduction in pressure was considered negligible. The oil itself was neutral to litmus and had no appreciable effect on nerve or muscle.

The Amount of Pressure Necessary to block the Impulse.

Our work was next directed toward determining the number of pounds' pressure necessary to block the impulse in nerve and muscle. The technique was as follows: For the experiments on nerve a gastrocnemius nerve muscle preparation was used. The pressure tube was placed in a moist chamber. The muscle was supported by a muscle clamp, and the nerve drawn through the lumen of the artery, which had previously been placed in the tube. The free end of the nerve rested on platinum electrodes. For experiments on muscle the sartorius was used. This muscle could be drawn through the artery and tube in the same way as nerve. One end lay on electrodes and the other was connected to a lever. In the case of tetanic stimuli no recording device was employed, the disappearance of the contraction being noted with the eye. In some experiments single break shocks from a stimulus selector were used and the contraction recorded with a muscle lever on a slow drum. In the majority of the experiments, however, the muscle was connected to a lever which on contraction of the muscle broke a circuit including an electric chronograph serving as a signal magnet. The moment of contraction was recorded on the plate of a spring myograph. The myograph broke the primary circuit of the induction coil in the usual way.

We were surprised in our first experiments to find the great resistance offered by nerve and muscle to compression. Pressures as high as 80 and 90 pounds did not at once block the impulse. Blocking, however, resulted after such pressures acted for some time. This emphasized the time factor, and we took it into account by starting with a given pressure, usually 40 pounds, and increasing it at the rate of 5 pounds every half-minute until block appeared. Table I presents the results of a series of seven experiments on the sciatic nerve.

In the first column are given the initial pressures. From 40 pounds the pressure was raised at the rate of 5 pounds every half-minute until block appeared at the number of pounds shown in the second column. The time of compression in minutes is given in the third column. In

all the experiments, unless otherwise mentioned, a Verdin coil was used, the secondary having 2000 turns of .7 mm. wire. The secondary coil in the experiments on nerve was kept 320 mm. from the primary. Maximal contractions were induced at 400–430 mm.; so the stimuli were well above the maximal. The stimuli were produced by the

TABLE I.

PRESSURES ON NERVE.

Pressures in pounds.		Time of compression.
Initial.	Final.	
		Min.
40	65	2
40	90	5
40	90	5
40	90	5
40	85	4.5
40	90	5
40	90	5
Average 40	85.7	4.5

knock-down key of the myograph and were uniform in strength. As can be seen from the table, an initial pressure of 40 pounds can on the average be increased to 85.7 pounds before the nerve impulse is blocked by the compression.

In Table II similar data are given for the sartorius muscle. The distance of the secondary coil from the primary was 200 mm., which gave a stimulus somewhat above maximal. All our experiments have shown that the average pressure necessary to block conduction in muscle is somewhat lower than it is in nerve. The range of pressures is somewhat wider, however, in the case of muscle. The greatest pressure required to produce block was 100 pounds, while 55 pounds was the lowest recorded. The average for all of our experiments on muscle is 75 pounds. The seven records in the above table were sub-

mitted because the muscles used were from the same frogs as the nerves in Table II.

Experiments were next made on curarized muscle for comparison with the non-curarized (Fig. 3).

TABLE II.

PRESSURES ON MUSCLE.

Pressures in pounds.		Time of compression.
Initial.	Final.	
		Min.
40	90	5
40	70	3
40	65	2.5
40	90	5
40	80	4
40	65	2.5
40	70	3
Average 40	75.9	3.8

Although the lowest pressure recorded, 55 pounds, is not below the lowest in non-curarized muscle, it will be noted that in no case was a pressure of more than 75 pounds required to block the impulse, and the average is 12 pounds below that for normal muscle.

From these experiments it seems that muscle is less resistant to pressure than nerve. This reduced resistance may, however, be due in part to the effect of the drug. While curare picks out the nerve endings specifically, it is by no means inert toward muscle. Possibly the results are but an expression of this action. We had hoped to find the difference between nerve and muscle in their reaction toward compression striking enough to give some idea as to which tissue was most affected in artificial heart block. The differences are not great enough to justify any conclusions, at least not until the pressures used in heart block are accurately known. It is sufficient to say

that in regard to pressure curarized muscle is less resistant than non-curarized, and non-curarized is less resistant than nerve.

TABLE III.

PRESSURES ON CURARIZED MUSCLE.

Pressures in pounds.		Time of compression.
Initial.	Final.	
		Min.
40	70	3
40	60	2
40	75	3.5
40	60	2
40	55	1.5
40	60	2
Average 40	63	2.3

An extensive series of experiments was also made in which tetanic stimuli were used to determine when block occurred. The same general facts as stated above were found to apply here also. Nerve proved the most resistant and curarized muscle least. In these experiments one leg of the frog was ligated before the injection of curare, and the sartorius of this leg was used as a control against the curarized muscle of the opposite leg. In every case the non-curarized muscle proved the more resistant.

From the first it was of course recognized that in determining the amount of pressure necessary to block the impulse two other factors were concerned — the length of time the pressure was applied and the strength of the stimulus. That a stronger stimulus increases the amount of pressure or the time that a constant pressure must act in order to produce blocking, must have been known to the previous workers, but none seem to have emphasized the fact, and most writers do not even note the stimulus used to determine the point of block. To find the relation of the amount of pressure and strength of stimuli

to the time required for block, certain arbitrary pressures and stimuli were selected.

It is sufficient to give the summaries of these various series of experiments. In twenty experiments on nerve under a constant pressure of 40 pounds the average time required to block the nerve impulse was 7.5 minutes. The longest time observed was 16 and the shortest 2.5 minutes. Twelve of the observations varied between 6 and 11 minutes. In a second series of five experiments 20 pounds' pressure was employed. The average time for block was 19 minutes. From this data it appears that with constant stimuli the time necessary to produce block is roughly proportional to the pressure applied.

The same general fact was easily shown in muscle, both normal and curarized. In ten experiments on normal muscle with the coil at 200 mm. distance, 40 pounds' pressure blocked the conduction on the average in 4.4 minutes. The time required for blocking with 20 pounds' pressure under the same conditions averaged 7 minutes. In curarized muscle 40 pounds' pressure blocked the impulse in 2.4 minutes, and 20 pounds' pressure in about double that time.

In both nerve and muscle the time required to interrupt conduction is proportional to the strength of stimulus. To demonstrate this we placed the nerve or muscle under a constant pressure, and whenever block appeared increased the strength of the stimuli until maximal contractions were again made by the recording muscle. One experiment on nerve subjected to 70 pounds' pressure gave the following results: maximal stimuli from the coil at distances of 320, 105, 82, 58, and 32 mm. were blocked in 3, 13.5, 31, 56, and 113 minutes respectively. The ability of the nerve impulse to pass through an area of compression therefore varies with the strength of the stimulus. The term "block" is really a relative one. It would be interesting to know whether the relation between the strength of stimulus and the pressure or time of compression is the same as that found by Greene[10] between strength of stimulus and the action current.

The pressure required for block did not seem to vary with the size of the muscle in any constant direction. A muscle 3 cm. long and weighing 87 mg. required 70 pounds' pressure for block in a given time, which was exactly the same amount required by a muscle 3.5 cm. long and weighing 214 mg. No doubt the size of the muscle influences

[10] Greene: This journal, 1898, i, p. 104.

the distribution of pressure to some degree, but it would not seem considerable enough to be a factor in our experiments.

Does Compression increase the Irritability of Nerve?

Various results have been obtained by previous workers in attempting to answer this question. Efron and Zederbaum reported a constant increase in irritability on compression. Ducceschi found it but rarely, and Calugareanu not at all. Increased irritability in this connection has meant the power of the nerve to augment the strength of the impulse as it passes through the area of compression. That this occurs Efron and Zederbaum showed by readings from the position of the secondary coil. Zederbaum found that increased irritability begins to appear when a weight of 75 gm. is placed on the nerve, and steadily increases until the weight reaches 500 gm. In his experiments with the nerve unweighted the stimulus just necessary to give a muscle twitch was produced by the secondary coil at a distance of 308 mm. from the primary, while with a weight of 500 gm. the coil gave the same effect at a distance of 332 mm.

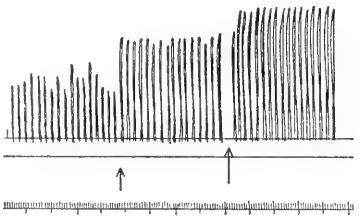

Figure 2. — Tracing showing increase in height of gastrocnemius after application of 20 pounds' pressure to sciatic nerve. First arrow marks point of compression. Second arrow marks removal of pressure and increase of strength of stimuli to the maximal.

In our work we attempted to show this increase in irritability as follows: We set the secondary coil at a point where submaximal stimuli were produced, and then by means of a stimulus selector sent in submaximal break shocks at the rate of 1 every 2.5 seconds. The muscular contractions were recorded in the usual way on a slow drum. After a short series of contractions were recorded pressure was applied to the nerve. Fig. 2 presents one of these tracings. In this case the admission of 20 pounds' pressure to the nerve brought about some change which resulted in an increased height of contraction. This does not always occur, but we have found it very frequently with low pressures. A pressure of 40 pounds usually blocks the impulse from a submaximal stimulus at once. Pressures of from 10 to 25 pounds,

on the other hand, frequently show the phenomenon described above.

Ducceschi was the first to point out that we are not here dealing with a real increase in irritability. He looks upon the impulse as merely being augmented by others set up in the compressed area. Increased irritability in the strict sense would mean some change in the lability of the protoplasm so that it responds more readily or completely to its stimulus. There seems to be no reason for believing that the nerve is rendered more irritable in this sense. More likely it is a matter of summation in the muscle. Compression itself is a form of stimulation. This is seen in certain cases by the production of an incomplete tetanus when the pressure is applied rapidly. It may be assumed that impulses are constantly being received by the muscle from the compressed area of the nerve. These impulses are summated with those from the single shock, and an increase in amplitude of the contraction results. In the same manner compression might result in the summation of sensory impulses. This is probably the explanation of hyperæsthesia in cases where a tumor or other growth has exerted pressure on a nerve trunk.

Fatigue Effects.

Recovery occurred both in nerve and muscle after compression provided the pressure was not too long maintained. Time records were not kept of all experiments, but from a large number of observations it may be said that in general nerve recovered from block under a constant pressure of 40 pounds in from three to thirty minutes. In those experiments in which the pressure had been raised rapidly recovery was also rapid and at times almost instantaneous. The longer the pressure was applied the slower was the return of function after the removal of compression. Muscle recovered more rapidly than nerve, and curarized muscle more rapidly than non-curarized. These findings were in line with the amount of pressure and length of time required to produce block in the same tissues.

The recovery, however, was not a complete one. Conduction never became entirely normal, as could be shown in two ways. In the first place, a second block could be produced with a much smaller pressure than at the beginning of the experiment. Forty pounds applied a

second time almost invariably suspended conduction at once. If the block was not immediate, the time necessary to produce it was much reduced.

In the second place fatigue effects could be produced such as those recently described by Tait [11] after subjecting the nerve to cold. This author found that, after nerve had been frozen and allowed to recover,

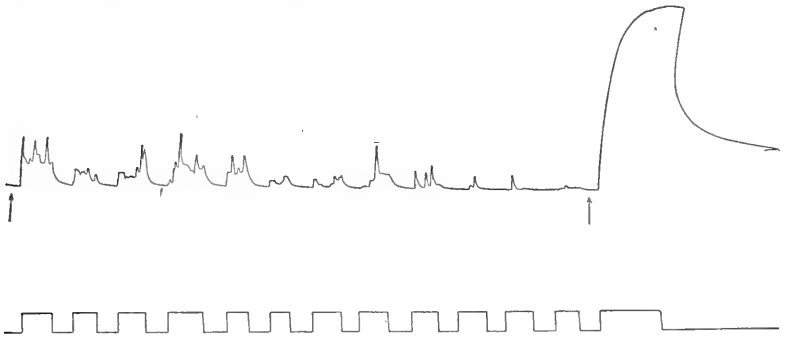

FIGURE 3. — Tracing showing fatigue effects of pressure on nerve. Harvard coil. Beginning with the first arrow a series of tetanic stimuli was sent in above the compressed area. At the second arrow, by means of a commutator, the stimuli were sent in between the muscle and the compressed portion.

on stimulation a series of tetani could be run, each lower than the last until the nerve was completely fatigued. Fig. 3 illustrates this phenomenon in our experiments. The nerve was compressed for thirty minutes with a pressure of 40 pounds. The nerve was then removed from the apparatus, placed in physiological saline and allowed to recover for two hours. The muscle was then connected to a muscle lever and the nerve laid over two electrodes, one peripheral and one central to the compressed portion. A series of short tetani was then sent in above the compressed area. As can be seen in the tracing, incomplete tetanic curves were produced, the last stimulation from the central electrode showing almost complete fatigue. That this was not due to fatigue of the muscle was shown by the complete tetanus from the same stimuli applied nearer the muscle than the compressed area. Fatigue of nerve can thus be demonstrated after compression quite as well as after freezing.

[11] TAIT: Quarterly journal of experimental physiology, 1908, i, p. 79.

Is the Velocity of the Nerve Impulse influenced by Compression?

The idea that pressure might slow the nerve impulse was suggested by the increased A–V interval which is found in artificial heart block. We may say at once that we do not have a satisfactory answer to this question, because we were unable to attack it in the most feasible way,

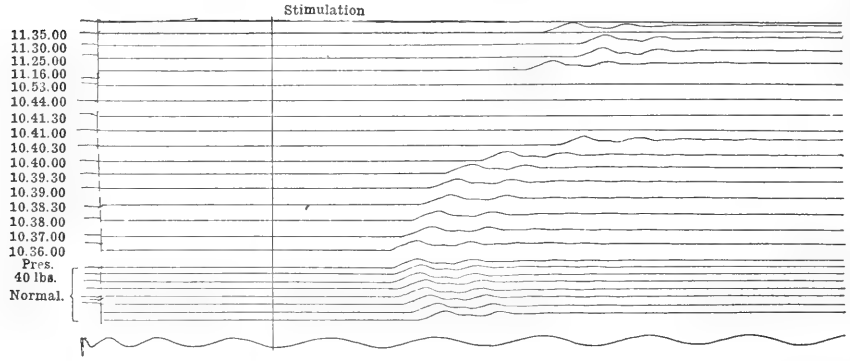

Figure 4. — Tracing showing increase in latent period and time of conduction after the application of 40 pounds' pressure to nerve. Pressure admitted at 10.35½. Block in 5½ minutes. Recovery some time between 12 and 35 minutes.

namely, by recording the wave of negative variation before and after its passage through the compressed area. We did find, however, that the interval between stimulation and contraction increased from the time pressure was applied until block made its appearance. This interval is the latent period of the muscle plus the time of conduction through the nerve. Fig. 4 shows the gradual lengthening of this interval when the nerve is subjected to pressure. The latent period was recorded on the spring myograph by an electric chronograph, the current to which was broken by the contraction of the muscle. The normal interval between stimulation and contraction as shown by the eight lower curves is about 1.25 seconds. This increases after 40 pounds' pressure is admitted to almost 3 hundredths before block appears. Ducceschi [12] states that there is no change in the muscular curve other than a reduction in amplitude, but we have found this

[12] Ducceschi: *Loc. cit.*

increased period between stimulation and contraction constantly, both in nerve and muscle.

This increased delay is probably due in most part to a longer latent period of the muscle as a result of a decrease in the strength of the impulses which affect it. We failed to lengthen the interval by doubling the area of nerve compressed, which seems to show that the nerve itself is not greatly concerned. By gradually reducing the strength of stimuli to a nerve or muscle the same increase in latent period may be shown. It thus seems evident that compression is a most delicate way of reducing the strength of the nerve impulse gradually and slowly. As the impulse weakens, the latent period of muscle lengthens, and conduction itself becomes slower in the nerve. Tigerstedt[13] has shown that the latent period of muscle may increase from .007 to .02 second as the impulse diminishes. Piper[14] found a slightly lower velocity of conduction in nerve for impulses from stimuli near the threshold value. These results do not eliminate the possibility of changes in velocity in the compressed area itself, but a study with the string galvanometer would be necessary for their determination.

Summary.

1. A method has been described which makes possible the fluid transmission of pressure to nerve and muscle.

2. The average time required to block the nerve impulse in the frog's sciatic by means of a constant pressure of 40 pounds was found to be 7.5 minutes. Under the same conditions 4.4 minutes were required to establish block in fresh sartorius muscles and 2.4 minutes in curarized. In both nerve and muscle the time necessary to interrupt conduction was proportional to the pressure applied and the strength of the stimulus.

3. Immediately after the application of pressures not exceeding 20 pounds to nerve, stimuli of a given strength frequently caused an increased height of contraction in the recording muscle. There was probably a summation in the muscle of impulses set up in the compressed area with those due to the electrical stimulus.

[13] Tigerstedt: Archiv für Anatomie und Physiologie, Sup. 1885, p. 165.

[14] Piper: Archiv für die gesammte Physiologie, 1909, cxxvii, p. 480.

4. Fatigue of the nerve was demonstrated after recovery from compression.

5. No change in velocity of conduction in the compressed region has as yet been detected. The muscular contraction, however, was reduced in height, and the interval between stimulation and contraction prolonged as block made its appearance.

THE EFFECTS OF STRETCHING THE NERVE ON THE RATE OF CONDUCTION OF THE NERVOUS IMPULSE.

By A. J. CARLSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

SIX years ago, the author, in collaboration with Dr. Jenkins, reported some observations on the pedal nerves of the slug (Ariolimax), which seemed to show that when these nerves are stretched within their physiological limit there is an actual extension of the conducting substance in the nerve, and a delay of conduction proportional to the degree of extension, the actual rate of conduction of the impulse thus remaining the same in the two conditions of the nerve.[1] These facts seemed to us "evidence on the side of the view that the conducting substance in this nerve is in a liquid condition, or at least in a semi-liquid condition." The following year experiments on a marine worm (Bispira) were reported.[2] These confirmed our results on the slug to the extent that stretching the worm increased the conduction time in the same length of nerve cord, as compared to the conduction time in the relaxed or unextended worm. Because of the impossibility of accurate measurements of the length of the nerve cord in the worm when not extended, I could not determine whether or not the delay in conduction of the impulse was proportional to the degree of extension of the nerve cord, that is, whether the stretching within the physiological limit actually affected the conduction rate. Estimates were made on this point that indicated the same conditions as were found in the pedal nerve of the slug, but the uncertain element is the length of the nerve cord in the non-extended worms, and for that reason little value was attached to those estimates. The very obvious conclusion was intimated that "the results do not point to any substance in the

[1] Jenkins and Carlson: Journal of comparative neurology and psychology, 1904, xlv, p. 85.

[2] Carlson: This journal, 1905, xiii, p. 351.

nerve as being concerned in the impulse, except that in case the neuroplasm is of a more fluid consistency than the neurofibrillæ, they speak in favor of the former as the substance involved."

Three years later Bethe[3] reported some experiments on the leech (Hirudo), which seemed to question our results on the slug and the marine worm. Contrary to our results, Bethe finds in the leech that stretching the nerve cord within the physiological limit does not affect the conduction time; and he concludes, therefore, that in this stretching there is no actual extension of the conducting substance, but only a straightening out of the kinks of the neurofibrillæ. These results of Bethe and his attempt to account for our previous findings on the basis of experimental errors are the occasion for the present note. It was planned to carry specimens of Ariolimax to the recent physiological congress in Vienna for actual demonstration, as there seems no better way to settle a disputed point as to facts. The animals arrived from California in good condition, but they did not seem to do well in our laboratory in Chicago, and those brought along succumbed on the way to Vienna.

The problem resolves itself into two essential questions, namely, (1) Does the stretching of the nerve within the physiological limit alter the conduction time? By "physiological limit" we understand the degree of stretching to which the nerve or nerve cord may be subjected in normal activity and which does not alter the excitability of the nerve or the intensity of the nervous impulse. (2) Is there any relation between the degree of stretching of the nerve and the amount of change in the conduction time?

As regards the first point, a recent repetition of my previous experiments has confirmed the results then reported. I wish to call attention to a few typical tracings (Figs. 1–4) secured last August on animals kindly sent me by Dr. Jenkins of Stanford University. The technique is essentially the same that was employed in earlier work. The stimulating electrode remains fixed at the same point of the pedal nerves near the pedal ganglia. The tracings are left in the same condition as when taken off the kymograph. The difference in the latent time is apparent on direct inspection, but those interested may draw the requisite lines and measure the actual delay of the conduction

[3] Bethe: Archiv für die gesammte Physiologie, 1908, cxxii, p. 1.

through the stretched nerve. The time signal is the same on all the tracings, namely, 50 d. v. per second.

The records in Figs. 1 and 2 are practically perfect. The distance of the muscle lever from the stimulation signal shows that the tonus

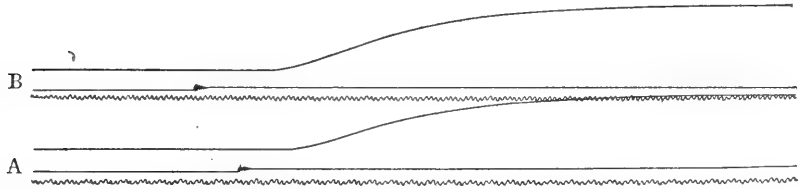

FIGURE 1. — Four sevenths the original size. Tracings from the foot musculature of the slug (Ariolimax) on stimulation of the pedal nerves at a fixed point near the pedal ganglia. *A*, nerves relaxed; *B*, nerves stretched. Delay of conduction in the stretched nerve.

of the reacting musculature (posterior end of the foot) is the same at the time of stimulation in each parallel series, and the nearly uniform amplitude of the contractions shows that the intensity of the nervous impulses reaching the reacting muscle in the two conditions of the

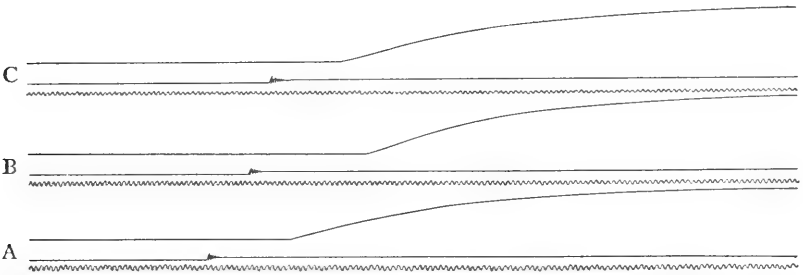

FIGURE 2. — Four sevenths the original size. Tracings from the foot musculature of the slug (Ariolimax) on stimulation of the pedal nerves at a fixed point near the pedal ganglia. *A*, *C*, nerves relaxed; *B*, nerves stretched. Delay of conduction in the stretched nerve.

nerve is practically the same. The records in Fig. 3 are less satisfactory, because the amplitude of the contraction is somewhat greater with the nerve in the stretched condition, while the tracings in Fig. 4 are typical of poor results, in other words, rejected series. The delay in the conduction time in the stretched condition is obvious, to be sure, but the tonus of the muscle and the amplitude of the contraction are too

variable. It is needless to say that in reaching the conclusions in our earlier work, only records like Figs. 1, 2, and 3 were admitted, as is shown by the sample tracings then published.

What are the sources of error to be guarded against in these experiments, and are the results possibly due to such errors as have been overlooked? The variable degree of tonus of the foot musculature is a troublesome factor. The excitability, and hence the latent period, of

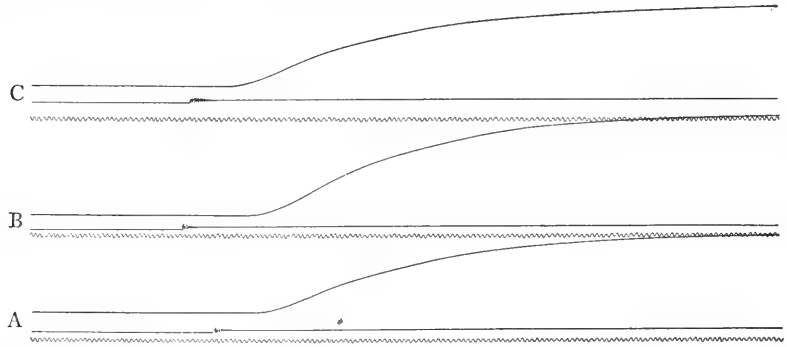

FIGURE 3. — One half the original size. Tracings from the foot musculature of the slug (Ariolimax) on stimulation of the pedal nerves at a fixed point near the pedal ganglia. *A*, *C*, relaxed nerve; *B*, stretched nerve. Delay in conduction in the stretched nerve.

the slug muscle probably varies with the muscle tone, as is the case in the vertebrates. Bethe assumes that we were ignorant of, or failed to consider, this elementary fact in the physiology of nerve and muscle, and so he imagines that we shifted the position of the preparation to suit any variations in the muscle tonus, and that in the readjustment we failed to place the writing point of the lever perpendicular to that of the stimulating signal! Perhaps we erred in assuming that physiologists would take these things for granted. Between preparation of the animal and the taking of the first record, and between each succeeding record, time was allowed for the foot muscle to reach a certain uniform degree of relaxation, as is evidenced by the distance of the muscle curve from that of the stimulating signal. That does not mean a complete cessation of the tonus relaxation, but the further relaxation is so slow that the muscle curve drawn on the recording surface, travelling at the speed necessary for these measurements, is practically a straight line (see tracings). Hence there is no unusual error in determining the beginning of the elevation of the muscle lever.

The strength of the stimulus (break induction shock) was always submaximal. Good preparations will yield successive submaximal contractions of fairly uniform amplitude. If maximal or supermaximal stimuli had been used, there might have been in the stretched nerve considerable variations in the excitability and the intensity of the impulse, with the amplitude of the muscle contraction remaining practically the same. With the muscle in similar condition of tonus

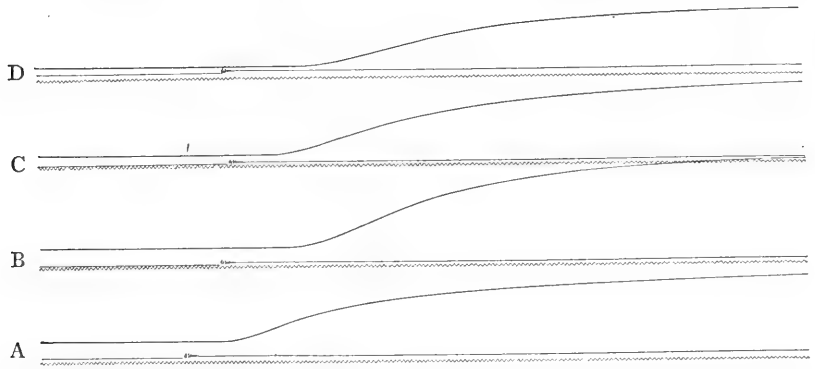

FIGURE 4. — About one half the original size. Tracings from the foot musculature of the slug (Ariolimax) on stimulation of the pedal nerves at a fixed point near the pedal ganglia. *A*, *C*, relaxed nerve; *B*, *D*, stretched nerve. Typical curves of the rejected series of experiments.

and degree of fatigue, and the intensity of the stimulus to the nerve constant and submaximal, if the contractions are of fairly uniform amplitude it seems safe to conclude that the excitability of the nerve and the intensity of the nervous impulse also remain fairly constant. Our criterion for the physiological limit of nerve stretching is, therefore, (1) the absence of effects on the intensity of the nervous impulse and on the excitability; (2) absence of actual excitation (over distention of the nerve results in muscle contraction; (3) approximation of the degree of stretching to that attaining in the animal when normally crawling. This latter is admittedly an approximation. I do not think that I can come closer to it than about 2 cm.

One source of error was overlooked by us in the earlier work, as well as by Bethe in his critical review of our data. In the slug preparation it is necessary to stretch the whole length of the nerve from the farthest electrode point to which the central end of the nerve is tied. In consequence, when the nerve is stretched, the portion of it between

two points of the electrodes is also stretched, and in consequence the point or region of actual excitation of the nerve is necessarily a little farther from the muscle than with the nerve in the relaxed state. There is no way of eliminating this error even when recognized. But although the rate of propagation of the impulse in these nerves is only 30–40 cm. per second, the delay in the conduction in the stretched nerve is too great to be accounted for by the slight shifting of the point of stimulation that actually takes place when the points of the electrodes are only 1 mm. apart.

Lastly, Bethe argues that the complexity of the slug nerve-muscle preparation renders the results doubtful. Biedermann and Bethe have shown that there is a peripheral ganglionic plexus in the foot musculature of many gasteropods. The pedal nerves evidently enter this ganglionic plexus, and in some gasteropods stimulation of the pedal nerves may give contraction or relaxation of the foot musculature, or a combination of contraction and relaxation. I have observed this both in Helix and in Limax, but never in Ariolimax, although I have made no special study of it in this slug. I have always failed to obtain relaxation of the foot musculature, even when in great tonus, by stimulation of the pedal nerves with single induction shocks or the interrupted current. And even in Limax the stimulation of the pedal nerves invariably gives a definite and uniform contraction of the posterior end of the foot and the dorsum, provided these musculatures are relaxed at the time of the stimulation. There is, in all probability, in the foot musculature of Ariolimax a ganglionic plexus similar to that in other gasteropods, but I fail to see how this vitiates the results under discussion. Such a mechanism might have made the latent periods of successive stimulations of the pedal nerve as variable as the latent time of many reflex reactions, and thus rendered this particular preparation unavailable for this work. But since this is not the case, we need not concern ourselves with this ganglionic mechanism in the present inquiry. As regards the first point, then, the data seem conclusive. Extension of the nerve increases the conduction time without altering the intensity of the impulse or the excitability of the nerve fibres. Bethe admits that when the leech is stretched beyond the physiological limit the conduction time is increased. It is not clear to me whether Bethe holds that this delay is proportional to the degree of

stretching.[4] Is there any relation between the degree of nerve stretching and the amount of increase in the conduction time? I have no new data touching this point. Bethe endeavors to show that our previous data are wide of the mark. As regards the data on the marine worm, it was distinctly stated that they were only estimates. But the results on the slug preparation seem more convincing. The sources of error in the measurements were recognized. It is difficult to measure with accuracy the length of the nerve in the relaxed condition. It is difficult to keep the electrodes nearest the muscle on the same point of the nerve when alternately stretching and relaxing it. And a further factor is the rapid slowing of the conduction in the nerve, whether stretched or not. Under these conditions individual variations are obviously inevitable. And we made it a special point to give three typical experiments in detail, one exceptionally good (Table III) and two fair (Tables I and II). I fail to see how any of the sources of error involved could work constantly in one direction. The average is, therefore, more nearly correct than any single pair of measurements. The average figures in our sixteen acceptable experiments, being 44 pairs of records on the stretched nerve, and 49 pairs of records on the relaxed nerve, are:

	Length of nerve.	Rate of conduction.
Stretched	8.15 cm.	34.6 cm. per second.
Relaxed	4.14 "	37.1 " " "

The inference seems obvious that the delay in the conduction is proportional to the degree of nerve extension, so that the actual rate of conduction remains practically constant.[5]

Attention may now be invited to Bethe's own results, particularly to the published tracings and to the experimental methods. When an attempt was made to extend the observations on the slug preparations to the worm phylum, I tried all available species of annelids, including the leech (Aulastomum) and the earthworm, but I finally settled on the marine worm Bispira as the only workable species. In my hands the leech proved the least suitable of all. And Bethe's

[4] "Erst bei Dehnung über die physiologische Länge tritt eine Verlängerung der Uebertragungszeit ein. *Die Geschwindigkeit der Reizleitung in einem gegebenen Stück Bauchmark ist also proportional seiner augenblicklichen Länge*" (p. 32).

[5] BETHE'S analysis (p. 8) of one of our experiments cited in detail (Table III, p. 89) is erroneous.

own curves and figures show that the species used by him (Hirudo) makes no exception. The anterior or reacting end of the leech is in almost constant motion, so that it is nearly impossible to secure consecutive contractions of the same amplitude, or sufficient duration of rest to obtain a horizontal line from the recording lever prior to the contractions evoked by the stimulations. That being the case, the determination of the exact point of rise of the lever, or the latent period, becomes a matter of more or less guesswork, as is evident on Bethe's published tracings (Fig. 10, p. 25). Such tracings can, at best, be used as the basis only for a rough estimate of the rate of conduction, as was pointed out seven years ago by Dr. Jenkins and the writer.[6]

In the second place, the stimuli, both proximal and distal, are sent through the entire worm by means of the pins fixing the preparation to the apparatus. This renders it difficult to determine the actual point or level of stimulation of the nerve cord. And lastly, the Jacquet chronograph was used as time signal in most of the experiments, rendering the measurements of even so large time elements as 0.01′ only approximate estimations. It would seem, then, that Bethe's data do not even prove his contention for the leech, and much less can they be used to refute the results on the slug and on Bispira, objects much better suited to such experiments.

The interpretation of the phenomenon is still a matter of conjecture, except in so far as it points to a fluid condition of the conducting substance. I have failed in my attempt to extend it to the spinal nerves of vertebrates, probably because these can be stretched only within narrow limits before changes in excitability and actual stimulation are produced. When the experimental difficulties are overcome, the phenomena will probably be demonstrated in the visceral nerve plexuses in the vertebrates. It probably also obtains as regards the excitation wave in muscle cells subject to relatively great tonus variations. As we have seen, even Bethe admits that when the nerve cord is extended beyond the physiological limit there is a delay of the conduction, but his figures are not sufficiently correct to determine whether the delay is directly proportional to the degree of stretching.

[6] Jenkins and Carlson: Journal of comparative neurology and psychology, 1903, xiii, p. 265.

THE PRODUCTION OF GLYCOSURIA BY ADRENALIN IN THYROIDECTOMIZED DOGS.

By FRANK P. UNDERHILL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut.*]

THE failure of adrenalin to induce glycosuria after removal of the thyroids (the parathyroids being intact) reported by Eppinger, Falta, and Rudinger [1] was not corroborated by Underhill and Hilditch.[2] In the experiments of the latter adrenalin called forth the appearance of sugar in the urine of all the thyroidectomized animals tested.

In a reply by Falta and Rudinger [3] the following criticisms concerning the work of Underhill and Hilditch are offered: (1) with a single exception the latter authors injected larger quantities of adrenalin than were employed by Eppinger, Falta, and Rudinger. The positive outcome of the single exception is given the doubtful explanation that some thyroid secretion might still be in circulation such a short time (two days) after thyroidectomy. On the other hand, when in the same animal adrenalin in the proper dosage called forth glycosuria eighty-one days after removal of the thyroids, Falta and Rudinger point out that "Es darf aber nicht vergessen werden, dass bei Hunden sich gar nicht so selten akzessorische Schilddrüsen längs des Oesophagus nach abwärts finden, welche bis zum Pericard herabsteigen können."[4] (2) Falta and Rudinger claim that comparison can be made with their experiments only when the irritability of the thyroidectomized animal to an electrical current has been determined

[1] Eppinger, Falta, and Rudinger: Wiener klinische Wochenschrift, 1908, p. 241; Zeitschrift für klinische Medizin, 1908, lxvi, p. 1, and 1909, lxvii, p. 1.

[2] Underhill and Hilditch: This journal, 1909, xxv, p. 66.

[3] Falta and Rudinger: Zentralblatt für die gesamte Physiologie und Pathologie des Stoffwechsels, 1910, xi, p. 81.

[4] Falta and Rudinger: *Loc. cit.*, p. 83.

in an endeavor to discover latent tetany. Underhill and Hilditch made no such observations. (3) Finally, according to Falta and Rudinger, Underhill and Hilditch made no autopsies to demonstrate whether perchance thyroids or accessory thyroids were still present in these animals.

In reply to the criticisms outlined above it should be stated, first of all, that apparently Falta and Rudinger read only a review [5] of the work by Underhill and Hilditch. In the original article [6] there are given at least two instances, out of a possible four, in which it may be seen that the same or a smaller quantity of adrenalin than that employed by Eppinger, Falta, and Rudinger induced glycosuria in dogs after thyroidectomy. Falta and Rudinger explain sugar excretion in the single exception noted by them (Dog A [7] or Dog I) by assuming that the adrenalin was administered too soon after removal of the thyroids (two days). This explanation will not suffice, however, for the appearance of dextrose in the urine when adrenalin was injected according to the Eppinger, Falta, and Rudinger standard of dosage in the case of Dog D.[8] This injection occurred six days after thyroidectomy, and the statement is made by the above-mentioned authors that at least three days after thyroidectomy adrenalin failed to produce glycosuria.

Falta and Rudinger admit in the case of Dog A (Dog I), in which glycosuria was caused eighty-one days after removal of the thyroids, that there can be no question here of a latent tetany, but they intimate that accessory thyroids may be present. At the time of the publication of our former investigation it was considered undesirable to kill Dog A (Dog I) in order to determine the presence of accessory thyroids. Since then, however, an autopsy has been performed upon this animal with this end in view. Every piece of tissue in any way bearing a resemblance to thyroid tissues was carefully preserved and sent for identification to Professor H. Gideon Wells of the University of Chicago. In his report concerning these tissues Professor

[5] UNDERHILL: Zentralblatt für die gesamte Physiologie und Pathologie des Stoffwechsels, 1909, x, p. 641.

[6] UNDERHILL and HILDITCH: *Loc. cit.*

[7] In the article by UNDERHILL and HILDITCH the animals were called A, B, C, etc. In the review by UNDERHILL Dog A was called Dog I, Dog B was designated Dog II, etc.

[8] UNDERHILL and HILDITCH: *Loc. cit.*

Wells makes the statement that he finds no evidence of thyroid tissue or accessory thyroids; nothing except parathyroid tissue was in evidence. During the present summer an autopsy was also performed upon Dog C (Dog III). The result demonstrated the presence of two somewhat hypertrophied parathyroids but no trace of thyroid

TABLE I.

Dog.	Time after thyroidectomy.	Adrenalin chloride per kilo injected.	Dextrose in urine.
		mgm.	gm.
A [1]	16 months	March 3 1.0	0.0
		March 15 1.0	4.79
21 [2]	21 days	1.0	0.76
22 [3]	4 days	1.0	3.67
23 [4]	3 days	1.0	14.38

[1] This animal was Dog A employed in experiments by UNDERHILL and HILDITCH. The dog never showed any signs of abnormality. The body weight was 12.1 kilos.

[2] A dog of 8 kilos in splendid nutritive condition. Both thyroids were removed, leaving two parathyroids intact. Fed a mixed diet. Autopsy revealed absence of thyroid tissues.

[3] Well-fed dog of 13.2 kilos. No evidences of thyroid tissues on autopsy.

[4] Dog of 12 kilos, in good condition. No thyroid tissues on autopsy. None of these animals showed any abnormality.

tissue, nor could any thyroid tissue be found at the autopsy of Dog D. The results of the autopsy of Dog B (Dog II) were noted in our former communication.

The criticism of Falta and Rudinger concerning the points just discussed are not extremely potent in view of the autopsy findings now reported, together with the observation that all dogs gave glycosuria after removal of the thyroids on treatment with adrenalin, and that two of the four reacted positively with the same dosage employed by Eppinger, Falta, and Rudinger. Nevertheless, in order to decide the question even more definitely further experiments have been undertaken the results of which may be seen in Table I. The methods employed were identical with those outlined in our former commu-

nication, except that in the observations here reported all adrenalin injections were made subcutaneously. It may be seen from these data that adrenalin chloride administered subcutaneously to dogs in the dosage of 1 mgm. per kilo body weight is capable of provoking the appearance in the urine of significant quantities of dextrose, three, four, and twenty-one days, and thirteen months after thyroidectomy. In no case were there any abnormal manifestations, nor could any thyroid tissues be found on autopsy.

In the present investigation, as in the previous one, we have deemed it unnecessary to determine the response of the thyroidectomized animal to electrical stimulation in order to discover latent tetany. The observation that none of the animals selected behaved in an abnormal manner, together with the fact that Dog A (Dog I) and Dog C (Dog III) of our previous experiments lived more than sixteen months without tetany, that like conditions obtained for Dog D, killed ten days after thyroidectomy because of an abscess at site of injection, and that Dog 21 of the present investigation was allowed to live more than twenty-one days after removal of the thyroids — these facts all speak against the idea that latent tetany was present.

The criticism that adrenalin injection was made too soon after operation in the case of Dog A (Dog I) of our former investigation will not hold for our present experiments, since in no case was adrenalin introduced under three days after removal of the thyroids. A survey of the work of Eppinger, Falta, and Rudinger reveals that in several instances their own injections were made three and four days after thyroidectomy.

In the paper by Falta and Rudinger two new experiments[9] are detailed designed to corroborate former statements. Concerning the first experiment the question may well be asked, "Why was the dose of adrenalin, 10.5 mgm. (less than 1 mgm. per kilo for a 13-kilo dog) divided into two portions (6 mgm. and 4.5 mgm.) and these injected on two separate days?" Such a procedure does not add weight to the statement of Eppinger, Falta, and Rudinger that 1 mgm. adrenalin per kilo body weight administered subcutaneously or intraperitoneally into thyroidectomized dogs is incapable of causing glycosuria. The failure of these quantities of adrenalin to provoke the appearance of sugar in the urine can be duplicated at times in

[9] Falta and Rudinger: *Loc cit.*, p. 82.

normal dogs. In fact, even 1 mgm. per kilo often fails to induce glycosuria (see Dog 22, Table II), and in Dog A (Dog I) without thyroids (see Table I) it may be seen that this dose failed on March 3, whereas on March 15 it caused the excretion of 4.79 gm. dextrose. From our experience with normal dogs we have been led to the conclusion that, although in general 1 mgm. adrenalin per kilo body weight administered subcutaneously or intraperitoneally is capable of causing glycosuria, animals are frequently encountered in which this dose provokes no glycosuria. On the other hand, these same animals at other times behave in the usual way and react to doses of 1 mgm. adrenalin per kilo.

The second experiment of Falta and Rudinger shows that 5 mgm. adrenalin injected intraperitoneally before removal of the thyroids caused a slight glycosuria. After thyroidectomy the same quantity injected intraperitoneally failed. Later 10 mgm. introduced subcutaneously into two places also failed to provoke glycosuria. In several experiments we have noted the absence of dextrose in the urine following the intraperitoneal introduction of 5 mgm. adrenalin into normal dogs smaller than the one employed by Falta and Rudinger. The fact that 5 mgm. in this instance failed to produce glycosuria does not necessarily mean that this has been due to thyroid removal. When the subcutaneous injection was given, the animal weighed 16.2 kilos, and yet only 10 mgm. adrenalin were administered. If Falta and Rudinger desired to offer evidence in support of their former statement that 1 mgm. adrenalin per kilo will not cause glycosuria in thyroidectomized dogs, why did they not inject enough adrenalin to comply with their own conditions? Again in a footnote the following is given concerning the dog of the second experiment: "Der Hund bekam vor dem 2. Versuch auch Pituitrinum infundibulare. Dieses hat nach unseren Untersuchungen keinen Einfluss auf den Kohlehydratstoffwechsel."[10] Nevertheless, in an experiment the results of which may be so significant it would have been much better to have eliminated this last unnecessary conflicting factor.

The experiments of Falta and Rudinger do not support the original statement of Eppinger, Falta, and Rudinger that 1 mgm. adrenalin per kilo administered subcutaneously or intraperitoneally fails to

[10] Falta and Rudinger: *Loc. cit.*

provoke glycosuria in thyroidectomized dogs, since in neither of the protocols reported is there any indication that these authors introduced 1 mgm. adrenalin per kilo body weight.

In an article by Grey and de Sautelle [11] the conclusion is drawn that after thyroidectomy in dogs glycosuria evoked by adrenalin is much smaller than in the normal animal. The method of procedure adopted by these investigators was as follows: Dogs were kept upon a fixed meat diet for several days. They were then injected with adrenalin, after which thyroidectomy was performed. During recovery from the operation a mixed diet was fed. Then meat was again given and adrenalin administered a second time. In the two experiments recorded less sugar in the urine was obtained after thyroidectomy, as a result of adrenalin injection, than was excreted by the normal dog.

The results obtained by these authors, namely, the appearance of sugar in the urine of thyroidless dogs after administration of less than 1 mgm. adrenalin per kilo, stand in direct opposition to those reported by Eppinger, Falta, and Rudinger, but they are in perfect harmony with the observations of Underhill and Hilditch. Grey and de Sautelle were also unable to find any thyroid tissue at autopsy.

On the other hand, the experiments cited hardly warrant the conclusion drawn by the authors, namely, that after thyroidectomy the glycosuria, produced by adrenalin in the normal animal, is greatly reduced. The investigation was evidently carefully planned and executed, but was based upon an assumption the correctness of which is questionable. Consequently the conclusion drawn is not firmly established. From the data presented it is apparent that these authors assumed that if the same normal dog is kept under constant conditions and given equal doses of adrenalin at two different times the quantity of sugar excreted after these injections should be approximately the same. If that assumption was not made, then the experiments are purposeless. All experimental evidence, however, points against such an assumption, for the same normal dog under constant conditions does not necessarily excrete the same quantity of sugar with the same dosage of adrenalin given at two different times. If a normal dog will not invariably respond to adrenalin

[11] Grey and de Sautelle: Journal of experimental medicine, 1909, xi, p. 659.

twice alike, it is fallacious to attribute a lessened elimination of sugar to the loss of the thyroids without at least the support of a great number of experiments all showing the same marked tendency. In an experiment having as its object the study of the influence of the thyroids upon carbohydrate metabolism, it is, therefore, apparent that *quantitative* changes in sugar excretion should be given little weight.

As corroboratory to the views just expressed the data in Table II are submitted. In these experiments the plan followed was very similar to that outlined by Grey and de Sautelle, and although the details differ somewhat the end aimed at, to keep conditions of diet constant, was attained. The animals had been fed upon meat for several days before the experiments began. They were then fed upon a constant mixed diet for a period of five days. Then adrenalin was given subcutaneously, — 1 mgm. per kilo body weight. On the day of the injection the usual quantity of water was given but no food. As a rule sugar elimination ceased within twenty-four hours after adrenalin administration. The dogs were then fed upon meat until five days before the second adrenalin injection. During these five days the mixed diet was again given. As before, no food was offered on the injection day. After the second adrenalin administration the thyroids were completely removed, but at least two parathyroids, one on each side, were left intact. This was confirmed at autopsy. During the period of recovery from the operation the dogs received the meat diet. Five days before the third adrenalin injection the mixed diet was fed, and as previously no food was given on the day of injection. By such a régime the animals remained practically constant in weight. In every instance 1 mgm. adrenalin per kilo was administered subcutaneously in the region of the lower ribs. The subcutaneous injection possesses the following advantages: animals do not die so frequently as with the intraperitoneal injection, and are not so likely to vomit or have diarrhœa or bloody urine. In this particular point we differ from Grey and de Sautelle, since their injections were made intraperitoneally. The principle, however, is identical in the two cases, since the mechanism involved in each case is the same. Furthermore, there is no basis for assuming that adrenalin given intraperitoneally will show a different behavior concerning the point under discussion than adrenalin administered subcutaneously.

The data presented in Table II demonstrate conclusively that adrenalin administered twice to the same normal animal under like conditions does not necessarily provoke the same degree of glycosuria in the two instances. Moreover, in every case reported adrenalin induced glycosuria after thyroidectomy, and the quantity of sugar

TABLE II.

SUGAR IN URINE IN GRAMS.

Dog.	Before thyroidectomy.		After thyroidectomy.
	First injection.	Second injection.	
A	...	...	March 3 0.0 March 15 4.79
X[1]	1.27	0.16	Died during operation
21	9.70	1.20	0.76
22	0.0	3.40	3.67
23	1.22	4.64	14.38

[1] This animal was a dog of 7.0 kilos. The details concerning the other dogs are given in the footnotes of Table I, p. 333. In each experiment 1 mgm. adrenalin per kilo was injected subcutaneously.

eliminated after the operation was not uniformly decreased. In fact, in two of three experiments detailed more sugar was excreted by the thyroidectomized dog than appeared in the urine of the normal dog.

CONCLUSIONS.

Renewed investigation concerning the efficiency of adrenalin in provoking glycosuria in thyroidectomized dogs leads to a reiteration of our former conclusion that adrenalin chloride administered subcutaneously in doses of 1 mgm. per kilo body weight causes a significant glycosuria in dogs deprived of both thyroids but retaining at least two parathyroids. The criticisms of Falta and Rudinger with respect to our former experiments have in no way invalidated this conclusion.

In the investigation by Falta and Rudinger, put forth in support of the conclusions deduced by Eppinger, Falta, and Rudinger, they have failed to comply with the conditions laid down by the latter. Consequently the results offered by Falta and Rudinger cannot be accepted as proof that adrenalin administered to thyroidectomized dogs in doses of 1 mgm. per kilo is incapable of causing glycosuria.

The observations of Grey and de Sautelle are in harmony with our own results and stand in direct opposition to the position taken by Eppinger, Falta, and Rudinger. The validity of the conclusions drawn by Grey and de Sautelle, however, may be questioned, since the investigation was based upon an assumption the correctness of which has not yet been established.

Experiments are reported demonstrating that adrenalin administered subcutaneously to normal dogs in doses of 1 mgm. per kilo causes a widely varying degree of glycosuria. The same quantity of adrenalin introduced into thyroidectomized dogs under like conditions is capable of inducing as great or even a greater glycosuria than occurs with the normal animal.

STUDIES IN EXPERIMENTAL GLYCOSURIA. — VI. THE DISTRIBUTION OF GLYCOGEN OVER THE LIVER UNDER VARIOUS CONDITIONS. POST MORTEM GLYCOGENOLYSIS.

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IN studying the influence of various conditions on the glycogenic function of the liver, it is customary to make a comparison of the amount of glycogen contained in a portion removed just prior to the experimental condition under investigation with the amount found in another portion removed during or immediately after it. The difference between these values indicates the amount of glycogenolysis in a given time, and this difference is conveniently expressed as a percentage of the original amount of glycogen present. This percentile glycogenolysis, as we may call it, is then compared with that occurring in a liver kept for a similar time as nearly as possible under normal conditions.

Although this method has been frequently employed by various workers, there is some considerable uncertainty regarding several fundamental data on which the calculations involved in it depend. These are especially with regard to:

1. The distribution of glycogen over the liver, particularly during ether anæsthesia and following the taking of food.

2. The average course of *post mortem* glycogenolysis (*i. e.*, its time of onset and the velocity with which it proceeds).

Having employed the above-described principle for estimating glycogenolytic activity in connection with some of the work which has been in progress in this laboratory during the past two years, and finding that the above-mentioned fundamental values might vary considerably, quite independent of the experimental conditions under investigation, we have thought it important to repeat many of the observations, more especially with the object of ascertaining to what cause these accidental variations are due.

I. The Distribution of Glycogen over the Liver immediately after and some Time after Death

Regarding the previous work in this connection the following articles are of importance: Külz,[1] using the Brücke-Külz method, found in the dog's liver divided into four parts the following percentages of glycogen: 5.18, 5.05, 5.0, and 4.96; the greatest difference was 0.26, or 4.24 per cent. In another dog's liver divided into three parts the percentages were 2.45, 2.25, and 2.19; greatest difference, 0.26, or 11.9 per cent.

Cramer,[2] working on rabbits' liver divided into three parts, found the following percentage amounts of glycogen: 0.9038, 0.9528, and 0.9438; the greatest difference was 0.490, or 5.1 per cent.

Schöendorff[3] found in the dog's liver a percentage of 18.82 in one portion and 18.33 in another; difference, 0.49, or 2 per cent.

Grube,[4] at the instigation of Pflüger, undertook a more thorough investigation of this question. He used dogs that had been starved from one to five days and then fed with a mixed diet containing an excess of carbohydrate. The dogs were killed by hæmorrhage at a variable time after feeding, and the following results were obtained:

[1] Külz: Zeitschrift für Biologie, 1886, xxii, p. 183.

[2] Cramer: Zeitschrift für Biologie, 1888, xxiv, p. 85.

[3] Schöendorff: Archiv für die gesammte Physiologie, 1903, xcix, p. 191.

[4] Grube: Archiv für die gesammte Physiologie, 1905, cvii, p. 483.

1. Dog fed for three days after a period of five days' starvation. Liver divided into four parts. Average amount of glycogen, 16.6 gm.; greatest difference, 0.86 gm., or 5.4 per cent.

2. Dog killed twelve hours after feeding, following two days' fast. The entire liver was minced, and four portions of the mixed mince analyzed. Average percentage, 7.4; greatest difference, 0.055, or 0.76 per cent.

3. Dog killed fourteen hours after feeding, following thirty-six hours' fast. Entire liver minced, and four parts of mixed mince taken for analysis. Average percentage, 6.4; greatest difference, 0.039, or 0.6 per cent.

4. Dog killed fourteen hours after feeding, following a twenty-four-hour fast. The peripheral portions of four different lobes were analyzed separately. Average percentage of glycogen, 9.73; greatest difference, 0.098, or 1.01 per cent.

5. Dog killed six hours after feeding, following a twenty-four-hour fast. Portions from three different lobes were analyzed. Average percentage of glycogen, 0.19 (one result was however very low, *viz.*, 0.139); greatest difference, 0.048, or 24.3 per cent. It is suggested by Grube that the unusually low value which one of the portions in the liver of Dog V showed is probably due to this portion having contained a large amount of connective tissue, blood vessels, etc. It is to be noted that it is only in Experiments 1, 4, and 5 that the glycogen in different lobes is compared.

Sérégé [5] found in dogs that during the first two hours following the taking of food (that is to say, during digestion in the stomach) there was more glycogen in the left half of the liver than in the right; during the next six to eight hours (that is to say, during digestion in the intestine) the distribution was reversed, there being more glycogen in the right lobes than in the left. From twelve hours on, the liver again contained more glycogen in the left lobes. Sérégé employed the Fränkel method (the precipitation of proteins by trichloracetic acid), which Pflüger has shown to be quite unreliable.

Lépine,[6] without however giving any experimental evidence, considers it is definitely settled that the distribution of glycogen over the liver is irregular.

These results, taken as a whole, would indicate that the distribution of glycogen over the liver does not usually vary by more than 5 per cent; the greater variation of 12 per cent, observed by Külz,

[5] Sérégé: Comptes rendus de la Société de Biologie, 1905, lviii, p. 521.

[6] Lépine, R.: Le diabète sucré, 1909, p. 99 (Paris, Félix Alcan).

being exceptionally great, and the high variation in Grube's last result being partly due to the experimental error incidental in estimating such small quantities of glycogen as this liver contained.

In the present research the portions of liver for analysis were removed as simultaneously as possible, cut into thin slices, pressed free of blood between filter paper, and treated by Pflüger's process for the estimation of glycogen.

The following are, briefly, the results:

Rabbit I. — Fed on preceding day with carrots; killed by stunning. Liver immediately excised and placed in incubator.

Percentage amount of glycogen (dextrose) in:

Quadrate lobes (immediately after death)	11.05	(20 min. later)	10.31
Left lobes (2 min. after)	10.80	(20 min. after)	10.21
Difference	.0.25		0.10
Difference in per cent of larger amount	2.26		0.97

Rabbit II. — Same procedure followed as in Rabbit I. Percentage amount of glycogen (dextrose) in:

	Immediately after death.	Twenty min. later.	Fifty min. later.
Quadrate lobe	8.840	8.111	6.733
Left lobe	8.793	7.960	5.932
Difference	0.047	0.151	0.801
Difference in per cent of larger amount	0.53	1.86	11.8

Dog I (Exp. 97). — Specially fed the day before on cane sugar; killed by bleeding. Liver left *in situ*, but animal kept on warm tank. Temperature of front lobes, 30° C.; temperature of back lobes, 36° C.

Percentage amount of glycogen (dextrose) in:

	Before death.	Ten min. later.	Twenty min. later.
Front lobes	7.195	7.664	7.370
Back lobes	6.750	7.025	6.234
Difference	0.445	0.639	1.136
Difference in per cent of larger amount	6.2	8.3	15.4

Dog II. — Dog fed with bread and meat; died while under anæsthetic during experiment of injecting N/8 lactic acid into vena pancreatico-duodenalis.

Percentage amount of glycogen (dextrose) in:

	Before death.	One hr. later.	Two hrs. later.	Three hrs. later.	Four hrs. later.	Five hrs. later.	Six hrs. later.
Left lobe	4.675	4.016	3.726	3.610	3.756	3.450	3.233
Right central	4.620	4.165	3.866	3.570	3.230	3.186	3.033
Difference	0.055	....	....	0.040	0.526	0.264	0.200

Dog III. — Dog not specially fed; killed in course of another experiment. Liver excised.

Percentage amount of glycogen (dextrose) in:

	Immediately after death.	Twenty min. after.
Left lobe	2.506	2.118
Left central	1.650	....
Right central	1.635	....
Right lobe	1.551	....
Greatest difference	0.955	....

Greatest difference in per cent of largest amount 38.2

Dog IV. — Dog not specially fed; had been used (under deep anæsthesia) in three-hour experiment on blood pressure.

Percentage amount of glycogen (dextrose) in:

Left lobe (back)	3.290	Caudate (front)	2.990
Left lobe (front)	3.140	Right central	3.180
Left central	3.120	Right lobe	3.070
Caudate (back)	2.760	Spigelian	2.800

Greatest difference 0.530

Greatest difference in per cent of largest amount 16.1

Dog V. — Dog fed with bread and meat; died of hæmorrhage in course of operation for making Eck fistula. At time of death had been under anæsthesia for about half an hour. The liver was placed in the incubator at 40° C. between the periods of removal of portions for analysis.

Percentage amount of glycogen (dextrose) in:

	Immediately after death.	Fifteen min. later.	Thirty min. later.	Forty-five min. later.
Left lobe	3.78	2.98	2.62	2.38
Right central	3.82	2.89	2.61	2.40
Difference	0.04	0.09	0.01	0.02
Difference in per cent of larger amount	1.05	3.02	0.38	0.82

Several conclusions of practical importance regarding the distribution of glycogen over the liver can be drawn from these observations:

1. When the liver is removed from an animal (rabbit or dog) that has just been placed under anæsthesia, the percentage amount of glycogen in the different lobes does not differ by more than about 6 per cent, the difference being usually much less than this. These differences are probably due to varying amounts of connective tissue and of blood in the different portions of the organ.

2. At varying periods after death, the *post mortem* disappearance of glycogen proceeds at first to an equal degree in the different portions of the liver, provided the liver be excised and kept at equitable temperature (Rabbit I, Dogs II and V).

3. If the liver be left in the body after death, *post mortem* glycogenolysis proceeds at a variable rate in the different portions of it, so that considerable differences are found in the percentage amount of glycogen in the different lobes. These results are partly due to differences of temperature between the deep and the superficial lobes (Dog I).

4. When, prior to death, the animal has been for some considerable time on its back under anæsthesia, a considerable difference in the glycogen content is found in the different portions of the liver. What it is that occasions these differences is not quite clear, but it is probably associated with unequal blood supply, for it will be shown later that the rate of glycogenolysis in liver tissue is very much influenced by the amount of blood in the viscus (Dogs III and IV).

II. The Distribution of Glycogen over the Liver at Varying Periods after Feeding with Carbohydrate-Rich Food.

It has already been pointed out that Sérégé claims to have found an unequal distribution of glycogen in the liver during the different periods of digestion. The results on which this conclusion is based have been seriously called in question by Pflüger,[7] who further quotes Grube's observations (Grube, *loc. cit.*) as absolutely refuting Sérégé's conclusions. This is not, however, the case, for Grube's observations do not bear directly on this point. The shortest period after feeding

[7] Pflüger, E. F. W.: Das Glycogen, 2d ed.

during which Grube determined the amount of glycogen in the different portions of the liver was six hours, but in that case only traces of glycogen were found, so that nothing final can be concluded from the result. The next period was twelve hours, and in this, as in the later periods, equality of distribution was observed, thus disproving at least one of Sérégé's conclusions, namely, that after absorption is over the left lobes are richer in glycogen than the right.

It is undoubtedly of great importance to definitely decide this question, not only because of its practical bearing, but on account of its physiological interest. Should Sérégé's observations be corroborated, then would it be evident that the mechanism which controls the deposition of glycogen in the liver is not a perfectly acting one: it would indicate that one portion of the viscus becomes partially filled with glycogen before any more of this substance is stored in other portions. Sérégé attributes this inequality of distribution to the fact that the blood of the mesenteric vein proceeds more or less directly to the right lobes without becoming mixed with that of the splenic vein which proceeds to the left lobes. During absorption therefore the right lobes receive the greater proportion of absorbed food stuff. Although, on *a priori* grounds, such a piece-meal deposition of glycogen in the liver is improbable, yet the possibility of its occurrence must be admitted, and there is nothing in Grube's results which absolutely refutes the hypothesis. The following experiments were performed in connection with this question :

Five dogs, each weighing about 10 kilos, and as nearly as possible alike in age and breed, were starved for five days. On the sixth day each dog was fed by the stomach tube with soup having cane sugar dissolved in it, corresponding quantities being given to each dog. The following was the further plan of the experiment:

8.30 A.M.	Each dog given about 250 gm. cane sugar in soup.
9.30 "	Again given some sugar.
10.30 "	Again given some sugar.
11.30 "	Dog I killed by chloroform.
1.20 P.M.	Dog II killed by chloroform.
2.20 "	Remaining dogs given some sugar.
3.20 "	Dog III killed by chloroform.
5.20 "	Dog IV killed by chloroform.
8.30 A.M.	Dog V killed by chloroform.

The following are the results:

Dog I. — Weight, 9.950 gm. Time since food first given, three hours. Time since food last given, one hour. Liver weight accidentally lost.

Percentage amount of glycogen (dextrose) in:

Left lobe	3.500 3.530	3.515 (1)[8]
Left central	3.549 3.630	3.594 (3)
Caudate	3.501 3.470	3.485 (1)
Right central	3.310 3.360	3.335 (2)
Right lobe	3.050 3.040	3.045 (1)

Greatest difference 0.549

Greatest dif. in percentage of largest amount . 15.5

Dog II. — Weight, 11.400 gm.; liver weight, 418 gm. Time since fed, three hours. Weight of liver, 3.66 per cent of body weight.

Percentage amount of glycogen (dextrose) in:

Left lobe	4.600 4.670	4.635 (2)
Left central	4.269 4.073	4.171 (5)
Caudate	4.104	4.104
Right central	4.149 4.149	4.149 (0)
Right lobe	4.134 4.119	4.126 (1)

Greatest difference 0.531

Greatest difference in percentage of largest amount 11.2

Dog III. — Time since first fed, five hours. Weight, 10.200 gm. Weight of liver, 341 gm. Weight of liver, 3.34 per cent of body weight.

Percentage amount of glycogen (dextrose) in:

[8] The figures in parentheses indicate the approximate percentage error between the duplicates.

Left lobe	3.446 3.516	3.481 (2)
Left central	3.468	3.468
Caudate	3.312	3.312
Right central	3.135 3.054	3.094 (3)
Right lobe	3.054	3.054
Greatest difference		0.427
Greatest difference in percentage of largest amount		12.2

Dog IV. — Time since last fed, three hours, but seven hours since main feeding. Weight, 9.300 gm.; weight of liver, 409 gm. Weight of liver, 4.39 per cent of body weight.

Percentage amount of glycogen (dextrose) in:

Left lobe	9.105 9.682	9.393 (5)
Left central	9.852 10.200	10.026 (4)
Caudate	10.310 10.087	10.198 (2)
Right central	9.700 9.742	9721 (1)
Right lobe	8.970	8.970
Greatest difference		1.228
Greatest difference in percentage of largest amount		12.04

Dog V. — Time since last fed, eighteen hours. Weight, 10.800 gm.; weight of liver 395 gm. Weight of liver, 3.65 per cent of body weight.

Percentage amount of glycogen (dextrose) in:

Left lobe	7.450 7.600	7.525 (2)
Left central	6.612 6.804	6.708 (3)
Caudate	6.770 6.680	6.725 (2)
Right central	7.014 6.588	6.801 (5)
Right lobe	6.700	6.700
Greatest difference		0.825
Greatest difference in percentage of largest amount		10.9

It is perfectly evident that in all the above observations more glycogen was found in the left than in the right lobes. This difference cannot be attributed to the supposed inequality of blood supply to the different lobes during absorption, for then, according to Sérégé, the right lobes should have contained more glycogen than the left in Nos. 1, 2, 3, and 4. The observed differences are undoubtedly due to the occurrence of *post mortem* glycogenolysis in the liver during the process of removal and weighing of portions for analysis. In the above observations the portions of liver were removed in the order in which the results are reported, and it took from twenty to thirty minutes to make all the necessary weighings. After the portions of liver had been weighed they were placed in flasks and potash added simultaneously to all, but since, as we shall show later, the *post mortem* process is more rapid in the intact viscus than in sections of it, there was opportunity for considerable variation in the amount of glycogen between the first and the last removed portions. It would have been more accurate to cool the liver in ice immediately after its removal. This was done in the following two experiments:

Dog VI. — Starved five days. Fed with 10 gm. per kg. cane sugar and killed by chloroform six hours later. The liver was immediately excised and placed in freezing mixture. Wedge-shaped portions were then removed and analyzed, beginning with left lobe.

Percentage amount of glycogen (dextrose) in:

Left lobe	10.83 10.86	10.845 (0.3)
Left central	10.98 10.80	10.890 (1.6)
Caudate	10.86 10.56	10.710 (2.7)
Right central	11.34 11.36	11.350 (0.17)
Right lobe	11.70 11.52	11.610 (1.5)
Greatest difference		0.900
Greatest difference in per cent of largest amount		7.6

Dog VII. — Treated in same way as Dog VI, but killed in twelve hours after feeding and portions of liver first of all removed from right lobes.

Percentage amount of glycogen (dextrose) in:

Right lobe	14.25 13.88	14.065 (2.5)
Right central	14.84 14.55	14.700 (2—)
Caudate	14.70 14.98	14.840 (2—)
Left central	14.28 13.99	14.135 (2)
Left lobe	14.43 14.46	14.445 (0.2)
Greatest difference		0.78
Greatest difference in per cent of largest amount		5.25

In the light of these last two experiments (6 and 7) it is evident that the differences in glycogen content of the various lobes observed in Dogs I, II, III, and V were largely due to *post mortem* glycogenolysis. The experimental error in the case of Dog IV was higher than usual, but even in this case a great part of the observed difference is undoubtedly due to the same cause. In the last two experiments (6 and 7) the differences observed are about the same as those already given as the normal variation due to inequality of connective tissue and blood (see pp. 344, 345).

Taking the results as a whole, we may conclude that even when every precaution is taken against unequal *post mortem* change the distribution of glycogen over the liver is not perfectly uniform. It may vary by from 5 to 7 per cent, being, however, no greater during absorption than at other times. It is impossible from these investigations to refute Sérégé's statement [9] that during absorption from the intestine glycogen is deposited more quickly in the right lobes than in the left (indeed some of our results would seem to confirm it, *cf.* No. 6), but it is clear that the differences, if they exist, are of small magnitude, being entirely masked in most of our experiments by *post mortem* glycogenolysis.

[9] In Dog VI it would at first sight appear that Sérégé's observations are confirmed (*i. e.*, that during absorption from the intestine glycogen is deposited more quickly in the right than in the left lobes). The difference is, however, too small to warrant any such conclusion.

III. Post Mortem Glycogenolysis

In certain preliminary observations on the rate of disappearance of glycogen from the liver after death, we were struck with the fact that it is an extremely variable process. The causes of the variations are obscure, and it was deemed essential for further progress to subject them to a more thorough investigation. In a previous contribution by one of us (in collaboration with H. O. Ruh) [10] in which the rate of *post mortem* glycogenolysis in the liver during stimulation of the splanchnic nerve is compared with that occurring without such stimulation, the following proviso is made in connection with the discussion of results: "Before drawing any final conclusions from the experiments here recorded, it is evident that we must be furnished with more reliable and extensive data regarding the course of *post mortem* glycogenolysis." It is pointed out in that connection that very little indeed is known about this process, either as regards the time of onset, or as to when it attains its maximum velocity.

In practically every form of hyperglycæmia increased production of dextrose by the liver is the initial cause of the condition. It is true that in the severer forms of so-called experimental diabetes (pancreatic, phloridzin, etc.), dextrose goes on accumulating in the blood after all the glycogen in the liver has been used up, but even in the late stages of at least some of these conditions, a serious derangement of the glycogenic function of the liver is known to exist, as evidenced by the fact that glycogen is no longer deposited in this viscus even when large amounts of carbohydrate food are ingested. What is it, therefore, which makes this glycogenic function so susceptible to derangement? Although we have at present no justification for believing that the cause of *post mortem* glycogenolysis is the same as that which brings about this process in experimental diabetes, yet it is evident that more accurate information regarding the former process — the cause of its onset, the nature of the conditions which accelerate or retard it, etc. — cannot but be of value to us in arriving at the exciting cause of the *ante mortem* process.

In a previous investigation by one of us on the cause of asphyxial glycosuria it was shown that in an incubated mixture of minced liver and blood glycogenolysis is much accelerated by the presence

[10] Macleod, J. J. R., and Ruh, H. O.: This journal, 1908, xxii, p. 397.

of an excess of carbon dioxide. It was further pointed out that it is probably by increasing the acidity of the mixture that carbonic acid produces this effect. After death acid substances (lactic acid) develop in the tissues (including the liver — Magnus Levy), and the thought immediately presents itself that the onset of *post mortem* glycogenolysis is dependent upon the development of acid. Before concluding that such is the case, however, much more must be known about the nature of the *post mortem* process than at present exists; its exact time of onset, its course, whether it is associated with an increase in the amount of glycogenase in the liver, etc.

With regard to the onset of glycogenolysis, Pavy and Bywaters [11] have recently expressed a similar view to the above. There is nothing in their paper, however, which corroborates it beyond the observation that the acidity of alcoholic extracts of liver increases after death and that injection of sodium carbonate solution in the portal vein prevents *post mortem* glycogenolysis.

As already pointed out in the paper just referred to, the variations in hepatic glycogenolysis that can be brought about experimentally during life may likewise be associated with varying degrees of acidity in the liver cells; thus the hyperglycogenolysis following muscular work may, like the increased respiratory activity, be due to the presence in the blood of acid products of muscular contraction.

a. **Comparison of the rate of glycogenolysis in the liver during ether anæsthesia and after death.** — Dogs fed on the previous day with bread, meat, and cane sugar were used for these experiments. Each animal was anæsthetized with ether and a piece of liver removed as quickly as possible from one of the right lobes, after which the dog was placed on the warm operating table and the anæsthesia maintained, other portions of liver being removed at regular intervals for a period of about one and a half hours. The animal was then bled to death and kept on the warmed operating table, portions of liver being removed as previously. Each portion of liver after removal was cut in thin slices, pressed between filter paper to remove blood, weighed and dropped into an equal volume of 60 per cent KOH. The glycogen determinations in the different portions were conducted

[11] Pavy, F. W., and Bywaters, H. W.: The journal of physiology, 1910, xli, p. 168.

simultaneously, every detail of the process being exactly the same for each portion.

The following table depicts the results of three such experiments:

TABLE I.

No. of experiment.	Condition of animal.	Per cent glycogen (as dextrose) in portions of liver removed every fifteen minutes.	Percentage amount of glycogen (dextrose) disappeared during each period.	Percentage amount of glycogen disappeared for equal periods before and after death.	Velocity constants.[1]
		gm.	gm.	per cent.	
3	Ether anæsthesia	6.36	...	...	....
		6.46	...	...	....
		5.76	0.70	...	.001537
		5.54	0.22	...	.001407
		4.98	0.56	1.38	.001769
	Dead (hæmorrhage)	4.16	0.82	...	.00250
		3.93	0.23	...	.00236
		3.51	0.42	...	....
		3.77	0.16	1.21	.00187
3*a*	Ether anæsthesia	9.17	...	...	....
		8.92	0.25	...	.00077
		8.22	0.70	...	....
		8.60	...	...	....
		7.92	0.15	...	.00105
		7.55	0.37	1.25	.00112
	Dead (chloroform)	7.41	0.14	...	.00102
		6.94	0.47	...	.00115
		6.95	...	...	.00100
		6.56	0.40	0.99	.00106
5	Ether anæsthesia	3.90	...	...	....
		3.31	0.59	...	(.00474)
		3.13	0.18	...	.00317
		3.28	...	...	.00243
		2.67	0.23	1.23	.00274
	Dead (hæmorrhage)	1.86	0.71	...	.00427
		1.45	0.41	...	.00477
		1.32	0.13	...	.00448
		1.065	0.255	1.605	.00470

[1] Velocity constants calculated from the equation $K = \frac{1}{T} \log. \frac{C}{C_1}$, where C_1 is the amount of glycogen left after the time T.

Several conclusions of importance can be drawn from these results:

1. In an anæsthetized animal (dog) there is a rapid disappearance of glycogen from the liver.

2. The rate of disappearance varies markedly in different lobes.

3. There is sometimes an acceleration in the rate of glycogenolysis when the etherized animal is killed.

The figures in the first two columns of the table reveal a remarkable irregularity in the rate of disappearance of glycogen from the various lobes of the liver during ether anæsthesia. Such a result shows clearly that in the anæsthetized animal the usual method for determining the rate of hepatic glycogenolysis is utterly unreliable (Croftan [12]). The irregularity in glycogenolysis is maintained after death, which, as we shall see later, is not the case when death is produced by stunning, or in some other sudden way. It cannot of course be inferred that normal hepatic glycogenolysis likewise proceeds in an irregular manner. We have already seen (p. 351) that while glycogen is being rapidly stored up in the liver there is an almost uniform rate of accumulation in the different lobes. The above observations indicate that when glycogen is being rapidly broken down quite another condition obtains, the process being markedly variable in intensity in different lobes.

With such irregularity in the rate of hyperglycogenolysis in the various lobes it is necessary, in comparing the rate of glycogenolysis before and after death, to employ values in which the variations will be as much minimized as possible. This has been done in two ways:

1. By finding how much glycogen (per 100 gm. liver) has disappeared for periods of one hour before and after death.

2. By calculating the velocity constant for the different time periods observed. The latter value will of course be influenced by the irregularity above noted, but since it expresses the glycogenolysis as a ratio of the original amount of glycogen present, the error will be less marked, and the calculated value will furnish a reliable criterion of the velocity with which glycogenolysis is proceeding at any given moment. It allows for the decreasing amount of glycogen capable of glycogenolysis.

By both methods of calculation it is evident that in Experiment 5 death caused an acceleration in the glycogenolysis. Under ether

[12] Croftan, A. C.: Archiv für die gesammte Physiologie, 1909, cxxvi, p. 407.

alone the process was gradually getting slower to become greatly accelerated immediately after death. The velocity constant in Experiment 3 also shows *post mortem* acceleration, but in Experiment 3 *a*, throughout which glycogenolysis was distinctly slow, no change was produced by death. This experiment differed from the others in the fact that death was produced by an overdose of chloroform instead of by hæmorrhage. The chloroform, in the blood left in the liver, probably retarded the glycogenolysis.

b. The time of onset of post mortem glycogenolysis and the reaction velocity. — To investigate these questions, the animal should be suddenly killed without previous administration of anæsthetic. This has been done in rabbits by stunning in the present research. In the case of the dogs used, the ether anæsthesia was as short as possible.

The particular questions which we have sought to answer are these:

1. At what period after death does glycogenolysis become marked?

2. Does this process, after it appears, attain its maximum intensity gradually or quickly?

3. Having attained its maximum intensity, does it remain constant at this or gradually fall away?

The velocity constant at different stages of the reaction supplies us with the most useful information from which to answer the above questions. This constant will be greater when the conditions are most favorable for the glycogenolytic process, and in calculating it allowance is made for the progressive decrease in glycogen.

In making use of the velocity constant for this purpose it is important to consider how this behaves during the hydrolysis of glycogen solutions *in vitro* by means of diastase. Researches of such a nature have been conducted by Philoche.[13]

When very strong preparations of diastase were used (1 to 50), it was found that glycogen was completely converted into maltose in about fifty hours in a 2 per cent solution. When feebler diastase preparations were used, however, the process ceased long before this stage. Investigation showed that between glycogen and maltose in the hydrolytic process are large amounts of dextrines into which glycogen is readily transformed, but from which, after some time, maltose ceased to be produced. This inhibition in the process was not due to adsorption or destruction of the diastase. Under the circum-

[13] Philoche, Ch.: Journal de chimie physique, 1908, vi, p. 359.

stances it was impossible for Philoche to calculate the velocity constant. If we take for comparison sake the velocity constants as determined for the action of diastase in starch, we find that these gradually decline. When large amounts of substrat are present, however, the first part of the process forms a linear curve and the latter part a logarithmic.[14]

Taylor[15] has computed the velocity constant in the case of the liver of clams kept at a constant low temperature. He found it to progressively decrease, even although he employed for its computation the disappearance of glycogen and not the appearance of reducing sugars.

In the present research we have determined the constant in the liver of two specially fed dogs, killed as quickly as possible by ether and hæmorrhage. After death in the case of one of these the liver was quickly excised and cut in very small pieces and placed in an incubator at body temperature. Portions were removed for estimation of glycogen at periods of one hour each. We recognize that some source of error is incurred by using weighed amounts of liver for the values. This source of error might have been lessened by making nitrogen estimations and using these, as Taylor has done, for the standard of amount of substance taken. For our purpose, however, as the results show, such precaution is unnecessary.

The following table depicts the results:

t (hours).	C_1 (gm. glycogen in 100 gm. liver).	$\frac{1}{t}$ log. nat. $\frac{C}{C_1}$.	t (hours).	C_1 (gr. glycogen in 100 gm. liver).	$\frac{1}{t}$ log. nat. $\frac{C}{C_1}$.
0	(C 4.345)	....	5	2.924	.0343
1	3.826	.0554	6	2.615	.0367
2	3.620	.0396	7	2.265	.0404
3	3.480	.0321	8	2.015	.0417
4	3.100	.0366	9	1.800	.0425

The process was most rapid during the first hour after death, then slowed off until the seventh hour after death, when it began to increase again. This increase is probably due to commencing putrefaction. The results do not, however, show any evident decrease in the activity of the ferment after the first hour.

[14] Brown and Glendinning, T. A.: The journal of the Chemical Society, 1902, lxxxi, p. 388.

[15] Taylor, A. E.: The journal of biological chemistry, 1908, v, p. 315.

In another experiment conducted in the same manner with the difference that the liver instead of being cut into small pieces was left intact the following results were obtained:

t (hours).	C_1 (Gm. glycogen in 100 gm. liver).	$\frac{1}{t}$ log. nat. $\frac{C}{C_1}$.	t (hours).	C_1 gm. (glycogen) in 100 gm. liver.	$\frac{1}{t}$ log. nat. $\frac{C}{C_1}$.
1	4.165	.04490 [16]	4	3.230	.03883
2	3.866	.03684	5	3.186	.03227
3	3.576	.03697	6	3.033	.03045

In this case there is a progressive decline in the constant.

We may conclude from the results that the *post mortem* process when once established proceeds at a practically uniform rate for several hours.

At what period after death does the process set in, and how long does it take to attain its maximal velocity? — It has proved a most difficult task to obtain data from which these questions can be answered. The periods of time intervening between the removal of the pieces of liver must be so short and the observed changes in glycogen contents are so small that after allowing for the experimental errors involved in such determinations (see p. 346) there is considerable uncertainty in the observations. It is only in animals killed without previous anæsthesia that these observations are of any value. Of the several experiments which we have conducted there are only two, on rabbits, which we will publish at the present moment:

RABBIT I.

t (min.).	C_1.	$\frac{1}{t}$ log. nat $\frac{C}{C_1}$.
0	10.800	....
20	10.210	.00120
40	9.466	.00142
60	9.100	.00123

RABBIT II.

0	8.793	....
20	7.960	.00215
50	6.733	.00232

[16] Portions of liver all taken from large left lobe. Controls taken from right lobes gave same results.

These preliminary results indicate that the process starts within twenty minutes after death and that by this time it has attained its maximal velocity.

The only studies of the same nature as the above which we can find recorded in the literature are by Pavy,[17] Dalton,[18] and Seegen.[19] The most important results were obtained by Pavy. In well-fed rabbits this investigator found that if a portion of liver were removed immediately after death by pithing, and instantly cooled in a freezing mixture, it did not yield on analysis any more dextrose than that usually found in the other tissues (up to 0.2 per cent). If a few minutes were allowed to elapse before the portion of the liver was removed, a great increase in dextrose (up to 1.29 per cent) was noted. By leaving the liver until next day, a further increase (up to 3.68 per cent) had occurred. The amount of sugar present in the liver on the following day is of course quite unreliable as indicating the degree of glycogenolysis which had meanwhile occurred, because considerable glycolysis must have taken place. Pavy concluded that the glycogenolytic process must become greatly slowed after a few minutes because 10 to 12 parts per 1000 sugar had accumulated during this time out of a glycogen supply which could yield, say, 50 parts per 1000. This would mean "that the whole [of the glycogen] would disappear in about three quarters of an hour if the production of sugar took place at the rate above mentioned." The question cannot, however, be so cursorily dismissed, for it is clearly shown by our results that for several hours after death the process is still at its maximum velocity.

There is no doubt, as our observations testify, that the rate of *post mortem* glycogenolysis varies considerably in different animals, even when they are of the same species. These variations have prompted us to investigate some of the factors which influence the *post mortem* process.

c. **Analysis of some of the factors which determine the speed of post mortem glycogenolysis.** — There are two factors which are known to have an influence on the rapidity of the glycogenolytic process in the liver. These are (1) the amount of blood in the viscus, and (2) the

[17] Pavy, F. W.: The physiology of the carbohydrates, London, 1894, p. 138.

[18] *Cf.* Bernard Claude: Leçons sur le diabète, 1877, p. 351.

[19] Seegen, J.: Die Zuckerbilding in Thierkörper, 2 ed. (Berlin), 1900, p. 62

connection of the liver with the nervous system. To study the relative importance of these factors we have proceeded as follows:

An Eck fistula was established in a specially fed anæsthetized dog. Portions of liver were removed from two lobes, and each of these portions was further subdivided into three parts. In one of these the glycogen was determined, another portion was placed in the incubator at body temperature, and the third portion was placed in blood in the abdomen of the animal. At the end of an hour the glycogen content was determined in:

1. The portion placed in the incubator.
2. The portion placed in blood in the abdomen.
3. The liver left *in situ* in the animal's body.

The liver left *in situ* was in some cases isolated from the nervous system by cutting all hepatic nerves; in other cases these nerves were left intact.

The results of these observations are given in Table II.

The figures recorded in Table II are chosen from experiments in which there was no uncertainty as to the accuracy of the results. The temperature of the incubator varied somewhat in different cases and the variations are noted. It is seen that the glycogen disappeared in every case more rapidly in the portions of liver that were bathed in blood in the abdomen than in the portions that were placed in the incubator, and from which as much blood as possible had been pressed out. This difference is of course readily understood. It is due to the presence of glycogenolytic ferment in the blood. Exactly similar results have been recorded by Bial.[20] Pavy [21] also observed that the addition of blood to washed liver materially increases the amount of sugar produced. As illustrating the importance of the presence of blood in accelerating the process, the following experiment is of interest. One half of the liver was washed free from blood by perfusing 0.9 NaCl solution through the branches of the portal vein running to it. Portions of liver were then removed from the washed lobes and placed in 0.9 NaCl solution in the incubator, and portions from the unwashed lobes were placed in blood at the same temperature. These were incubated for one hour, at the end of which

[20] Bial: Archiv für die gesammte physiologie, 1894, lv, p. 434.

[21] Pavy: *Loc. cit.*, p. 147.

time it was found that 17.5 per cent of the glycogen had disappeared from the blood-free lobes, whereas 22.1 per cent had disappeared from the lobes incubated in blood (Experiment 134).

TABLE II

PERCENTILE GLYCOGENOLYSIS IN ONE HOUR IN THE LIVER OF THE DOG, AS AFFECTED BY THE AMOUNT OF BLOOD LEFT IN IT AND CONNECTION WITH THE NERVOUS SYSTEM (RESULTS GIVEN AS DEXTROSE).

No. of experiment.	Per cent glycogen at start.	Per cent glycogen disappeared: A. From portion of liver placed in incubator.	B. From portion of liver placed in blood in abdomen.	C. From liver left *in situ*.	Difference between A & C	Difference between B & C	
123	0.7857	21.4 (Temp. 39.5)	28.0	57.4	36.0	29.4	Hepatic nerves cut.
128	10.9425	7.8 (Temp. 42°C.)	10.0	26.4	19.6	16.4	
129	9.2300	....	10.1	23.4	...	13.3	
121	5.0800	35.3 (Temp. 39°C.)	...	65.0	29.7	...	Hepatic nerves intact.
122	8.605	....	...	28.3	...	...	
124	4.249	12.5 (Temp. 42–44)	19.4	34.7	22.2	15.3	
125	2.240	....	22.7	41.0	...	18.3	
130	4.286	....	29.8	39.4	...	9.6	
Average of last three						14.4	

Much more marked, however, are the differences between the glycogenolysis in the amputated portions placed in blood and in the liver left *in situ*. It is difficult to see how these differences can be dependent upon differences in the amount of blood, for the portions placed in the abdomen were thoroughly cut up so that the blood might come in intimate contact with the liver tissue. The blood in the abdomen was collected from the femoral artery and the pieces of liver were placed in it before it became coagulated. It was thought that this difference might be due to nervous influence which would act on the liver for at least some minutes after the portal circulation had been

cut off. To test this hypothesis, certain of the observations were conducted with the hepatic nerves intact, and others with these nerves entirely severed. There was no difference in the results of these two types of experiments; that is to say, the difference between the glycogenolysis in the intact liver, and in an isolated portion kept in blood in the abdomen, was just as great when the liver was disconnected from the nervous system as when these connections were intact.

We must conclude, therefore, that the exaggerated glycogenolysis which occurs in intact liver as compared with that occurring in disconnected portions of the same liver is not due to any influence of the nervous system. It may be dependent upon the more thorough intimacy of contact between blood and liver cell existing in the intact viscus as compared with that which is possible in the case of isolated portions of liver placed in blood.

The practical importance of these results in connection with all researches in which comparison is made of the rate of glycogenolysis under different conditions cannot be too strongly emphasized. Indeed it was while making observations on the influence of stimulation of the great splanchnic nerve on *post mortem* glycogenolysis that the necessity of such observation became apparent to us.[22]

d. **The influence of stimulation of the great splanchnic nerve on the rate of post mortem glycogenolysis.** — In order to demonstrate the influence of nervous control over the glycogenic function, one of us in conjunction with H. O. Ruh [22] has already published experiments in which the rate of glycogenolysis was compared in livers during stimulation of the great splanchnic nerve, with that which occurs when this nerve is not stimulated. The comparisons seemed to show a more rapid glycogenolysis when the nerve was stimulated. It is evident, however, from the observations above reported that a comparison between the glycogenolysis in different livers is not a very reliable criterion on account of the considerable variations in speed with which this process proceeds in different livers. It must also be remembered in all these experiments that what we are really studying is the rapid *post mortem* process, and it may well be that this is in itself as rapid as can be, so that any nervous influence over it is entirely masked. To control the previous results, another series of ex-

[22] Macleod, J. J. R., and Ruh, H. O.: This journal, 1908, xxii, p. 397.

periments were therefore undertaken in which the above-observed differences in the rate of glycogenolysis in intact and isolated livers were compared with the same differences when the great splanchnic nerve was stimulated.

The dogs were prepared as described above with the difference that the great splanchnic nerve was stimulated at frequent intervals during the hour intervening between removal of the portions of liver for analysis.

The following table depicts the results:

TABLE III.

PERCENTILE GLYCOGENOLYSIS IN LIVER IN SITU DURING STIMULATION OF THE GREAT SPLANCHNIC NERVE, COMPARED WITH THAT IN PIECES OF THE SAME LIVER PLACED IN BLOOD IN THE ABDOMEN.

No. of experiment.	Per cent glycogen at start.	Per cent glycogen disappeared:		
		A. From pieces of liver placed in abdomen.	B. From intact liver.	Difference between A & B.
131 [1]	6.997	17.9	30.2	12.3
133 [1]	6.934	28.6	50.1	21.5
135 [2]	10.942	8.06	22.8	14.2
Average				16.0

[1] Blood pressure low, with slight rise on stimulation.
[2] Blood pressure normal, marked rise on stimulation.

The average difference in these experiments between the glycogenolysis in the intact and isolated liver is practically the same as in the previous observations where the nerve was not stimulated.

These results show that artificial stimulation of the splanchnic nerve does not accelerate post mortem glycogenolysis in intact liver. — We are aware that this conclusion is out of harmony with that contained in the previous article referred to above, but when we allow for the undoubted variations in speed with which glycogenolysis proceeds in

different livers, even when these are treated alike, it is readily seen that the method of procedure adopted in the earlier work is unreliable and uncertain. We recognized this while doing the previous work and recorded the results without drawing any final conclusions from them. *Post mortem* glycogenolysis proceeds with such rapidity that the influence of nerve control is undemonstrable on it. So far, therefore, the only means of demonstrating this control is in the intact animal by making observations on the reducing power of the blood before and during stimulation of the nerve.

Conclusions.

The percentage amount of glycogen in the different lobes of the liver varies by about 5 per cent. This variation is partly due to errors in the method of estimation (Pflüger) and partly because of an unequal amount of connective tissue and of blood in different portions of the organ. These differences become much greater (*a*) under ether anæsthesia; (*b*) when the liver is left *in situ* in the dead animal.

The differences are not materially affected by absorption of carbohydrate food from the intestine.

After death in an ætherized animal there is usually, but not always, an acceleration in the rate of glycogenolysis; this varies in different lobes.

It has, so far, been impossible to determine the exact time of onset of *post mortem* glycogenolysis, but it is certainly well established within twenty minutes after death.

Once established, *post mortem* glycogenolysis proceeds at a uniform speed for several hours after death, being dependent solely on the amount of glycogen remaining in the viscus (temperature, etc., being constant). The value of K in the equation for reaction velocity remains therefore practically constant for several hours after death.

Post mortem glycogenolysis is much more active in intact than in cut up liver.

In cut up liver glycogenolysis is much more rapid when the liver is in contact with blood than when it is blood free.

The greater glycogenolysis in intact liver is not due to any influence which the nervous system might have during the few minutes after death in which it could still exert an influence.

Stimulation of the great splanchnic has no constant influence on the course of *post mortem* glycogenolysis.

THE METABOLISM OF DOGS WITH FUNCTIONALLY RESECTED SMALL INTESTINE.

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THE necessity of removing varying lengths of intestine from man has made imperative extensive investigations concerning the influence of such surgical procedures upon nutritional processes. This is particularly true with respect to the mechanisms involved in digestion and absorption. For obvious reasons the experimental demonstration of the effects incident to resection of portions of the enteric tract has been made for the most part upon the dog and cat. Of particular importance in this connection have been the observations recorded by Harlay,[1] Senn,[2] Trzebicky,[3] Monari,[4] De Filippi,[5] Erlanger and Hewlett,[6] Flint,[7] and others.[8] From these investigations it may be stated that extirpation of portions of the small intestine generally entails a decreased absorption of the nitrogenous and fatty constituents of the food. The extent of lessened absorption depends upon the relative length of intestine removed, and also upon the period which has elapsed after the operation, that is, whether compensation has been established. The composition of the food

[1] Harlay: Proceedings of the Royal Society, London, (B), 1899, lxiv, p. 255.

[2] Senn: Experimentelle Beiträge zur Darmchirurgie, Basle, 1892, quoted.

[3] Trzebicky: Archiv für klinische Chirurgie, 1894, xlviii, p. 54.

[4] Monari: Beiträge zur klinischen Chirurgie, 1896, xvi, p. 479.

[5] De Filippi: Archives italiennes de biologie, 1894, xxi, p. 445.

[6] Erlanger and Hewlett: This journal, 1901, vi, p. 1.

[7] Flint: Transactions Connecticut State Medical Society, 1910, p. 283. A complete discussion of the earlier literature upon this subject both in connection with the human subject and with the lower animals may be found in this article.

[8] London and Dmitriew: Zeitschrift für physiologische Chemie, 1910, lxv, p. 213; Carrel, Meyer, and Levene: This journal, 1910, xxv, p. 439.

may play a significant rôle, since it has been established that large quantities of fat bring about a much poorer utilization of food nitrogen than occurs when smaller quantities of fat are ingested. Diminished utilization of fat is particularly noticeable in these experimental animals. With respect to carbohydrate absorption the observations are somewhat at variance, since in some instances it has been reported that the fæces held reducing substances, and in other experiments none were found.

In the investigation to be reported study of three problems was held in view, (1) the absorption of foodstuffs after functional removal of varying lengths of small intestine, (2) a study of food absorption at different intervals after the operation, and (3) the determination of carbohydrate utilization under varied conditions.

Experimental.

Description of the animals employed. — The dogs used in these experiments were placed at our disposal through the kindness of Professor Joseph Marshall Flint, and for convenience will be designated Dog A, Dog B, and Dog No. 12. In these three instances a portion of the small intestine was short-circuited.

Dog A was a water spaniel of 8.3 kilos in splendid nutritive condition. Two weeks after the operation, when she came into our possession, January 26, 1910, the wound was well healed. The stools of this animal were fairly well formed, and throughout the entire period of observation diarrhœa was not once noticed. On autopsy about nine months later it was found that the entire intestine was 412.5 cm. long and that 162.5 cm. or 39 per cent had been short-circuited.

Dog B was a mongrel bitch weighing 13.5 kilos when she came into our possession, January 26, 1910. The animal was in fair nutritive condition, but suffered from persistent diarrhœa, discharging copious liquid stools of exceedingly foul odor. On her entrance to the laboratory two weeks after the operation the wound was well healed. On autopsy about nine months later measurement showed the entire length of intestine to be 525 cm., of which 350 cm. had been short-circuited, — 66 per cent.

Dog No. 12 was the animal called Dog No. 12 in Professor Flint's report. She was received into the laboratory January 8, 1909, al-

most six months after the operation. At that time she weighed 7.4 kilos; a distinct loss of weight which was never regained. Diarrhœa was persistent and the appetite was ravenous. On autopsy it was found that of the 324 cm. of small intestine 235 cm. or 73 per cent had been short-circuited.

Methods. — In general the methods followed were those usually employed in metabolism experiments in this laboratory. Urine was collected in twenty-four-hour periods by catheterization. The water content of the fæces was determined by drying them upon the water bath under acid alcohol and preserving them air-dry. Carbohydrates in the fæces were estimated according to the method of Tsuboi.[9] Food was given in two portions daily. One meal was at nine o'clock in the morning, the other at four o'clock in the afternoon.

Description of Experiment I.

In this experiment Dog A and Dog B were employed. For several days previous to the investigation the animals had received an adequate mixed diet. The experiment was planned to determine the ability of these dogs to maintain nitrogenous equilibrium upon sufficient mixed diets the composition of which was to be radically altered at intervals with respect to the content of fat and carbohydrate. As originally planned, each period of the experiment was to extend over five days. Owing to fæcal contamination of the urine of Dog B, it was practically impossible to obtain urine and fæces unmixed for five consecutive days, with the exception of the first period. Accordingly in the other periods balances have been made covering the consecutive days on which the urine was uncontaminated. Although it was out of the question at times to include the excreta in the balance, food was given as usual and all other conditions remained constant, so that when a balance of less than five days is reported the results are probably fairly representative of the animal's condition throughout the entire period. The periods of this experiment have been designated Periods 1, 2, 3.

Diets. *Dog A.* — The diet for Period 1 consisted of 100 gm. meat, 50 gm. cracker meal, 20 gm. lard, 10 gm. bone ash, and 150 c.c.

[9] Tsuboi: Zeitschrift für Biologie, 1897, xxxv, p. 68.

water, the total nitrogen content of which amounted to 4.39 gm., or .56 gm. nitrogen per kilo. The estimated fuel value was 505 calories, or 64 calories per kilo.

In Period 2 the diet was made up of 100 gm. meat, 75 gm. cracker meal, 36 gm. lard, 10 gm. bone ash, and 230 c.c. water, containing 4.81 gm. nitrogen and 750 calories, or 0.62 gm. nitrogen and 96 calories per kilo.

The composition of the food in Period 3 was as follows: 100 gm. meat, 100 gm. cracker meal, 10 gm. bone ash, and 300 c.c. water. The diet contained 5.23 gm. nitrogen, and a calculated fuel value of 520 calories, or 0.69 gm. nitrogen and 67 calories per kilo body weight.

Further details concerning Experiment I, Dog A, may be found in Tables I and III, pages 370 and 372 respectively.

Diets. *Dog B.* — In the first period this dog was placed upon a diet consisting of 200 gm. meat, 80 gm. cracker meal, 30 gm. lard, 20 gm. bone ash, and 300 c.c. water. This diet contained 8.44 gm. nitrogen and had an estimated fuel value of 837 calories, or 0.63 gm. nitrogen and 63 calories per kilo body weight.

In Period 2 the diet was made up of 200 gm. meat, 120 gm. cracker meal, 50 gm. lard, 30 gm. bone ash, and 400 c.c. water, and contained 9.11 gm. nitrogen and 1180 calories (calculated), or 0.71 gm. nitrogen and 93 calories per kilo.

The diet in Period 3 had the following composition: 200 gm. meat, 160 gm. cracker meal, 30 gm. bone ash, and 450 c.c. water. The food contained 9.78 gm. nitrogen with an estimated fuel value of 875 calories, or 0.79 gm. nitrogen and 70 calories per kilo body weight. For further details of Experiment 1, Dog B, see Tables II and III, pages 371 and 372 respectively.

Discussion of Results of Experiment I.

Throughout the entire first experiment, Dog A maintained body weight and furnished large positive nitrogen balances (see Tables I and III). Nitrogen utilization was practically the same as that of a normal animal upon a mixed diet. The effect of increasing carbohydrate and fat intake had little influence upon nitrogen equilibrium, although fat utilization on this diet was somewhat diminished. A further increase is to be noted when lard was entirely removed from

TABLE I

EXPERIMENT I. — DOG A, 39 PER CENT INTESTINE RESECTED.[1]

Date.	Body weight.	Nitrogen in food.	Urine.		Fæces.				
			Volume.	Total nitrogen.	Weight. Moist	Weight. Air-dry.	Water content.	Nitrogen.	Ether extract.
1910.	kilos.	gm.	c.c.	gm.	gm.	gm.	per cent.	gm.	gm.
Jan. 29	7.8	4.39	160	1.95	3.3	2.7	18		
" 30	7.7	4.39	160	3.06	...	...	...		
" 31	7.7	4.39	180	3.15	...	29.0	...	2.66	11.96
Feb. 1	7.7	4.39	100	2.70	36.5	22.5	38		
" 2	7.7	4.39	100	3.21	59.6	38.0	36		
PERIOD 2. — FOOD: 100 GM. MEAT; 75 GM. CRACKER MEAL; 36 GM. LARD; 10 GM. BONE ASH; 230 C.C. WATER.									
Feb. 3	7.7	4.81	200	3.21	...	...	...		
" 4	7.7	4.81	200	2.93	77.2	46.7	39		
" 5	7.7	4.81	200	4.08	...	26.8	...	3.68	27.35
" 6	7.7	4.81	200	3.03	40.8	21.0	48		
" 7	7.7	4.81	200	3.43	53.8	28.7	46		
PERIOD 3. — FOOD: 100 GM. MEAT; 100 GM. CRACKER MEAL; 10 GM. BONE ASH; 300 C.C. WATER.									
Feb. 8	7.7	5.23	230	4.26	36.8	17.9	51		
" 9	7.6	5.23	240	4.02	55.6	26.0	53		
" 10	7.6	5.23	240	3.90	...	...	...	3.67	9.80
" 11	7.7	5.23	280	3.84	32.6	20.4	37		
" 12	7.7	5.23	310	4.26	97.5	43.7	55		

PERIOD 1. — FOOD: 100 GM. MEAT; 50 GM. CRACKER MEAL; 10 GM. LARD; 10 GM. BONE ASH; 150 C.C. WATER.

[1] In all experiments the urine showed an acid reaction to litmus.

TABLE II.

EXPERIMENT I. — DOG B, 66 PER CENT INTESTINE RESECTED.

Date.	Body weight.	Nitrogen in food.	Urine.		Fæces.				
			Volume.	Total nitrogen.	Weight.		Water content.	Nitrogen.	Ether extract.
					Moist.	Air-dry.			
1910.	kilos	gm.	c.c.	gm.	gm.	gm.	per cent.	gm.	gm.
PERIOD 1. — FOOD: 200 GM. MEAT; 80 GM. CRACKER MEAL; 30 GM. LARD; 20 GM. BONE ASH; 300 C.C. WATER.									
Jan. 29	13.3	8.44	550	9.24	23.9	9.7	59		
" 30	13.0	8.44	350	6.90	93.8	52.7	44		
" 31	13.0	8.44	420	5.40	104.7	50.2	52	5.33	29.26
Feb. 1	12.9	8.44	320	8.16	119.8	45.8	62		
" 2	12.7	8.44	310	8.16	73.2	36.8	50		
PERIOD 2. — FOOD: 200 GM. MEAT; 120 GM. CRACKER MEAL; 50 GM. LARD; 30 GM. BONE ASH; 400 C.C. WATER.									
Feb. 5	12.5	9.11	350	7.86	...	...	...		
" 6	12.6	9.11	410	8.24	153.5	81.5	47	3.41	15.66
" 7	12.6	9.11	450	7.89	114.2	64.5	43		
PERIOD 3. — FOOD: 200 GM. MEAT; 160 GM. CRACKER MEAL; 30 GM. BONE ASH; 450 C.C. WATER.									
Feb. 10	12.4	9.78	430	7.80	155.7	59.3	62	3.37	5.48
" 11	12.3	9.78	360	7.86	129.7	50.2	61		

the diet and carbohydrate intake again augmented. Carbohydrate utilization was perfect upon all diets of this experiment.

With Dog B there was a gradual but steady loss of body weight upon diets which would have been entirely adequate for a normal animal of the same weight. In the first period (five days) a negative balance of 0.99 gm. nitrogen or minus 0.19 gm. nitrogen per day was

obtained. During this period nitrogen of the food was utilized to the extent of 87 per cent. The fat utilization was 85 per cent, while that of the carbohydrate was perfect — that is, no trace of carbohydrate could be demonstrated in the fæces. In the second period (three

TABLE III.

SUMMARY. — EXPERIMENT I.

Periods.	Food.	Excreta.			Balance.	
		Urine.	Fæces.	Total.	Per period.	Per day.
	Dog A, 39 per Cent					
	Nitrogen.					
	gm.	gm.	gm.	gm.	gm.	gm.
1	21.95	14.07	2.66	16.73	+5.22	+1.04
2	24.05	16.68	3.68	20.36	+3.69	+0.74
3	26.15	20.28	3.67	23.95	+2.20	+0.44
	Dog B, 66 per Cent					
1	42.20	37.86	5.33	43.19	−0.99	−0.19
2	27.33	23.99	3.41	27.40	−0.07	−0.02
3	19.56	15.66	3.37	19.03	+0.53	+0.26

days) the dog was in almost perfect nitrogenous equilibrium, a total negative balance of only 0.07 gm. or 0.02 gm. nitrogen per day being obtained. During the period both carbohydrate and fat intake had been markedly increased, nitrogen increase being slight. In spite of these increases the utilization of the three components of the diet remained practically unchanged. The third period (two days) reveals a positive nitrogen balance of 0.53 gm. nitrogen, or plus 0.26 gm. nitrogen per day. No lard was fed during this period, but carbohydrate was much increased. The utilization of nitrogen was not quite so good as in previous periods. Fat utilization was greatly diminished. A portion of this apparent diminution may probably be ex-

plained by the presence in the fæces of ether soluble intestinal excretory products which in the absence of truly unutilized food fat causes a distortion of the percentage utilization. Although the carbohydrate intake was twice that of the first period, no trace of sugar-

TABLE III.

SUMMARY EXPERIMENT I.

Utilization.	Fat (ether extract). Food.	Fæces.	Utilization.	Carbohydrate. Food (calculated).	Fæces.	Utilization.
INTESTINE RESECTED.						
per cent.	gm.	gm.	per cent.	gm.	gm.	per cent.
87	125.50	11.96	90	172	0	100
84	207.55	27.35	86	273	0	100
86	29.55	9.80	66	364	0	100
INTESTINE RESECTED.						
87	199.45	29.26	85	292	0	100
87	181.59	15.66	91	262	0	100
82	22.36	5.48	75	233	0	100

yielding substances could be detected in the fæces. A point of interest in connection with the fæces is the variable water content.

From these experiments upon animals with different lengths of intestine put out of function shortly after the operation only small differences can be detected in the ability of the two dogs to utilize their food, although in one case, Dog A, only 39 per cent of the entire small intestine was not functionating, whereas with Dog B 66 per cent was non-functional. Furthermore, notable increases in fat intake appeared to cause little or no change in fat, nitrogen, or carbohydrate utilization. Large increases in carbohydrate did not result in impaired utilization. The carbohydrate utilization was

very much better than that of normal dogs upon practically the same diets. For instance, unpublished experiments of Dr. Mary D. Swartz make it evident that only 90 to 95 per cent of ingested carbohydrate is utilized in the normal dog. Only in the case of fat can the utilization be called poor.

TABLE IV.

EXPERIMENT II. — DOG A, 39 PER CENT INTESTINE RESECTED.

Date.	Body weight.	Nitrogen in food.	Urine.		Fæces.				
			Volume.	Total nitrogen.	Weight		Water content.	Nitrogen.	Ether extract.
					Moist.	Air-dry.			
1910.	kilos.	gm.	c.c.	gm.	gm.	gm.	per cent.	gm.	gm.
Period 1. — Food: 100 gm. Meat; 50 gm. Cracker Meal; 10 gm. Lard; 10 gm. Bone Ash; 150 c.c. Water.									
May 25	10.5	4.43	75	3.77	23	13	43		
" 26	10.4	4.43	80	3.70	32	17	47	1.60	5.88
" 27	10.4	4.43	65	3.28	38	25	34		
Period 2. — Food: 100 gm. Meat; 75 gm. Cracker Meal; 36 gm. Lard; 10 gm. Bone Ash; 230 c.c. Water.									
May 28	10.5	4.88	85	3.44	16	7	56		
" 29	10.5	4.88	110	3.51	49	27	45		
" 30	10.6	4.88	180	3.42	41	22	46	3.44	13.22
" 31	10.5	4.88	160	3.28	43	21	51		
June 1	10.5	4.88	220	3.33	68	35	48		

DESCRIPTION OF EXPERIMENT II.

At the completion of Experiment I the dogs were allowed to run in large airy cages and were fed upon adequate mixed diets. As time progressed, it became noticeable that Dog B developed an almost insatiable thirst. Diarrhœa was persistent, the stools discharged being notably clay-colored. A constant but gradual loss of weight also

occurred. On the other hand, Dog A appeared to be perfectly normal and steadily gained in weight. The rest period for these animals extended from February 12 to May 23. From this time until June 2,

TABLE V.

EXPERIMENT II. — DOG B, 66 PER CENT INTESTINE RESECTED.

Date.	Body weight.	Nitrogen in food.	Urine.		Fæces.				
			Volume.	Total nitrogen.	Weight. Moist.	Weight. Air-dry.	Water content.	Nitrogen.	Ether extract.
PERIOD 1. — FOOD: 200 GM. MEAT; 80 GM. CRACKER MEAL; 30 GM. LARD; 20 GM. BONE ASH; 300 C.C. WATER.									
1910.	kilos.	gm.	c.c.	gm.	gm.	gm.	per cent.	gm.	gm.
May 23	10.2	8.50	205	7.83	137	50	64		
" 24	10.4	8.50	150	6.39	223	67	70		
" 25	10.4	8.50	175	7.78	140	59	58	6.93	66.96
" 26	10.2	8.50	190	7.37	166	60	64		
" 27	10.2	8.50	150	7.05	165	64	64		
PERIOD 2. — FOOD: 200 GM. MEAT; 120 GM. CRACKER MEAL; 50 GM. LARD; 30 GM. BONE ASH; 400 C.C. WATER.									
May 28	10.2	9.22	170	6.99	118	32	73		
" 29	10.2	9.22	175	6.96	315	106	65		
" 30	10.0	9.22	165	6.96	262	110	59	8.63	102.37
" 31	10.0	9.22	155	7.26	372	150	60		
June 1	10.0	9.22	185	7.56	63	25	60		

two periods of five days each were carried out upon diets practically identical with those of Experiment 1. The food fed in these two periods corresponded with that given in the first two periods of Experiment 1. Owing to slight differences of nitrogen content of the meat, the total nitrogen intake varied slightly from that in the previous experiment. See Tables IV, V, and VI.

Discussion of Results of Experiment II.

During the second period of observation (Table VI) Dog A furnished only positive nitrogen balances. This animal had 39 per cent of its small intestine short-circuited. Fat utilization may be fairly com-

TABLE VI.

Summary. — Experiment II.

Periods.	Nitrogen.					
	Food.	Excreta.			Balance.	
		Urine.	Fæces.	Total.	Per period.	Per day.
	Dog A, 39 per Cent					
	gm.	gm.	gm.	gm.	gm.	gm.
1	13.29	10.75	1.60	12.35	+0.94	+0.31
2	24.40	16.98	3.44	20.42	+3.98	+0.79
	Dog B, 66 per Cent					
1	42.5	36.42	6.93	43.35	−0.85	−0.17
2	46.1	35.75	8.63	44.38	+1.72	+0.34

pared with that of a normal dog on a mixed diet and was better than in Experiment I several months earlier. Increase of fat intake had little if any influence upon utilization of any of the foodstuffs. Carbohydrate utilization remained perfect. The body weight of Dog A was 7.8 kilos at the beginning of Experiment I, and at the end of Experiment 2 had increased to 10.5 kilos — a gain of 2.7 kilos.

It is at once apparent from an inspection of Table VI, Dog B, that at a period three months after functional resection of two thirds of the intestine fat utilization was much lower than shortly after the operation. Increasing fat intake within somewhat narrow limits did not markedly impair utilization of any of the foodstuffs. Carbohydrate utilization was still perfect. Nitrogen utilization, however, was somewhat lowered and appeared to undergo a still further slight diminution

by increase in fat intake. At this later period of observation the dog furnished a slight negative nitrogen balance for the first five days and a positive nitrogen balance for the second period. The body weight at the beginning of Experiment I was 13.3 kilos and at the end of Experiment II was 10.0 kilos — a loss of 3.3 kilos.

TABLE VI.

SUMMARY. — EXPERIMENT II.

	Fat (ether extract).			Carbohydrate.		
Utilization.	Food.	Fæces.	Utilization.	Food (calculated).	Fæces.	Utilization.
per cent.	gm.	gm.	per cent.	gm.	gm.	per cent.
INTESTINE RESECTED.						
87	75.48	5.88	92	109	0	100
86	206.20	13.22	94	273	0	100
INTESTINE RESECTED.						
83	201.28	66.96	66	292	0	100
81	301.90	102.37	66	437	0	100

DESCRIPTION OF EXPERIMENT III.

The food received by Dog No. 12 was the usual mixture of raw meat, cracker meal and lard which was fed several days previous to the actual period of observation and in sufficient quantities to maintain a normal dog in nitrogenous equilibrium. Water was given *ad libitum*. Preliminary trials demonstrated the separate collection of urine and fæces to be almost impracticable owing to the persistent diarrhœa. To overcome this obstacle the animal was fed small quantities of finely ground agar-agar with the food — a procedure which resulted in the passage of stools which while not formed were also not fluid. The number of defæcations was just as great as without

the agar, but the character of the stools was so entirely altered that contamination of the urine was prevented. In all probability the insoluble agar produced this change in the texture of the fæces by imbibing the water from the intestinal contents.

Utilization of nitrogen, fat, and carbohydrate. — In the first observation with this dog it was planned to bring about nitrogenous equi-

TABLE VII.

EXPERIMENT III, PERIOD 1. — DOG NO. 12, 73 PER CENT INTESTINE RESECTED.

Date. 1909.	Body weight.	Nitrogen in food.	Urine.				Fæces.				
			Volume.	Total nitrogen.	Ammonia nitrogen.	Indican fehlings sol. = 100.	Weight.		Water content.	Nitrogen.	Ether extract.
							Moist.	Air-dry.			
Jan.	kilos.	gm.	c.c.	gm.	gm.		gm.	gm.	per cent.	gm.	gm.
14	7.0	8.92	130	6.48	0.34	10	210	36	83		
15	7.0	8.24	125	6.00	0.55	10	232	37	84		
16	7.0	8.24	220	8.40	0.70	12	...	67	...	8.70	18.70
							79	14	82		
17	6.8	8.24	210	6.86	...	12	...	7	...		

librium as nearly as possible and then to determine the utilization of the different foodstuffs. To accomplish this purpose a few days previous to the real observation the dog received a diet consisting of 200 gm. meat, 120 gm. cracker meal and 10 gm. lard containing 8.92 gm. nitrogen and furnishing approximately 775 calories, or 1.27 gm. nitrogen and 110 calories per kilo body weight, amounts which would be far in excess of the requirements for a normal dog of this size. This diet was continued through the first day (January 14) of observation and was then reduced, since the animal appeared to have difficulty for the first time in devouring these large amounts of food. The new diet contained 200 gm. meat, 80 gm. cracker meal, 10 gm. lard, and 10 gm. agar. The nitrogen content amounted to 8.24 gm. This diet was eaten readily.

During the four days of observation, the results of which may be seen in Tables VII and IX, it is apparent that the dog was not in a condition of nitrogenous equilibrium in spite of the previous ingestion of large quantities of food, the nitrogenous balance for the four days

being minus 2.80 gm., or minus 0.7 gm. nitrogen per day. Turning to the utilization of nitrogen and fat, it may be observed that both were poor, nitrogen being utilized to the extent of only 74 per cent fat

TABLE VIII.

EXPERIMENT III, PERIODS 2 AND 3. — DOG NO. 12, 73 PER CENT INTESTINE RESECTED.

Date.	Urine.			Fæces.				
	Volume.	Total nitrogen.	Indican fehlings solution = 100.	Weight.		Water content.	Nitrogen.	Ether extract.
				Moist.	Air-dry.			
1909.	c.c.	gm.		gm.	gm.	per cent.	gm.	gm.
PERIOD 2. — FOOD PER DAY: 100 GM. MEAT; 25 GM. GELATIN; 80 GM. CRACKER MEAL; 10 GM. LARD; 10 GM. AGAR-AGAR. TOTAL NITROGEN = 8.24 GM.								
Jan. 22	120	6.38	7	112	20	82		
" 23	160	6.57	9	...	48	..		
" 24	175	7.49	8	201	51	80	7.19	15.80
" 25	250	10.10	8	86	18	80		
" 26	160	6.72	10	159	24	84		
PERIOD 3. — FOOD PER DAY: 50 GM. GELATIN; 80 GM. CRACKER MEAL; 10 GM. LARD; 10 GM. AGAR-AGAR. TOTAL NITROGEN = 8.96 GM.								
Jan. 27	450	8.88	11	146	26	82		
" 28	430	7.44	8	...	35	..		
" 29	170	7.58	8	161	29	82	8.91	24.90
" 30	160	7.51	9	...	32	..		

to the extent of 72 per cent. These figures agree well with those of Erlanger and Hewlett. In spite of the extremely foul odor of the fæces the quantity of indican eliminated through the urine was not excessive. The figures for ammonia nitrogen are high when compared to the output of the normal dog. Another point of interest was the rather large percentage of water contained in the fæces of this animal. Normally the water content of the air-dry fæces rarely

exceeds 75 per cent, whereas those passed by this animal had a water content of 80 to 85 per cent (see also Table VIII). It is not unlikely that the water content of the fæces may have been increased by the presence of agar, which would imbibe a certain quantity of water and prevent its absorption. It is hardly probable, however, that this is the sole explanation since the fluidity of the fæces passed when

TABLE IX.

SUMMARY. — EXPERIMENT III, DOG NO. 12, 73 PER CENT INTESTINE RESECTED.

Periods.	Nitrogen.							Fat (ether extract).		
	Food.	Excreta.			Balance.		Utilization.	Food.	Fæces.	Utilization.
		Urine.	Fæces.	Total.	Per period.	Per day.				
	gm.	gm.	gm.	gm.	gm.	gm.	per cent.	mg.	gm.	per cent.
1	33.64	27.74	8.70	36.44	−2.80	−0.70	74	67.60	18.70	72
2	41.20	37.26	7.19	44.45	−3.25	−0.64	82	96.50	15.80	84
3	35.84	31.41	8.91	40.32	−4.48	−1.12	75	59.20	24.90	58

no agar was given was such that a large water content was obvious. By varying the amount of carbohydrate even up to four times the requirement for a normal dog of the same weight, no change in the utilization of this foodstuff could be observed. It has been demonstrated repeatedly in this laboratory that a diet sufficient for a normal dog of this weight may consist of 200 gm. meat, 30 gm. cracker meal, and 25 gm. lard. Dog No. 12 received approximately this diet, then the cracker meal was increased to 80 gm. and the lard reduced to 10 gm. Finally, the cracker meal was still further increased to 120 gm. In all cases carbohydrate utilization was complete.[10] In these experiments designed to test carbohydrate utilization agar was not given with the food for obvious reasons.

THE INFLUENCE OF GELATIN FEEDING UPON THE ELIMINATION OF URINARY INDICAN.

In a previous communication[11] it was demonstrated that the replacement of meat by gelatin in a mixed diet results in a diminution

[10] *Cf.* FLINT: *Loc. cit.* [11] UNDERHILL: This journal, 1904–1905, xii, p. 176.

in the excretion of indican in the urine of the dog. This observation is in accord with the now well-established origin of indican. In Table VIII (Periods 2 and 3) are given the results of observations made with a view of determining whether a dog with a short-circuited intestine would behave in a manner similar to a normal animal when a portion or all the meat of the diet was replaced by gelatin. It is obvious from these figures that the substitution of gelatin for meat was without significant influence upon urinary indican elimination. With this animal only negative nitrogen balances were obtained. In the second gelatin period nitrogen utilization amounted to 75 per cent, which is comparable to the utilization obtaining when meat was fed (see Table IX, Period 1). The fat utilization in the first gelatin period (see Table IX, Period 2) when some meat was fed was much better than when the meat was entirely replaced by gelatin. In the first instance utilization amounted to 84 per cent; in the second gelatin period (see Table IX, Period 3) only 58 per cent of the fat was utilized. It would appear that meat in the diet of this animal had a tendency to aid fat utilization.

Summary.

From the foregoing observations it is apparent that as much as 39 per cent of the small intestine of a dog may be short-circuited without causing significant detrimental changes in the utilization of the various foodstuffs, and the animal may gain in weight. This statement is equally true when observations are made either at a period shortly after operation or at a period several months later.

When as much as 66 per cent of the small intestine has been functionally resected, the nutritive condition of the animal presents an entirely different aspect. Under these conditions fat utilization is particularly decreased and the dog displays a decided tendency to furnish negative nitrogen balances. A small though steady loss of weight is especially noticeable. Food utilization is in general apparently much better immediately after the operation than at a later period. In neither animal did a material increase in fat intake cause significant change in the utilization of this or other foodstuff.

When about three quarters of the small intestine of the dog has been short-circuited, food utilization for the most part is seriously

impaired, at least at a period several months after the operation. This is particularly true for fat utilization. Indican elimination through the urine is not materially altered under these conditions by replacement of meat in the diet with gelatin, an observation directly opposed to that obtained with the normal dog.

The animal with a short-circuited intestine displays a greater ability to utilize carbohydrate than does the normal dog. Even though the carbohydrate intake may be much, in one case several times, greater than the normal animal requires, carbohydrate utilization is complete whether the test is made shortly after the operation or months later. This observation may prove of practical importance in the dietary treatment of the human subject who has undergone extensive intestinal resection.

THE INFLUENCE OF THE PRECEDING DIET ON THE RESPIRATORY QUOTIENT AFTER ACTIVE DIGESTION HAS CEASED.

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IN laboratories where calorimetric measurements are impracticable much use has been made of a study of the respiratory exchange as a general index of the total metabolism and of the energy transformations in the body. Up to the present time practically all of the contributions to our knowledge of the respiratory exchange in experiments of short duration have been derived from experiments made with the Zuntz apparatus by Zuntz and his co-workers. Since there is a great difference of opinion as to the practicability of using a respiration apparatus which employs a special appliance for breathing, such as a mouthpiece, nosepiece, or mask, especially when the subjects are untrained, it is particularly fortunate that we have so large a number of observations made by the most skilled manipulators of the Zuntz apparatus, including Zuntz, Loewy, Mueller, Caspari, and particularly Durig and Magnus-Levy. By means of these observations our knowledge of the respiratory exchange with men at rest and under different conditions of muscular activity has been much amplified.

Using the numerous methods for determining carbon dioxide in air, experiments have been made by different investigators on the carbon-dioxide excretion of man, since the measurement of this factor presents no serious difficulty. As has been frequently pointed out, however, the carbon-dioxide excretion in a given time cannot be considered an accurate index of the katabolism, since the amount of energy produced in the body for every gram of carbon dioxide exhaled varies widely, depending upon whether the carbon dioxide is derived from the carbon of protein, fat, or carbohydrate. For every gram of carbon dioxide resulting from the oxidation of protein in the body

there are liberated 2.9 calories; for every gram of carbon dioxide resulting from the oxidation of fat, 3.4 calories; and for every gram of carbon dioxide resulting from the oxidation of carbohydrate, 2.57 calories.

The ordinary diet of mankind contains varying amounts and varying proportions of fat and carbohydrate, and it has been customary to assume that under ordinary conditions the carbohydrate is for the most part burned first. For periods following inanition this has been completely disproved.[1] During periods when no food is given it is clear that the combustion must of necessity be largely of body fat, although here again the carbohydrate material in the body stored in the form of glycogen may be such as to furnish by its oxidation a considerable proportion of the total carbon-dioxide excretion in the course of a day. Thus it was found in fasting man that as much as 181 gm. of glycogen may be burned in the body in the first day of complete inanition.[2]

Certain methods of studying the respiratory exchange take into consideration the important factor of oxygen consumption, this being particularly true of the Zuntz apparatus. When the energy per gram of oxygen absorbed is considered, it is found that the differences appearing in the carbon dioxide, and depending upon the character of the material katabolized, do not exist in the case of oxygen. With oxygen the calorific value of 1 gm. remains nearly the same irrespective of whether the oxygen is used to burn protein, fat, or carbohydrate. Consequently, in lieu of calorimetric experiments, an accurate measurement of the oxygen intake is taken as a reasonably exact index of the total energy transformations during the time of an experiment.

This index of the total katabolism is, however, by no means so satisfactory as an apportionment of the metabolism between the protein, fat, and carbohydrate, — an apportionment that may be made if one knows the nitrogen excretion during the period of experimenting, the carbon-dioxide exhalation, and the oxygen consumption. Given these three factors, it is possible, by means of the method devised by Zuntz, to compute the energy transformations by so-called "indirect calorimetry." It is not possible here to enter into a discussion of the

[1] BENEDICT: Carnegie Institution of Washington, Publication No. 77, 1907, p. 532.

[2] BENEDICT: *Loc. cit.*, p. 464.

comparison between direct and indirect calorimetry in short experiments, as an extended series of experiments on this point is now in progress in this laboratory.

Entirely aside from the energy transformations, however, one of the chief advantages of obtaining the respiratory exchange by a method which permits an estimation of the oxygen consumption in addition to the carbon-dioxide production is that it enables the apportionment of the katabolism between carbohydrate and fat. In studying questions regarding the relative efficiency of fat and carbohydrate as a source of muscular work, such an apportionment is of prime importance, as has already been demonstrated in discussing the transformations of material during diseases resulting from disturbances of metabolism, such as diabetes.[3]

The calculation of the total katabolism expressed in terms of protein, fat, and carbohydrate may be carried out with reasonable accuracy when the output of nitrogen and carbon dioxide and intake of oxygen are known. A general picture of the character of the metabolism, showing the nature of the material burned inside the body without a quantitative estimation of the exact amounts burned, may be obtained by a simple inspection of the ratio of carbon dioxide to oxygen, namely, the respiratory quotient. When carbon in carbohydrate is burned, the volume of carbon dioxide resulting from the combustion is exactly equal in volume to the oxygen absorbed, and hence the ratio of carbon dioxide to oxygen is 1. When fat is burned, a very much larger volume of oxygen is consumed than of carbon dioxide eliminated, since a portion of the oxygen is used to oxidize the hydrogen of the fat molecule and form water. With a diet rich in fat, therefore, the ratio is usually found to be not far from 0.71. These calculations may be approximately verified by feeding animals pure fat and pure carbohydrates. It is a little more difficult to feed pure protein, and the calculation of the theoretical respiratory quotient for protein varies somewhat with different writers. It is usually accepted, however, as being not far from 0.81.[4] Consequently, if the respiratory quotient is in the neighborhood of 1, it is obvious that the katabolism must be largely of a carbohydrate nature. If it is in the neigh-

[3] Benedict and Joslin: Carnegie Institution of Washington, Publication No. 136, 1910, p. 203.

[4] Benedict: This journal, 1909, xxiv, p. 350.

borhood of 0.7, it is likewise clear that it must be largely of fat. In practically all of the diets which man is accustomed to use, protein rarely forms more than 15 per cent of the total katabolism; hence, for many comparisons, the protein katabolism can be neglected, and the general conclusions drawn that respiratory quotients in the neighborhood of 0.7 to 0.75 indicate a predominantly fat katabolism, and respiratory quotients between 0.9 and 1 indicate a predominantly carbohydrate katabolism.

With these marked variations in the respiratory quotient, depending upon the nature of the material katabolized, it was soon seen that, to obtain results of any significance in a study of the respiratory exchange, the experiments should be made a sufficient length of time after the last meal to eliminate the question of the increased katabolism invariably following the ingestion of food. This increase in the katabolism is most marked after protein is eaten, but a positive increase is also observed when carbohydrate and fat are ingested. As a result of many experiments made by Magnus-Levy and others, it has been the consensus of opinion that twelve hours after the last food is taken the active work of digestion has ceased, and the katabolism is therefore assumed to be constant for each individual under like conditions of body activity. A distinction has been made between the value obtained twelve hours after the last meal and the value obtained after a fast of twenty-four or more hours, the first value being the so-called "Nüchternwert" of the Germans. As a matter of fact, however, a large number of experiments on fasting men [5] have shown that the katabolism is far from constant on the first two days of complete inanition, and that only on the third day is constancy approximated. Hence there must be a regular transition from the time the last food was taken until complete inanition takes place, the first step being the active process of digestion, and the second when body material (fat, and particularly glycogen) is drawn upon. After the glycogen has in large part been reduced there is, then, a period of relative constancy in the katabolism, for it is chiefly of fat, with a small draft upon the remaining store of body glycogen and a relatively constant protein katabolism throughout the whole period.

An examination of the literature of experiments made twelve hours

[5] Benedict: Carnegie Institution of Washington, Publication No. 77, 1907, pp. 456 *et seq.*

after the preceding meal shows that the respiratory quotient may be very considerably higher on one day than on another. Again we find series of experiments in which the respiratory quotient was remarkably constant, not only from day to day, but also from season to season. Inasmuch as in the majority of cases the respiratory quotient is relatively constant from day to day and from month to month with the same individuals, it has been the custom of many writers to determine this value once for all for a base line and consider it as an average value in subsequent experiments. For example, when studying the influence of the ingestion of food or muscular work upon katabolism, the values found twelve hours after the last meal are deducted from the values found under the conditions of the experiment, and the difference ascribed to the effect of the ingestion of food or to the effect of work as the case may be. Since these average values play an important rôle in many subsequent calculations, it can be seen that it is of the utmost importance to know to what extent they represent the true average, what variations may commonly be expected, and, if possible, to what the variations are due. A study of these average values has accordingly been made in this laboratory, and the results are here presented and discussed.

General Plan of the Experiments.

The primary object of this study was to note the effect of the preceding diet upon the respiratory exchange twelve hours after the last meal was taken. Consequently, the earlier experiments were so designed as to have the study of the respiratory exchange made approximately twelve hours after a meal [6] which had consisted either in large part of carbohydrates or, on the other hand, of relatively small amounts of carbohydrate. The diets, then, were distinctly carbohydrate-rich at one time and carbohydrate-poor at the other. In order to eliminate the possible influence of an excessive ingestion of protein, the protein in the diet of the preceding meal was in no instance materially increased, although care was taken to have the carbohydrate-poor diet contain enough fat to keep up the energy requirement for the day. As a rule, the meal on the evening preceding the experiment was usu-

[6] In no case was the experiment made less than twelve hours after the last meal.

ally eaten about six o'clock. The subject then came to the laboratory the next morning without eating, and immediately lay down upon the couch, preparatory to a series of experiments.

This method of studying the influence of the preceding diet has a number of disadvantages. In the earlier experiments the subject was simply instructed to make the last meal of the day before either carbohydrate-rich or carbohydrate-poor. No attempt was made to control the diet exactly and, indeed, subsequent calculation based upon the reported amounts eaten shows that there were enormous variations in the amount of carbohydrate ingested, — variations that were not suspected by the subject. Experiments subsequent to October 1, 1910, were made on a somewhat different plan. In most of these the diet on the evening before was controlled with reasonable rigidity. In a number of cases actual weighings of all food materials were made, and the diet so apportioned as to secure approximately exact amounts of carbohydrate, large or small.

It is obvious that the difficulty was to be found more particularly in the meals in which low carbohydrates were desired. Usually it could be assumed with every guarantee of certainty that the subjects would eat large amounts of carbohydrate if so instructed, but the proper selection of a carbohydrate-low diet was not easy for inexperienced individuals. The admirable plan instituted and followed by Atwater in his researches at Wesleyan University in Middletown, Conn., of having all experiments preceded by a three-day period in which the same diet was ingested as on the experimental day, would have been followed here had the time and the expense allowed it. Under these conditions and these only can we be certain of the condition of the body and the plane of nutrition prior to the experiment. This is particularly the case if it happened, as it is more than likely that it did happen, that the subjects had been on certain days engaged in active muscular work and perhaps had eaten an insufficient lunch at noon owing to pressure of other work, thus necessitating a heavy draft upon body glycogen. Furthermore, although there might have been a large amount of carbohydrate ingested in the evening meal, it is not at all improbable that a considerable proportion of this may have been stored as glycogen, as has been suggested by Johansson.[7]

[7] Johansson and Hellgren: Festschrift für Olof Hammarsten, Upsala, 1906, vii, p. 8.

On the whole, however, these experiments were designed to be so extensive and to include so many individuals as to eliminate these accidental possibilities as far as possible. The subjects for the most part were living upon diets that were reasonably uniform and regular.

Methods.

The respiration apparatus used in this investigation was devised by one of us [8] and had been fully tested prior to these experiments. With this apparatus the subject, lying upon a comfortable couch, breathes through two nosepieces of special design into a closed air circuit. By means of a small rotary blower the current of air in the ventilating pipe is kept moving at the rate of 35 litres a minute. After the expired air enters the air current, it is passed through a set of absorbers, the first containing sulphuric acid, which removes the water vapor given off from the lungs; the second containing soda lime, which absorbs the carbon dioxide in the exhaled air; and the last, sulphuric-acid, which removes the water absorbed by the air current as it passes through the moist soda lime. Since this dry air would be irritating to the mucous membrane in the nose and throat, moisture is added by passing it through an air-moistening device. The deficiency in oxygen is also made up by adding oxygen from a cylinder of the gas. The air is then in approximately normal condition, though free from carbon dioxide, and is returned to the subject for breathing.

The air in the ventilating current is kept at atmospheric pressure by means of a tension equalizer, in which a rubber diaphragm falls or rises as air is withdrawn or added to the system. Any loss in volume due to the absorption of oxygen by the subject is made up by the oxygen admitted from the cylinder, so that when the experiment is concluded, the volume of air in the system is the same as the initial volume. The amount of carbon dioxide given off by a subject may be determined by noting the difference in weight of the carbon-dioxide absorber before and after an experiment, also of the sulphuric-acid container in which the water from the soda lime is absorbed, and finding the algebraic sum of the two. The loss in weight of the oxygen cylinder, corrected for the small amount of nitrogen in the oxygen,

[8] Benedict: This journal, 1909, xxiv, p. 345.

gives the weight of the oxygen consumed by the subject. By this means the carbon-dioxide excretion and oxygen absorption are rapidly and accurately determined without the necessity of using a complex gas analysis apparatus.

A sample of the form used for recording the data of an experiment is reproduced here to give an idea of the simplicity of the calculations required to obtain the results. As will be seen, at the head of the form are recorded the number of the experiment, the date, name, or initials of the subject, and time of beginning and ending of the experiment, as well as its duration. In the left-hand division the records are made of the weights of the carbon-dioxide absorber, the water absorber, and the oxygen cylinder, together with the simple calculations for obtaining the weights of the carbon dioxide excreted and oxygen consumed. The calculations necessary to reduce these weights to volumes by means of the factors 0.5091 and 0.6998 are recorded at the right below the respiration record, also the calculation of the respiratory quotient. In the two divisions at the bottom the calculations for obtaining the amounts of carbon dioxide excreted and oxygen consumed in cubic centimetres per minute are given, while space is reserved below for any miscellaneous observations which may be worthy of record. The records of the pulse and respiration rates are given in the upper divisions. The time required for ten respirations is recorded as well as the rate of respiration per minute, and the average respiration and pulse rates.

Statistics of Experiments.

Although the statistics with regard to the body weight, age, etc., have no particular value in a study of the influence of the preceding diet on the respiratory quotient or on the character of the katabolism, they are included in Table I, as the experiments may be of interest in other connections.

Experiments with seven subjects are included in this report. With six of these, the experiments were made with the respiration apparatus; four of these six subjects were also subjects of respiration calorimeter experiments which are included in this report, together with respiration calorimeter experiments made with one other subject.

RESPIRATION EXPERIMENT NO. 1.

May 6, 1909.

Subject, J. R. . . { Start . . . 8.40.00 A. M.
End . . . 8.55.02 A. M.
Duration . 15 min. 2 sec.

Pulse, per min.	65		Resp. 10, 51 sec. = ...	12	
Pulse, per min.	64		Resp. 10, 50 sec. = ...	12	
Pulse, per min.	60		Resp. 10, 46 sec. = ...	13	
Average	63		Average	12	
H_2SO_4 No. 19 End	2153.70		Log. .5091 = ...	70680	
H_2SO_4 No. 19 Start	2151.89		Log. total CO_2 = ...	74974	
	1.81				
S. L. G End	5250.76		Log. vol. CO_2 = ...	45654	
S. L. G Start	5246.95		Log. vol. O_2 = ...	52972	
	3.81				
	1.81				
Total CO_2 .	5.62		Log. R. Q. = ...	92682	
O_2cyl. No. 27427 Start	7345.22				
O_2cyl. No. 27427 End	7340.40				
	4.82		Log. .6998 = ...	84496	
N. corr. . . .	.019		Log. total O_2 = ...	68476	
Total O_2 . .	4.839				
Respiratory Quotient: 84.			Log. vol. O_2 = ...	52972	
Log. vol. CO_2 = ...	45654		Log. vol. O_2 = ...	52972	
Log. time in min. = . . .	17705		Log. time in min. = . . .	17705	
Log. c.c. CO_2 per min. = .	27949		Log. c.c. O_2 per min. = .	35267	
c.c. CO_2 per min. = ...	190		c.c. O_2 per min. = ...	225	

Remarks: .....

All of the subjects were engaged in scientific work in the laboratory, and were thoroughly familiar with the methods of experimenting and the object of the research. Their intelligent co-operation was thus assured.

The experiments reported in this article are of two kinds. As previously stated, most of them were made with a respiration apparatus and only the carbon-dioxide output and oxygen intake were determined. To supplement these experimental researches with the respiration apparatus, a number of experiments with a respiration

TABLE I.

STATISTICS OF AGE, HEIGHT, AND AVERAGE WEIGHT OF SUBJECTS.

Subject.	Age.	Height.	Weight without clothing.
	years	cms.	kilos
F. G. B.	40	183	83
J. R.	27	182	66
Miss S.	27	172	66
J. J. C.	27	175	65
L. E. E.	30	179	60
V. G.	17	162	53
T. M. C.	31	166	49

calorimeter, which were made for another purpose, have been here included. In certain calorimeter experiments it is perfectly feasible to combine a test on the influence of the preceding diet on the respiratory exchange with some other special subject for investigation. Accordingly, in a number of such experiments, the subjects were provided with carefully controlled diets containing either large or small amounts of carbohydrate. These respiration calorimeter experiments, however, are presented only in abstract, as the values for the respiratory quotient alone are of significance in this connection.

The details of the respiration experiments with the different individuals are given in Table II, and those with the respiration calorimeter in Table III. The respiration experiments were made primarily for this study, and each daily series represents comparable experiments under like conditions of muscular activity and preceding diet. The experiments were usually of fifteen minutes' duration and followed each other at intervals of approximately thirty to forty-five minutes. While with certain of the subjects the uniformity in the

oxygen consumption from experiment to experiment was not so constant as could be desired, thereby indicating slight differences in muscular activity, it was believed that the large number of experiments here made, many of them on the same day, would more than offset the differences in muscular activity, and the average value for the whole day must represent very nearly the actual conditions of katabolism existing at the time the experiment was made. This is particularly the case in this discussion, as the respiratory quotient is the only factor of significance, and it has been maintained by Zuntz and his associates[9] that even moderately severe muscular work does not alter the respiratory quotient.

During the experiments the subjects were lying quietly upon a couch, and the greatest muscular relaxation was insisted upon. A close examination of the figures in Table II shows, however, that frequently the first experiment of a series gave results distinctly abnormal as compared with the subsequent experiments. This is explained by the fact that there may have been a somewhat larger carbon-dioxide exhalation due to irregular respiration, and that in subsequent experiments these irregularities disappeared.

Nitrogen Excretion.

In experiments in which the diet is undergoing marked alterations it is necessary to demonstrate rather than to assume that the protein intake on the day before is not abnormally increased or decreased, as such change would influence the respiratory exchange and mask any effect of a variation in the amount of carbohydrate in the preceding diet. Accordingly a study was made of the urine passed at the time of the experiments, that is, for the period representing the twelfth to the eighteenth hours after the last meal. The nitrogen excretion per hour in these experiments has been calculated from the results of the Kjeldahl determinations and presented in Table IV. It is here seen that, considering the subjects were for the most part subsisting on uncontrolled diets, there is a remarkable uniformity in the nitrogen excretion per hour in practically all of the experiments with the same individual, thus showing conclusively that there could not have been any marked alteration in the protein proportion of the diet the day

[9] Heinemann: Archiv für die gesammte Physiologie, 1901, lxxxiii, p. 441.

TABLE II.

RESPIRATORY EXCHANGE IN EXPERIMENTS WITHOUT FOOD FOLLOWING DIETS HIGH OR LOW IN CARBOHYDRATES.
(RESPIRATION APPARATUS.)

Subject and experiment number.	Date, and character of preceding diet.	Carbon-dioxide excretion per minute.	Oxygen consumption per minute.	Respiratory quotient.	Subject and experiment number.	Date, and character of preceding diet.	Carbon-dioxide excretion per minute.	Oxygen consumption per minute.	Respiratory quotient.	Subject and experiment number.	Date, and character of preceding diet.	Carbon-dioxide excretion per minute.	Oxygen consumption per minute.	Respiratory quotient.
		c.c.	c.c.				c.c.	c.c.				c.c.	c.c.	
F. G. B.	1910	218	259	0.84	L.E.E.	May 10	191	235	0.81	V.G.	Nov. 18	191	245	0.78
1	Nov. 11	225	258	0.87	6	(High)	191	231	0.83	1	(Low)	199	250	0.79
	(High)	225	253	0.89			204	243	0.84	2	Nov. 30	194	238	0.81
		229	253	0.91			214	257	0.83		(High)	190	226	0.84
		234	240	0.97	7	May 13	200	238	0.84			186	219	0.85
		226	263	0.86		(Low)	207	246	0.84			188	229	0.82
		231	254	0.91			209	239	0.88	J. R.	1909			
		229	256	0.89			206	252	0.82	1	Apr. 27	207	234	0.88
2	Nov. 15	187	247	0.76			209	235	0.89		(High)	185	226	0.82
	(Low)	192	247	0.78			210	232	0.91			210	239	0.88
		188	252	0.74	8	May 20	234	241	0.97	2	Apr. 29	217	246	0.88
T. M. C.						(High)	228	257	0.89		(High)	207	255	0.81
1	Nov. 14	176	209	0.84			224	256	0.88			187	238	0.79
	(High)	173	193	0.90			216	249	0.87	3	May 1	220	234	0.94
		165	182	0.91			233	252	0.93		(High)	219	230	0.95
		165	182	0.91			233	244	0.95			218	243	0.90
		166	187	0.89	9	May 22	230	240	0.96	4	May 6	190	225	0.84
		170	185	0.92		(High)	221	252	0.88		(High)	177	226	0.78
		179	187	0.96			228	249	0.92			201	227	0.89
		...	187	...			238	253	0.94	5	May 12	...	247	...
		174	185	0.94			249	263	0.95		(Low)	183	...	...
		171	183	0.93			248	253	0.98			182	243	0.75

2	Nov. 16	168	191	0.88	10	June 1	215	268	0.80			192	237	0.81
	(Low)	150	177	0.85		(High)	208	248	0.84			190	244	0.78
		148	183	0.81			218	277	0.79			198	245	0.81
		152	190	0.80			204	252	0.81	6	May 15	215	256	0.84
		156	192	0.81			212	273	0.78		(High)	198	252	0.79
J. J. C.							230	271	0.85			211	256	0.82
1	Nov. 22	204	228	0.89	11	June 9	219	248	0.88			214	245	0.87
	(High)	203	223	0.91		(High)	202	242	0.84			197	237	0.83
		200	231	0.87			200	252	0.80			204	234	0.87
		197	225	0.88			210	258	0.81	7	May 18	202	234	0.86
		208	229	0.89			228	260	0.88		(High)	190	235	0.81
		208	245	0.85			219	274	0.80			202	243	0.83
		198	225	0.88	12	June 16	204	263	0.77			192	241	0.80
		197	231	0.85		(High)	198	280	0.71			196	225	0.87
2	Dec. 6	189	239	0.79			211	264	0.80			222	240	0.93
	(Low)	190	237	0.80			218	257	0.85	8	May 21	219	244	0.90
		192	143	0.79			210	254	0.83		(High)	197	245	0.81
L. E. E.	1909					1910	223	273	0.82			219	238	0.92
1	Apr. 26	225	242	0.93	13	Nov. 26	220	259	0.85			199	244	0.82
	(High)	200	243	0.82		(High)	220	250	0.88			208	241	0.86
		212	244	0.87			222	258	0.86			201	233	0.86
		210	239	0.88			225	265	0.85	9	May 29	198	266	0.75
2	Apr. 28	222	236	0.94							(Low)	195	277	0.71
	(High)	203	233	0.87	14	Nov. 29	200	273	0.73			193	254	0.76
		211	236	0.89		(Low)	207	263	0.79			201	253	0.79
3	Apr. 30	211	234	0.90			211	274	0.77			205	259	0.79
	(High)	193	239	0.81			205	276	0.74			202	256	0.79
		203	248	0.82	15	Dec. 3	196	239	0.82	10	June 2	212	242	0.87
4	May 3	252	229	1.10		(Low)	194	235	0.83		(Low)	216	255	0.85
	(High)	217	236	0.92			201	239	0.84			203	247	0.82
		231	232	0.99	V. G.							211	258	0.82
5	May 7	246	243	1.01	1	Nov. 18	207	251	0.83			206	252	0.82
	(High)	218	232	0.94		(Low)	203	243	0.84			199	278	0.72
		217	232	0.94			196	239	0.82	..	....	..	..	..
6	May 10	181	236	0.77			215	245	0.88		....	..	..	..
	(High)	196	242	0.81			209	249	0.84		....	..	..	..

before. Hence the effect of the protein ingested in the last meal can be considered as negligible in this discussion. An examination of the figures shows that while there may be a general tendency for the

TABLE III.

RESPIRATORY QUOTIENT IN EXPERIMENTS WITHOUT FOOD FOLLOWING DIETS HIGH OR LOW IN CARBOHYDRATES. (RESPIRATION CALORIMETER.)

Subject.	Date.	Carbohydrates in last meal.	Respiratory quotient.	Subject.	Date.	Carbohydrates in last meal.	Respiratory quotient.
T. M. C.	1909 Feb. 4	High	.89	V. G.	1910 Oct. 26	High	.90
	1910 July 12	High	.88		Nov. 7	High	.94
	1909 Mar. 23	Low	.80		Oct. 24	Low	.82
J. J. C.	1910 Oct. 31	High	.92		Nov. 4	Low	.85
	Nov. 8	High	.89	Miss S.	Apr. 28	High	.85
	Oct. 27	Low	.85		June 21	High	.91
	Nov. 3	Low	.85		Apr. 4	Low	.84
L. E. E.	Dec. 9	Low	.81		Apr. 11	Low	.79

nitrogen excretion to be somewhat lower following a high carbohydrate diet, nevertheless there are so many irregularities that no general deduction on this point can be drawn.

DISCUSSION OF RESULTS.

The most important comparison to be made in connection with these experiments is the relationship between the amount of carbohydrate in the last meal of the preceding day and the respiratory quotient on the morning of the experiment. To make this comparison all the more clear, the results of all the experiments are brought together in Table V in such manner as to show which days were preceded by a high carbohydrate diet and which by a low carbohydrate diet. In this table the average respiratory quotient for each experimental day, both with the respiration apparatus and with the respiration calorimeter, is given. It is clear that the respiratory quotient

TABLE IV.

NITROGEN EXCRETION PER HOUR IN EXPERIMENTS WITHOUT FOOD FOLLOWING DIETS HIGH OR LOW IN CARBOHYDRATES.

Subject.	Date.	Carbohydrates in last meal.	Nitrogen excretion per hour.	Subject.	Date.	Carbohydrates in last meal.	Nitrogen excretion per hour.
	1910		gm.		1910		gm.
F. G. B.	Nov. 11	High	.400	L. E. E.	Nov. 26	High	.441
	" 15	Low	.491		" 29	Low	.567
J. J. C.	Oct. 27	Low	.450		Dec. 3	Low	.450
	" 31	High	.472		" 9	Low	.456
	Nov. 3	Low	.371	V. G.	Oct. 24	Low	.462
	" 8	High	.318		" 26	High	.316
	" 22	High	.383		Nov. 4	Low	.324
	Dec. 6	Low	.481		" 7	High	.274
	1909						
T. M. C.	Feb. 4	High	.531		" 18	Low	.487
	Mar. 23	Low	.494		" 30	High	.306
	July 12	High	.387		1909		
	1910			J. R.	Apr. 27	High	.458
	Nov. 14	High	.358		" 29	High	.464
	" 16	Low	.454		May 1	High	.488
	1909						
L. E. E.	Apr. 28	High	.625		" 6	High	.383
	" 30	High	.462		" 12	Low	.554
	May 3	High	.448		" 15	High	.473
	" 7	High	.471		" 18	High	.494
	" 10	High	.480		" 21	High	.449
	" 13	Low	.387		" 29	Low	.550
	" 20	High	.515		1910		
	" 22	High	.538	Miss S.	Apr. 4	Low	.257
	June 1	High	.531		" 11	Low	.431
	" 9	High	.658		" 28	High	.347
	" 16	High	.478		June 21	High	.300
				....	....	....	. ..

which was determined twelve hours after a meal consisting of a diet rich in carbohydrates was in general higher than when the last meal contained but a small amount of carbohydrates.

TABLE V.

AVERAGE RESPIRATORY QUOTIENTS IN EXPERIMENTS WITHOUT FOOD FOLLOWING DIETS HIGH OR LOW IN CARBOHYDRATES.

Subject.	High carbohydrate.		Low carbohydrate.		Subject.	High carbohydrate.		Low carbohydrate.	
	Date.	R. Q.	Date.	R. Q.		Date.	R. Q.	Date.	R. Q.
F. G. B.	1910 Nov. 11	0.89	1910 Nov. 15	.76	L. E. E.	1909 June 9	0.84	1909	..
J. J. C.	Oct. 31	0.92	Oct. 27	.85		" 16	0.80	....	..
	Nov. 8	0.89	Nov. 3	.85		1910 Nov. 26	0.86	1910	..
	Nov. 22	0.88	Dec. 6	.79	V. G.	Oct. 26	0.90	Oct. 24	.82
T. M. C.	1909 Feb. 4	0.89	1909 Mar. 23	.80		Nov. 7	0.94	Nov. 4	.85
	1910 July 12	0.88	1910 Nov. 16	.83		Nov. 30	0.83	Nov. 18	.83
	Nov. 14	0.91	....	..	J. R.	1909 Apr. 27	0.86	1909 May 12	.79
L. E. E.	1909 Apr. 26	0.88	1909 May 13	.86		" 29	0.83	" 29	.77
	" 28	0.90				May 1	0.93	June 2	.82
	" 30	0.84	1910 Nov. 29	.76		May 6	0.84	....	..
	May 3	1.00	Dec. 3	.83		" 15	0.84	....	..
	" 7	0.96	" 9	.81		" 18	0.85	....	..
	" 10	0.82	....	..		" 21	0.86	....	..
	" 20	0.92	....	..	Miss S.	1910 Apr. 28	0.85	1910 Apr. 4	.84
	" 22	0.94	....	..		June 21	0.91	" 11	.79
	June 1	0.81	....	..		....	..	....	..

A general average for each subject is given in Table VI. In this table the method of averaging may be open to criticism, as with two of the subjects, L. E. E. and J. R., an unequal number of experiments was used in obtaining the average values, the experiments following a high carbohydrate diet exceeding those which followed a diet low in

carbohydrates. With the other subjects approximately equal numbers of experiments were made on each nutritive plane, and the general averages of the table are therefore probably not far from representing the actual conditions at the time of the experiment.

In making experiments with so complicated an organism as the human body it is extremely difficult to foresee the exact conditions

TABLE VI.

AVERAGE RESPIRATORY QUOTIENTS WITH DIFFERENT INDIVIDUALS IN EXPERIMENTS WITHOUT FOOD FOLLOWING DIETS HIGH OR LOW IN CARBOHYDRATES.

Subject.	Respiratory quotient.		Subject.	Respiratory quotient.	
	High carbohydrate.	Low carbohydrate.		High carbohydrate.	Low carbohydrate.
F. G. B. . .	.89	.76	V. G. . . .	.89	.83
J. J. C. . .	.90	.83	J. R. . . .	.86	.79
T. M. C. . .	.89	.82	Miss S. . .	.88	.82
L. E. E. . .	.88	.82	..	..	..

for experiments in all instances and thus arrange ideal preliminary periods and preliminary body conditions. In all probability a serious error was made in these experiments in not insisting upon a longer preliminary plane of nutrition. We have here, therefore, only the general impressions derived from a large number of experiments, and can explain only inadequately the several anomalous experiments which stand out so prominently in the series.

The amounts of carbohydrate ingested with the last meal on the days with a rich-carbohydrate diet were in many instances very large, occasionally over 300 gm. Since, however, the subjects were accustomed to eat the hearty meal of the day at night, this is not an excessive amount of carbohydrate, and not more than might have been taken at many meals, since 300 gm. corresponds to about 1200 calories, or from one third to one half of the daily requirement. In certain instances the amounts of carbohydrate were from 400 to 500 gm. It is possible, therefore, that under these conditions we may have again to deal with a delayed carbohydrate absorption out of the intestinal tract, and it may be that the active digestion had not ceased, although

these experiments were for the most part made not twelve, but fourteen hours after the last meal.

The possibility of the high respiratory quotient after a high carbohydrate diet being due to the delayed absorption and combustion of carbohydrates in the alimentary tract, is somewhat difficult of proof. In fact, the evidence is rather against this theory, since in certain experiments, namely, with T. M. C., where a high carbohydrate diet was given, the high respiratory quotient continued throughout rather a lengthy series of experiments which lasted a good many hours. There was apparently no tendency for the respiratory quotient to fall rapidly, as would be expected at the termination of carbohydrate digestion in the alimentary tract. These experiments point out, as do almost no others, the necessity for long experiments, preceded by a plane of nutrition carefully studied beforehand.

Such evidence as has been accumulated in previous experiments in which carbohydrates have been ingested shows that the respiratory quotient is rarely above the initial value ten hours after the ingestion of relatively large amounts of carbohydrate, thus indicating a rapid absorption and digestion. Indeed, Magnus-Levy [10] states his inability to increase the respiratory quotient in a man that had been given a very rich carbohydrate diet (rice, etc.) for two days previous to the experiment. In the light of the experiments here presented Magnus-Levy's statement is very difficult to explain. It seems hardly probable that by reason of preliminary drafts upon body glycogen the store of glycogen in his subject was so lowered that two days of rich carbohydrate feeding could not replenish it.

Anomalous experiments of this kind occasionally appear, and it seems difficult to predict exactly what the respiratory quotient will be after a given diet with carbohydrate-rich and carbohydrate-poor food. Our experiments show that, in general, when a carbohydrate-rich diet is given, there is an increase in the respiratory quotient, although certain experiments seem to show no effect whatever. In certain of these experiments, however, it has been shown, after a careful calculation, that the diets on the evening before were not so rich in carbohydrate as the subjects thought. Thus, on one occasion when the subject, V. G., was given a so-called carbohydrate-rich diet, it

[10] Magnus-Levy: Archiv für die gesammte Physiologie, 1894, lv, p. 25.

was found that the total amount of carbohydrates ingested on the evening before was but 200 gm. Under these conditions the respiratory quotient did not vary materially from that in experiments with a carbohydrate ingestion of but 50 to 60 gm.

Obviously the previous body condition plays a very important rôle. The extent to which the body storage of glycogen has been drawn upon, the muscular activity of the day previous to the experiment, possibly the temperature of the surrounding air, the general diet of the individual for several days before, in fact, anything which contributes to a disturbance of the storage of glycogen in the body, may alter the influence of the ingestion of a carbohydrate-rich meal. If the glycogen storage in the body is at a low point, the ingestion of a carbohydrate-rich meal does not result in an increased respiratory quotient in accordance with the amount ingested, as a not inconsiderable proportion of the carbohydrate may be stored immediately as glycogen. Until this glycogen storage has been replenished the combustion of carbohydrate in the food may be delayed. On the other hand, with individuals subsisting without food and remaining quiet in a respiration chamber, the store of glycogen may last for some time. From these data we may infer, then, that muscular activity may play an important rôle in affecting the storage of glycogen.

Post-digestive Katabolism.

These experiments on the effect of the preceding diet on the respiratory exchange suggest many ideas with regard to a study of the character of the katabolism after active digestion has ceased. Recognizing the influence of the ingestion of food upon katabolism, it is of great importance and value to know what is the character of the katabolism after this digestive activity has ceased. Assuming that the carbohydrate, fat, and protein have been converted into their soluble forms and withdrawn from the alimentary tract, and active peristalsis, secretion, absorption, etc., have ceased, what is the character of the katabolism and how can it change? The katabolism under these conditions is obviously made up of the disintegration of the three main compounds of the body, — the protein, the fat, and the carbohydrate. As has been shown previously in the discussion and in the table giving the nitrogen excretion, the protein katabolism

remains essentially constant throughout all experiments. This is assuming, obviously, that the protein katabolism follows closely the curve of nitrogen excretion in the urine, an assumption that is commonly made if not absolutely correct. It is, then, clear that the changes must take place in the relative proportions of the fat and carbohydrate oxidized. Twelve hours after a rich meal of either fat or carbohydrate, the body contains a relatively large amount of fat which has not undergone proportionately any great change. Even with no food the amount of fat drawn upon during one day or in twelve hours would not materially deplete the storage. On the other hand, while the storage of carbohydrate is quantitatively very much less than fat, it is subject to fluctuations in its content which may amount to an enormous percentage of the original storage. It has been assumed that the amount of carbohydrates in the body of an average man is, roughly, not far from 400 gm. This is probably a low rather than a high estimate. If, as has been demonstrated, in twenty-four hours without food 180 gm. of carbohydrate can be drawn upon, it will be seen that nearly 50 per cent of this storage may disappear after twenty-four hours of complete inanition. It is obvious, therefore, that in a study of the character of the katabolism after active digestion has ceased, we must pay particular attention to the drafts upon body glycogen. Inasmuch as these drafts affect the total storage by a very large proportion, it is also easily seen that a determination of the amount of these drafts may be of great significance, and that the respiratory quotient as an index of the character of the katabolism may throw much light upon the storage of carbohydrates or glycogen existing in the body.

Any alterations in the relative proportion of carbohydrate and fat burned are due almost exclusively to the fluctuating amount of carbohydrate storage rather than of fat storage. The fat storage is very large and is sufficient to supply the body with energy for complete maintenance for many days. This being the case, then, we may again examine the transitional period from the end of active digestion until complete inanition takes place. At the end of active digestion it may be assumed that the body is fully charged with carbohydrate if a carbohydrate-rich diet has been given for some time before. The immediate drafts upon this carbohydrate are probably very rapid, and inside of twenty-four or forty-eight hours nearly one half may

have been used; accordingly the proportion of the total katabolism during this time supplied by carbohydrates may be very large. Indeed, it has been demonstrated in one experiment [11] to have amounted to 37.8 per cent of the total energy for the twenty-four hours following the twelve hours after the last meal. At the end of the first twenty-four hours there may be still a continued draft upon body glycogen which with man reaches, on the average, 40 gm. in twenty-four hours. After this second twenty-four hours is over, we have a relatively constant plane of metabolism which involves the katabolism of about 20 to 25 gm. of glycogen up to and including the seventh day. Experiments with fasting men determining the glycogen metabolism longer than seven days have not as yet been made. It would appear, therefore, that the characteristic transition between whole digestion and inanition was on the first and second days of inanition, and it is to be questioned whether the "nüchtern" value has any significance whatever, except as representing, possibly, the first moments after active digestion has ceased. It is obviously very difficult to distinguish between the exact moment when the end of digestion takes place and the drafts upon body glycogen begin to be made. It is highly probable that this is more or less of an interchangeable situation, and that toward the end of digestion there may already be drafts upon body glycogen. This would be the case if, as a result of muscular activity or even of minor muscular activity in bed at rest, the carbohydrates in the food were not sufficient to supply the drafts upon the previously stored glycogen.

On these grounds, therefore, we can assume that if all of the factors tending to increase the storage of glycogen are constant and all of the factors tending to decrease this storage are constant, there will be a sufficient equilibrium established in which the body would have a normal amount of glycogen present. If this glycogen is not drawn upon or added to, we would have a constant plane of nutrition, and one would expect that the respiratory quotient at this plane of nutrition would remain constant. This being the case, it is not difficult to realize that in many series of experiments, with a relatively constant diet and with approximately the same muscular activity from day to

[11] BENEDICT: Carnegie Institution of Washington, Publication No. 77, 1970, p. 497.

day, the respiratory quotient would not materially alter. Such a constancy in the respiratory quotient would be expected, particularly in experiments made on successive days and in the same period of the year, which would insure no change in the character of the diet. These are the conditions which undoubtedly obtained in the series of experiments reported by Reach.[12] His experiments, eleven in number, extended from June 4 to July 13, and show respiratory quotients which ranged only from 0.772 to 0.865, — certainly a striking uniformity.

In view of these considerations, therefore, it is questionable whether it is logical to speak of a particular state or plane of nutrition as corresponding to the "nüchtern," or whether this state followed by the fasting state actually exists. The most sharply defined condition is that of the first two days of inanition after ordinary diet, before the metabolism settles to a normal plane, which apparently continues for five days at least without marked alteration.

From the noticeable alterations in the respiratory quotient following diets of different character, it would seem feasible to conduct experiments with the special view of altering the glycogen content of the body. This could be done either by the ingestion of carbohydrate to increase the glycogen storage or to decrease it by a low carbohydrate diet accompanied by muscular work, or possibly by the influence of cold baths. Considering the importance that the storage of glycogen has taken in studying the metabolism of diabetics, it will be seen that the susceptibility of the body to glycogen storage may be of great value in the diagnosis and prognosis of disease. In fact, studies of the respiratory quotient by modern technique would lead one to believe that the significance of the relatively small amounts of glycogen in the body may prove to be of the greatest importance in studying many problems in normal as well as pathological metabolism. The influence of muscular activity, training, etc., with regard to the varying alterations of the glycogen storage in the body, should all prove problems of the greatest value. It is not impossible, for example, that "training" might prove to be a process of adjusting the body to a condition in which there will be excessive glycogen storage, and that this storage will subsequently be used for the muscular

[12] Reach: Landwirtschaftliche Jahrbücher, 1908, p. 1091.

work accompanying the strenuous muscular exercise. The body is thus prepared for the sudden drafts upon body material. Certainly, during muscular exercise there is a draft upon body material and perhaps the most labile substance is the glycogen. The relationship, therefore, between the glycogen storage and muscular activity is one that should prove of great interest.

THE RESPIRATORY EXCHANGE AS AFFECTED BY BODY POSITION.

By L. E. EMMES and J. A. RICHE.

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THE well-known influence of muscular activity upon the respiratory exchange has led investigators for purposes of comparison to determine the value for metabolism at the lowest attainable degree of muscular activity, usually when the subject is lying on a couch or sofa, and always some time after the preceding meal. This fundamental value has proved of much importance in determining the effect of various agencies upon metabolism, particularly muscular activity and the ingestion of food. In the majority of the experiments, the subjects have been lying awake in a state of enforced muscular rest. The values thus obtained are somewhat abnormal for the practical purpose of estimating the energy requirements of individuals, in that people usually do not lie absolutely quiet while awake; consequently their resting metabolism would be somewhat higher than that of experimental subjects voluntarily restricting muscular movements while lying on a couch. Investigators are at present not in accord as to the influence of sleep on metabolism, some maintaining that the metabolism is not lowered during sleep,[1] and others,[2] that it is distinctly lowered.

There are relatively few observations with regard to the metabolism of an individual sitting upright in a chair. Two experiments on Caspari[3] are reported in which there was no apparent alteration in the metabolism when the subject was sitting as compared with that

[1] Johansson: Skandinavisches Archiv für Physiologie, 1898, viii, p. 85.

[2] Benedict and Carpenter: Carnegie Institution of Washington, Publication No. 126, 1910, p. 242.

[3] Zuntz, Loewy, Müller, and Caspari: Höhenklima und Bergwanderungen, Berlin, 1906, Table XII.

when he was lying down, but the experiments were not made on a strictly comparable basis and cannot be used as a criterion.

Johansson [4] found in a series of experiments in which the carbon dioxide output alone was determined that the average values per hour when the subject was sitting were about 6 per cent greater than when he was lying down.

Benedict and Carpenter [5] have made a number of comparisons of the metabolism of individuals when lying in a bed calorimeter and when sitting in a chair inside of another type of calorimeter. These comparisons have been made in a number of ways. When the average of a large number of experiments with individuals asleep inside of a respiration chamber is compared with another large number of individuals sitting up in a chair, they find an increase in the sitting metabolism of some 35 to 40 per cent over that when the subject is lying down. This increase must not be considered as measuring the difference in metabolism between the body positions of sitting and lying, for the subjects when lying down were asleep during the night hours and therefore the metabolism was at a minimum. On the other hand, when sitting in the chair they were not always absolutely quiet, and, indeed, at times they might have moved considerably.

In a more recent series of experiments, as yet unpublished, Benedict and Carpenter [6] have compared the metabolism of individuals when lying quietly awake in the daytime in a bed calorimeter with that of individuals sitting very quietly in a chair calorimeter. The increase noted in these experiments is much less than in the first series referred to, the metabolism being on the average from 20 to 30 per cent greater with the chair calorimeter than with the bed calorimeter.

In experiments on muscular work it has been necessary at times to determine the metabolism while standing. This has recently been done by Reach [7] in Durig's laboratory. In his experiments the subject was not only standing free but also leaning forward or backward against a support.

[4] Johansson: *Loc. cit.*, p. 118.

[5] Benedict and Carpenter: *Loc. cit.*, p. 252.

[6] An abstract of these comparisons is given by Benedict and Joslin: Carnegie Institution of Washington, Publication No. 136, 1910, p. 175.

[7] Reach: Landwirtschaftliche Jahrbücher, 1908, p. 1100.

Zuntz has always used a position in which the subject lies on a couch with the greatest muscular relaxation. Johansson has almost invariably used a similar position with enforced muscular relaxation, but aside from the few experiments of Caspari and Johansson, no extended series of experiments has as yet been reported with men sitting upright in a chair. Individuals sitting up in a chair quietly reading might be considered by some persons to have even a lower metabolism than when lying in bed. It is believed, for example, that there is less work performed in respiration when the person is sitting up than when lying down, as in the lying position, the chest must be raised and lowered at each respiration. On the other hand, when sitting in a chair, there is invariably some muscular tension to hold the position, as is evidenced by the general relaxation and instability of the upper portions of the body when a person falls asleep sitting in a chair.

The experiments reported here were made for the purpose of studying the metabolism as affected by the body position. They were planned with a special view to finding out if the metabolism increased when the subject was sitting as compared with lying, particularly if in the sitting position the head was comfortably supported, the muscular activity was kept at the lowest possible point, and there were no muscular movements other than those of involuntary respiration. A comfortable armchair with a special headrest was used for the sitting experiments, the headrest being so adjusted that the nosepieces of the respiration apparatus described by Benedict [8] could be inserted without any discomfort to the subject. The study was made in connection with the series of experiments reported in the preceding paper [9] on the effect of the previous diet on the respiratory quotient twelve hours after the last meal.

The subjects came to the laboratory without breakfast and lay down upon the couch in preparation for a series of experiments. Usually three or four experiments were made in a series; the details are given in the preceding paper.[9] After the experiments were completed, the subject then changed his position and sat upright in the chair, and a second series of three experiments followed. The details of these latter experiments are given in Table I. But two subjects

[8] Benedict: This journal, 1909, xxiv, p. 364.

[9] Benedict, Emmes and Riche: This journal, 1911, xxviii, p. 383.

were used in these experiments, L. E. E. and J. R., and the series are so numbered as to show that they correspond with a similar group of experiments in the preceding article; thus, series 1 *a* with L. E. E. followed immediately series 1 with the same subject in the article of Benedict, Emmes, and Riche.

TABLE I.

RESPIRATORY EXCHANGE OF SUBJECTS WHILE SITTING IN CHAIR.

Subject, and number of series.	Carbon dioxide excretion per min.	Oxygen consumption per minute.	Respir'y quotient.	Subject, and number of series.	Carbon dioxide excretion per min.	Oxygen consumption per minute.	Respir'y quotient.
	c.c.	c.c.			c.c.	c.c.	
L. E. E.	213	277	0.77	J. R.	226	247	0.91
1 *a*	221	254	0.87	1 *a*	218	242	0.90
	213	274	0.78		212	254	0.84
	212	267	0.80		210	249	0.84
2 *a*	199	250	0.80	2 *a*	208	249	0.84
	208	261	0.80		205	250	0.82
	230	257	0.90		219	256	0.85
3 *a*	221	249	0.89	3 *a*	229	261	0.88
	215	242	0.89		226	251	0.90
	244	243	1.01		211	256	0.83
4 *a*	238	258	0.93	4 *a*	...	...	...
	230	255	0.90		202	252	0.80

No difficulty was experienced in carrying out these experiments; both the subjects for the most part were comfortable, and the metabolism was reasonably uniform. Only occasionally do we find noticeable differences in the carbon dioxide production or oxygen consumption in a series of two or three experiments. The greatest variation is that noted in the second experiment with L. E. E. (1 *a*), where the oxygen consumption was noticeably less than in either of the other two experiments. Usually the agreement is fully as satisfactory as could be expected under the conditions of the experiment.

A comparison of the metabolism when lying on a couch with that

when sitting in a chair is given in Table II, in which we have the values not only for carbon dioxide excretion and oxygen consumption per minute, but the percentage increase while sitting has also been computed,

TABLE II.

COMPARISON OF THE AVERAGE PULSE RATE AND RESPIRATORY EXCHANGE OF SUBJECT SITTING IN CHAIR AND LYING ON COUCH.

Subject, and number of series.	Body position.	Pulse rate per minute.	Carbon dioxide excretion.		Oxygen consumption.	
			Per minute.	Increase in chair.	Per minute.	Increase in chair.
			c.c.	per cent.	c.c.	per cent.
L. E. E. 1	Lying	59	212	...	242	...
" " 1 *a*	Sitting	71	216	1.9	268	10.7
" " 2	Lying	56	212	...	235	...
" " 2 *a*	Sitting	64	206	−2.8	259	10.2
" " 3	Lying	61	202	...	240	...
" " 3 *a*	Sitting	72	222	9.9	249	3.8
" " 4	Lying	58	233	...	232	...
" " 4 *a*	Sitting	68	237	1.7	252	8.6
J. R. 1	Lying	69	201	...	233	...
" " 1 *a*	Sitting	77	219	9.0	248	6.4
" " 2	Lying	67	204	...	246	...
" " 2 *a*	Sitting	74	208	2.0	249	1.2
" " 3	Lying	68	219	...	236	...
" " 3 *a*	Sitting	73	225	2.7	256	8.5
" " 4	Lying	63	189	...	226	...
" " 4 *a*	Sitting	70	207	9.5	254	12.4

using as the basis the metabolism of the subject while lying on the couch. For the carbon dioxide excretion, the values show a range from −2.8 per cent in series 2 *a* with L. E. E. to +9.9 per cent in series 3 *a* with L. E. E. For the oxygen consumption there is uniformly an increase, ranging from 1.2 per cent in series 2 *a* with J. R. to

12.4 per cent in series 4 *a* with J. R. The table also shows the average pulse rate determined in each series of experiments. An inspection of the data shows an increase in the pulse rate in the experiments while sitting.

Comparing all of the data with both subjects, we obtain the following results:

	Pulse.	Carbon dioxide c.c. per min.	Oxygen c.c. per min.
Lying	63	209	236
Sitting	71	218	254
Increase	8	9	18
Percentage of increase in metabolism		4.3	7.6

Inasmuch as the oxygen consumption is commonly considered as the best index of metabolism, it is seen that our experiments indicate an increase in metabolism amounting to 8 per cent when the metabolism in the lying position is compared with that when the subject is sitting upright. These values may probably be considered as subject to slight corrections. For example, the experiments with the couch were always made earlier in the morning than experiments with the chair; consequently, since the metabolism shortly after arriving at the laboratory is frequently somewhat larger than after the subject has been lying down for several hours, the metabolism measured on the couch would be slightly larger than the normal value determined at the period of the day when the sitting experiments were made. This would tend to lower somewhat the apparent increase in metabolism. We believe, however, that this error cannot be very large, and would not be more than 1 or 2 per cent of the total metabolism.

In the series of experiments reported by Benedict and Carpenter,[10] there was an average increase of some 35 to 40 per cent in the metabolism while the subject was sitting in a chair as compared with the metabolism when the subject was lying in bed asleep. As has been pointed out before, the validity of this increase is vitiated by the fact that when in bed the subjects were all asleep with consequent minimum metabolism, while in the chair they were somewhat active. On the other hand, in the series of experiments made by Benedict and

[10] Benedict and Carpenter: *Loc. cit.*

Carpenter for control tests in a study of diabetes,[11] the increase was not so large, amounting on the average to a total increase in metabolism of about 25 per cent. This increase is, however, more than twice as great as that found in the experiments reported here. The natural explanation for this is that the subjects of our experiments were absolutely quiet aside from the change in body position, while in the other experiments the muscular activity of the subject while sitting in a chair inside the calorimeter was certainly very much greater than that of either of the subjects in our sitting experiments.

A series of experiments on women, as yet unpublished, has recently been carried out in this laboratory by Carpenter, in which the chair calorimeter was first used, and then the bed calorimeter. These experiments showed an increase in metabolism in the chair calorimeter over that in the bed calorimeter amounting to 7 per cent. This value agrees much more closely with those obtained in our experiments, and the agreement is undoubtedly due to the fact that the series of experiments with the women were made under such conditions that extraneous muscular activity was minimized. The women, as a rule, were noticeably less restless than the men, particularly while in the chair calorimeter.

The values given by Benedict and Carpenter for the difference in metabolism between persons lying asleep and sitting in a chair awake, with a moderate degree of restlessness, may be of much practical value in estimating the energy requirements of convalescents. For experimental purposes, however, when the metabolism at a given condition of body rest is to be determined, it is also of value to know, as a result of experiments with the respiration apparatus, that the metabolism of a subject when sitting absolutely quiet in a chair without extraneous muscular activity represents a metabolism 8 per cent greater than that of a subject lying on a couch with similar muscular rest. The difference in metabolism is then due, primarily, to the difference in the internal muscular activity necessitated by the sustaining of body parts. This is in conformity with the well-known fact that the pulse rate of an individual when sitting is always noticeably higher than that when he is lying down.[12] From these tests

[11] Cited in abstract by BENEDICT and JOSLIN: *Loc. cit.*

[12] GUY reports the average pulse rate of 100 men, averaging 27 years in age, as 70.1 per minute while sitting and 66.6 while lying. See "Cyclopædia of anatomy

we could infer that if it were possible to so support the body of the subject in a sitting position that the pulse rate would be no greater than when the subject was lying down, the metabolism would be essentially the same in both positions.

and physiology," 1852, iv, p. 186, cited by LEONARD HILL in SCHAEFER'S Text-book of physiology, 1900, ii, p. 101.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE UNDER THE DIRECTION OF E. L. MARK. No. 220.

CONTRIBUTIONS TO THE PHYSIOLOGY OF REGENERATION. — V. REGENERATION OF ISOLATED SEGMENTS AND OF SMALL PIECES OF WORMS.

By SERGIUS MORGULIS.

THE question why an animal ceases to grow when it has once attained a certain size is of fundamental importance in biology, but thus far it has eluded every attempt at a solution. The question why one organism grows faster or slower than another is a still more difficult problem, a solution of which at the present time we cannot even approach, because our knowledge of even the simpler processes of growth is still very meagre. In the province of regeneration two problems similar to those stated present themselves to the investigator who deals with this phenomenon from a quantitative standpoint: why does regeneration come to an end, and why does an organ regenerate at different rates under changed conditions?

It is an old fact, but recently studied anew and greatly emphasized, that an amputated organ regenerates with varying degrees of intensity according to the position, or level, of the cut surface, the intensity diminishing in an antero-posterior direction. The tail of a salamander, for instance, regenerates fastest from (*i. e.*, when cut off at) the base and slowest from the tip, and all gradations are found between the two extremes. A phenomenon of such interest has naturally stimulated scientific thought, and a number of more or less ingenious hypotheses have been offered to explain it. These hypotheses have sought to interpret the different rates of regeneration on the basis of the different amount of nutritive substance available for the regeneration of a lost organ, or on the basis of a nervous impulse expressed in the form of functional activity, or on the basis of the varying degrees of pressure mutually exerted by developing parts, or, finally,

even on the basis of an occult formative stimulus. It would take us too far afield to discuss critically these various views, especially since they have been discussed in the second part of these Contributions (Morgulis, 1909[b]), to which the reader is referred for a detailed account. In the same publication was proposed an hypothesis suggested by the fact that in the linear growth of an organ successive portions are formed at gradually diminishing rates. The progressive diminution of the growth energy is undoubtedly due, it was there maintained, to the diminished reproductive power of the cells, which lose their original vitality and vigor with each successive generation. A linear organ presents regionally throughout its length various degrees of senility, the more senile segments being the more posterior ones; the different rates of regeneration from the corresponding regions are therefore explicable on the ground that the cells are endowed with different degrees of vitality. This hypothesis, supported directly by observations on regeneration from different levels (see Morgulis, 1909[a], pp. 600–610), and indirectly by the fact that young animals as a rule regenerate more vigorously than adult animals, presupposes furthermore a regional differentiation in the organs. The assumption of such a material basis seemed to be warranted by the experiments on Lumbriculus, where it was shown that the relative potentiality of regeneration is transmitted from the original piece to the regenerated piece (Morgulis, 1907[b], p. 219), and also by the fact that when a circular strip of tissue is cut off from a jelly-fish the rate of regeneration is the same both peripherally from the disc and centrally from the strip. The assumption has received further confirmation in the experiments on isolated segments of Podarke obscura, which will now be described.[1]

This worm, which is about three fourths of an inch long, is made up of 40 to 50 segments, each bearing a pair of parapodia. The individual segments are very small, but with the aid of a watchmaker's lens they can be easily distinguished. Between every two adjacent segments there is a more or less deep constriction; the area of junction of the segments is larger in the middle portion of the worm than in the posterior. The operation of removing single segments increases in difficulty as one passes from the tip of the tail towards the head. It is a comparatively simple operation to obtain single segments from

[1] The experiments were performed in the laboratory of the U. S. Bureau of Fisheries, Woods Hole, Mass., in the summer of 1909.

the hinder third of the animal, the chief difficulty there being the smallness of the segments. In the middle of the worm the segments are relatively large, but, the area of junction between segments being also large, one is not always successful in isolating them without much injury. It is practically impossible to obtain single segments from the anterior third of the worm, because the digestive tube in that region is rather large and muscular and therefore prevents the effective separation of two adjacent segments. In the few instances, however, where the isolation was accomplished, the segments soon died because they were badly mutilated.

It is necessary, above all, to conduct experiments with large numbers of single segments, as only a small per cent of those successfully removed survive. It is not easy, however, to fulfil the requisite conditions. In the first place, for the survival of the segments it is absolutely necessary that the wound should close as quickly as possible; this occurs in those instances where no, or at any rate very little, tissue from adjacent segments remains attached. A great many isolated segments die in consequence of their failure to close up the wound, either because the wound surface is too large or because a piece of the gut protrudes, thus preventing the margins of the wound from completely coalescing. Besides, attached fragments of tissue become easily infected with bacteria and thus cause death to the isolated segments. Secondly, on the day following the operation the segments must be cleaned of every trace of cast-off epidermis and adhering tissue; it is needless to say that, as a result of this delicate operation, many isolated segments perish. Finally, the continual and vigorous contraction of the segments is an important cause of mortality, because the stiff bristles of the parapodia, being alternately pulled in and out, break through the body wall and in fact frequently tear the segment to pieces. The process of regeneration of the isolated segments is in no manner different from that which I have already described for larger portions of the worm in the case of Podarke (Morgulis, 1909[a], p. 601). Moreover, what was found previously with regard to worms cut at different levels has now been fully corroborated by experiments on isolated segments belonging to different regions of the worm; namely, that the more anterior the level the sooner does the regeneration begin. The results, however, must be judged with much caution, because, as pointed out before, the cut

surface being smaller in posterior segments, the preliminary stages of regeneration — the closure of the wound — are accomplished there more easily. But in spite of that, as will be shown presently, isolated segments from an anterior region regenerate faster than those from a posterior region.

The results of the experiments with isolated segments agree among themselves in every essential respect, except that more segments may have survived in one instance than in another. Here are the results of an experiment. Three days after operation 21 isolated single segments from the middle of the worm and 40 single segments from the posterior portion remained alive. Of the former 7 (or 33.3 per cent) commenced to regenerate; of the latter only 8 (or 20 per cent). Five days after operation only 12 isolated segments from the middle of the body were alive, and of these 58 per cent had regenerated; and of the 32 surviving single posterior segments 13 (41 per cent) were found regenerating. Three days later still, *i. e.*, eight days after the segments were isolated, the former had regenerated on the average 3.3 new segments, while the latter had regenerated only 3 segments, the extremes in the number being 3 to 4 segments in the first case and 2 to 4 segments in the other. Although isolated segments were frequently maintained alive for two weeks, they never regenerated more than 4 new segments. The isolated segments are bound to die soon for lack of nutrition, because a head is not regenerated in this worm; but it is very noteworthy that such an exceedingly small part of an organism — not more than a millimetre in length — is capable not only of existing for the space of a fortnight without any food, but also of continually expending energy in its manifold motions and, in addition to its other functions, of building new segments out of its own substance. One who has watched these isolated fragments of the organism — themselves complete organisms — convulsively contracting and thus shifting about the bottom of the receptacle, who has seen the incessant but vigorous lashing of their ciliated surfaces, and who has observed the unfolding step by step of a new worm out of this clump of tissue cannot evade the urgent question, Whence is all this energy?

Even parts of a segment can be maintained alive, and these will proliferate new tissue. Of course, the difficulties here are so numerous and so great that experiments succeeded only rarely; therefore the regeneration of fragments could not be investigated satisfactorily.

However, I am convinced from the few successful instances that not only does the wound close over, but that also a growth of new tissue occurs; but whether or not such fragments of a segment can complete the missing portion is a question which, under the circumstances, must remain unanswered. Another question of great theoretical weight, namely, whether or not an isolated segment can regenerate again when the regenerated tissue has been removed, must remain unsolved on account of the impractibility of the experiment.

The regeneration of isolated segments is significant in several respects. In the first of these Contributions (Morgulis, 1909[a]) it was shown that the process of posterior regeneration is divisible into four distinct stages, each characterized by a different intensity of the regenerative energy, and that this is true of all regenerating worms regardless of the level of the cut. There is a difference between regeneration from an anterior and from a posterior level; for at no stage of the process does the organ regenerate with as great intensity from the posterior level as from the more anterior. Morgan (1906) thought that the regenerative potentialities are the same at all levels, although he foresaw that further researches might disclose a difference in the potentiality. The experiments on isolated segments do, I believe, prove the existence of a regional differentiation in the regenerative potentiality. I may mention in this connection that isolated segments from the very tip of the tail did not regenerate at all. These segments are extremely minute, but the smallness of their size has nothing whatever to do with their failure to regenerate. Of course, this point cannot be proven directly by experiment, but its cogency is nevertheless obvious from a consideration of what is known generally on this subject. As I showed previously, segments only a few times larger may regenerate even as many as four new segments; furthermore, the fact that regeneration is largely independent of food would make it highly doubtful whether the failure of the most posterior segments to regenerate is due to a lack of formative material. Besides, the greater regeneration from an anterior level completely refutes such a possibility, because in this case the greatest regeneration occurs where there is least material. According to the hypothesis which I have suggested (Morgulis, 1909[b], p. 435), the most posterior segments of the worm, being the remotest descendants from the original embryonic material, *i. e.*, the latest growth of the organism,

are also the most senile and therefore the least capable of regeneration. Similarly, in some animals the power to regenerate diminishes almost to complete disappearance as they grow from the embryonic to the adult condition.

The regeneration of isolated segments gains particular interest when compared with the regeneration of large portions of the worm. A single segment does not begin to regenerate as quickly as a piece embracing one half or two thirds of a worm, but it should also be remembered that in the single segments there are two wounds to be closed, whereas in the larger pieces there is only the posterior wound. Therefore the longer time required by isolated segments before the tail commences to regenerate may be due to the greater complexity of the preliminary stages of regeneration. The greatest number of new segments which an isolated segment can regenerate in the course of eight days is 4, or only two thirds of that which a piece 15 to 17 segments long regenerates within the same length of time; as has been intimated in the foregoing, the isolated segments never regenerate any more. When it is recalled, however, that large pieces of a worm, even in the course of weeks, do not regenerate more than about 12 to 14 new segments, it becomes obvious that an isolated segment regenerates relatively more than a piece 15 to 17 segments long. The study of the regeneration of isolated segments leaves no room for doubt that practically every segment of the worm Podarke is capable of producing out of its own substance a few new segments, and even as many as 4. A group of 15 to 17 segments must, accordingly, have sufficient formative material for some 40 to 60 new segments, of which, however, they actually regenerate only a small fraction, therefore the potential regenerative energy of each component segment is only partially utilized. The proportionately greater regenerative efficiency of isolated segments is of much importance in understanding the physiology of regeneration, but before discussing this topic I shall present some further results upon the regeneration of pieces varying in initial size of another worm, Lumbriculus.

In a number of worms the posterior halves were removed, and the anterior portions were divided into three groups. In the first group (*A*) the half-worms were merely decapitated; in the second group (*B*) the half-worms were cut into two parts, of nearly equal length, and in the third group (*C*) they were cut into four nearly equal parts; in all

three groups only the most posterior pieces were experimented upon. In other words, the level of the posterior cut is, roughly speaking, the same in all three groups, but according to their size the pieces represent about one half, one fourth, and one eighth of the entire worm,

TABLE I.

Group . . .	A.		B.		C.	
Segments . .	Old.	Regenerated.	Old.	Regenerated.	Old.	Regenerated.
August 12–22	43	64	36	55	19	48
	55	58	29	50	15	46
	44	58	33	47	12	44
	42	56	25	47	10	44
	44	55	27	44	11	42
	43	54	18	42	14	38
	44	51	21	41	17	37
	35	51	21	38	15	35
	37	47	21	38	14	35
	38	46	31	36	..	..
Average . . .	42.5	54	26.2	43.8	14.1	41
Ratio between old and regenerated segments	1.271		1.672		2.906	

as is shown diagrammatically in Fig. 1. When such pieces of Lumbriculus are allowed to regenerate for several days, one finds that the difference in the number of regenerated segments is not commensurate with the difference in the number of segments in the regenerating pieces. An inspection of Table I will make this clear. From this table it will be seen that the average number of old and of regenerated segments in the *A* pieces was 42.5 and 54 respectively; in the *B* pieces 26.2 and 43.8, and in the *C* pieces 14.1 and 41, Even these figures suffice to show the proportionately greater regeneration in pieces of

the smaller size; but the precise ratio is more clearly perceived when the number of regenerated segments is divided by the number of segments in the regenerating piece, which is equivalent to determining the number of segments regenerated on the average for each old segment; this number serves as the coefficient of regeneration for the corresponding group of pieces. We find that the coefficient of regeneration in the *A*, *B*, and *C* pieces was respectively 1.271, 1.672, and 2.906.

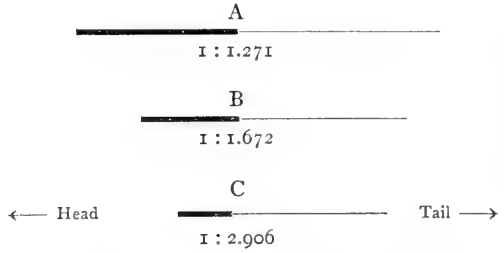

FIGURE 1. — The three diagrams, *A*, *B*, *C*, represent the average number of segments in the regenerating (heavy line) and in the regenerated (light line) portions of the worm for the groups *A*, *B*, *C*, respectively. The numerals under the diagrams show the ratio of the number of segments in the two portions.

In another experiment two groups of pieces, having the posterior cut approximately in the middle of the worm, and embracing respectively about one eighth (*A*) and one sixteenth (*B*) part of the worm, were left to regenerate for several days. The results of this experiment are recorded in Table II. Like those of the previous experiment, these show that the number of regenerated segments does not diminish in the same ratio as the number of segments in the regenerating pieces. Thus, in the *A* group the average number of old segments was 12.8 and of regenerated segments 34.5; in the *B* group it was 7.6 and 27 segments respectively. The coefficient of regeneration in group *A* is 2.706 and in group *B* 3.573; that is to say, there is manifested relatively more regenerative potentiality in the small pieces of the worm than in the larger ones.

We may infer from these experiments that two $^1/_{16}$ pieces of a worm regenerate considerably more than one $\frac{1}{8}$ piece, and that two $\frac{1}{8}$ pieces regenerate more than one $\frac{1}{4}$ piece, etc. Furthermore, as the coefficients of regeneration of the two experiments indicate, within the same length of time, every segment of the $\frac{1}{2}$ worm regenerates

1.271 new segments, of the ¼ worm 1.672, of the ⅛ worm 2.906 (in the second experiment 2.706), and, finally, of the $^1/_{16}$ worm 3.573 segments. Therefore the *rate* of regeneration in the small pieces is also

TABLE II.

Group	A.		B.	
Segments.	Old.	Regenerated.	Old.	Regenerated.
August 24–September 6	13.0	42	7.0	35
	12.0	41	9.0	33
	12.0	40	6.0	30
	13.0	38	9.0	27
	14.0	35	9.0	27
	11.5	35	7.0	27
	12.5	26	8.0	26
	14.0	19	7.5	20
	...	...	5.5	18
Average	12.8	34.5	7.6	27
Ratio between old and regenerated segments . . .	2.706		3.573	

greater than in the large pieces, the relative rate of output of the regenerative energy increasing as the initial size of the regenerating object decreases.

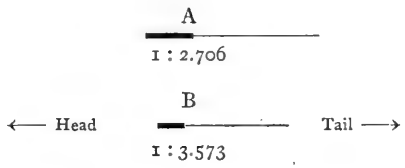

FIGURE 2. — See explanation of Fig. 1.

Now, with the two chief results established, namely, *that isolated segments regenerate proportionately more than groups of segments* (*Podarke*), *and that the smaller the piece the greater the rate of its regenera-*

tion (*Lumbriculus*), we may proceed to discuss their bearing upon some general problems.

Within the past fifteen years or so much biological discussion has centred about the problem of the potentiality of the developing egg and of the isolated blastomeres. This discussion, which has stimulated much thought and experimental work bearing fruitful results in the province of developmental mechanics, concerned itself chiefly with the qualitative study of the whole and partial egg. Unfortunately, we know much less with regard to the quantitative differences between the development of an egg or of its component blastomeres, and it would be highly desirable to fill this gap in our knowledge. The general conclusion following from the study of isolated blastomeres is that "a part is capable of doing that which the whole is set aside to do" (Morgan, 1896, p. 292). Moreover, as Morgan (1896, p. 291) has said, the egg fragments divide "beyond their normal cleavage limit"; this is very similar to the above results from the regeneration of isolated segments.

The bearing of the study of regeneration of isolated segments and of pieces of worms of different initial size upon the physiology of regeneration will be more clearly seen if some of the already established facts are recalled. It has been shown on several occasions that an organism is capable of regeneration many times in succession. Furthermore, the amount of regeneration after a *single* operation is considerably less than the amount regenerated for the same length of time following *two or more* operations (Morgulis, 1907[a], 1909[a], 1909[b], 1911[a], and 1911[b]). Another important fact recently brought to light is that regeneration usually ceases before the lost portion has been completely restored. Thus, in Podarke it was found (Morgulis, 1909[b], 1911[a]) that only 0.4 of the number of removed segments regenerated in the course of about eight weeks. As at that time the regenerative process is practically at a standstill, it is quite certain that not more than half as many segments regenerate as are removed. But the regeneration does not come to an end because the regenerative capacity of the organism has been exhausted, for by removing the new tissue the regeneration may be started again. From the experiments on isolated segments we may infer that had the sum of the regenerative potentialities of all segments been utilized, it would have sufficed not only to replace that which was lost, but to produce even an excess.

The explanation of this phenomenon, it seems to me, lies in the circumstance that the organism possesses a large regenerative potentiality, of which, however, only a small fraction is actually utilized in the process of regeneration. The results of the experiments with pieces of worms of varying sizes suggests that there is a factor which determines to what extent the regenerative potentiality may be utilized. From the fact that, while other conditions remain equal, the smaller the initial size of the regenerating object the more and the faster proportionately it regenerates, we may infer that the organism presents a certain amount of inertia, due to a tendency to maintain a definite state of equilibrium and of functional adjustment. This inertia, which forms a resistance to regeneration that must be overcome, varies directly with the size of the regenerating object.

I do not wish to be understood as attempting to translate a biological fact into terms of pure mechanics, although a comparison with the well-known behavior of inanimate objects is strongly suggested. In conclusion I would, however, mention some other facts which admit of the same interpretation. It was discovered by Chambers (1908), when he assorted eggs of a single frog according to their dimensions, that the smaller eggs developed faster; and Morgan (1906) also found that the rate of growth of the salamander Diemyctylus viridescens depends upon the size of the animal, being greater in the small individuals. We see in these instances the same relation between the initial size of the object and the phenomena of growth and development that has been established by the foregoing experiments on regeneration.[1]

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THE INFLUENCE OF COLD BATHS ON THE GLYCOGEN CONTENT OF MAN.

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KÜLZ [1] showed that a dog which had fasted twenty days still contained glycogen in the muscles, and he warned against the common use of the term "carbohydrate-free animal." He discovered that strychnin tetanus was a real means of producing an animal which was free from glycogen. Zuntz [2] demonstrated that fasting rabbits, deprived of glycogen by strychnin convulsions and subsequently narcotized with chloral hydrate, stored glycogen both in liver and muscles during the interval of quiet.

These experiments of Zuntz illuminated certain respiratory quotients obtained by him [3] as the result of exercise, in fasting men. The period during which mechanical work was done was characterized by a higher respiratory quotient than that obtained during a period of rest. The following table shows this:

	Respiratory quotient.	
	Rest previous to work.	Period of work.
Fourth day of starvation . . .	0.63	0.78
Fifth day of starvation	0.66	0.74
Sixth day of starvation	0.69	0.74

Durig [4] has obtained low respiratory quotients (0.71) during periods of rest in the Capanna Margherita, a hut on Monte Rosa (4560 metres), following periods of intensive exercise.

[1] KÜLZ: LUDWIG'S Festschrift, Marburg, 1891, p. 109.

[2] ZUNTZ: Archiv für Physiologie, 1893, p. 378.

[3] LEHMANN, MÜLLER, MUNK, SENATOR, ZUNTZ: VIRCHOW'S Archiv für pathologische Anatomie, 1903, Supplement to vol. cxxxi, p. 197.

[4] DURIG: Denkschrift der mathematisch-naturwissenschaftlichen Klasse der kaiserlichen Akademie der Wissenschaften, 1909, lxxxvi, p. 107.

Since 15 per cent of the total energy of a fasting man arises from protein and 85 from fat, it has been calculated that the respiratory quotient should be 0.722, were these materials oxidized in this relationship. The low quotients during rest would indicate the retention of a body rich in oxygen, perhaps glycogen, which could be oxidized in case of further exercise. A quotient of 0.75 indicates that about 9 per cent of the oxygen absorbed is utilized in the combustion of carbohydrates. Benedict [5] finds that the respiratory quotient of a fasting man varies between 0.74 and 0.75, from the second to the seventh day of starvation.

Magnus-Levy [6] has calculated that if a diabetic oxidizes 100 gm. of protein and 250 gm. of fat, and if from the protein about 60 gm. of dextrose arise (as first established by Reilly, Nolan, and Lusk [7]), the respiratory quotient would then be 0.699 instead of 0.722. Magnus-Levy also calls attention to the fact that owing to the loss of carbon dioxide through the skin this quotient is reduced by from 0.01 to 0.015, when it is obtained by the measurement of the gaseous exchange through the lungs only.

Magnus-Levy [8] himself has reported low quotients of 0.693, 0.697, 0.719, obtained from cases of severe diabetes. Similar quotients have also been found in severe diabetes by Benedict and Joslin.[9] Thus in case C. 19, Oct. 25, 1909, when the D:N ratio for the day may be calculated at 3.5:1 (which indicates a complete intolerance for carbohydrate), the quotients obtained during four intervals within about an hour's time were 0.70, 0.70, 0.68, 0.68, an average of 0.69.

Rubner [10] investigated the influence of baths upon a man and found that a douche at 16° raised the oxygen absorption 110 per cent above the normal, and increased the respiratory quotient from 0.87 to an average of 1.03, from which it was evident that the source of the metabolism during the douche was glycogen.

[5] Benedict: Metabolism in inanition, Carnegie Nutrition Laboratory, 1907, p. 451.

[6] Magnus-Levy: Archiv für Physiologie, 1904, p. 381.

[7] Reilly, Nolan, and Lusk: This journal, 1898, i, p. 395.

[8] Magnus-Levy: Zeitschrift für klinische Medizin, 1905, lvi, p. 86.

[9] Benedict and Joslin: Metabolism in diabetes mellitus, Carnegie Institution of Washington, 1910.

[10] Rubner: Archiv für Hygiene, 1903, xlvi, p. 390.

The writer [11] has shown that cold baths administered to fasting phlorhizinized dogs at first raised the D:N ratio, indicating a removal of residual glycogen, but subsequent baths were without influence. Ringer [12] has demonstrated that a dog so treated yielded no extra sugar in the urine on administration of adrenalin, which is further evidence of a complete exhaustion of the glycogen supply.

The question arises whether cold baths will not completely remove the glycogen content of a man, and to that end two individuals who had accustomed themselves during the summer to a swim of fifteen minutes' duration in the early morning in cold water, and who took daily cold douches as a matter of routine at other times, submitted themselves during the morning (without breakfast) to the action of two baths, each of six to ten minutes' duration. The water contained floating ice blocks and had a temperature of 10° C., the baths being given at intervals of about two hours. As will be seen below, the treatment was effective in exhausting the glycogen supply.

Experimental Part.

The apparatus used in these experiments is called the "small Benedict apparatus" for studying the respiratory exchange.[13] It was constructed in this laboratory by Mr. J. A. Riche, who had had large experience with its use in the Nutrition Laboratory at Boston. Mr. Riche also had control of the apparatus in the determination of the data here presented. The individual under investigation lies comfortably on a bed and respires through a nose piece.

The following protocols may be given:

Case A. — October 14, 1910. 2 P. M., usual luncheon. Lively ride one and a half hours on a horse, followed by cold douche. 7.30 P. M., dinner of fish and steak, no carbohydrates.

October 15. 6 A. M. Rose and took a cold douche; 6.45, took a cup of hot black coffee.

Period I. — 10.04 to 10.19 A. M. Normal resting period.

First Bath, 10.38 to 10.44. Immersed six minutes in bath with ice

[11] Lusk: This journal, 1908, xxii, p. 163.

[12] Ringer: Journal of experimental medicine, 1910, xii, p. 105.

[13] Benedict: This journal, 1909, xxiv, p. 345.

blocks; temperature, 10° C.; skin red, shivering. The knees and chest could not be simultaneously immersed, and therefore a movement was made which immersed the two alternately.

Period II. — 10.48 to 11.03. Period with shivering. Shivering lasted during eight minutes of the experimental period. Skin only partly dry, partly covered with a sheet; room temperature, 23°, window open. No discomfort, shivering seemed a normal compensation for heat loss.

11.05. Cup of hot coffee.

Period III. — 12.04 to 12.19 P. M. Normal resting period.

Second Bath, 12.29 to 12.37; temperature of bath, 12°. General shivering during the eight minutes of immersion.

Period IV. — 12.41 to 12.56. Period with shivering. Shivering lasted six to seven minutes. Urine showed neither sugar nor albumin as a result of the second bath.

Period V. — 1.26 to 1.41. Normal resting period.

Food, 1.45 to 1.55. Food taken, 1000 c.c. of milk with added cream, milk-sugar, and four raw eggs. The whole contained 8.25 gm. of nitrogen and 1212 calories, divided as follows: protein 201, milk-sugar 282, and fat 729; the diet had been prepared for a tuberculous patient.

Period VI. — 3.30 to 3.45. Normal resting period after food ingestion.

The results of these experiments are found in Table I. The calorific values are calculated on the basis of Zuntz's tables as given by Magnus-Levy,[14] it being assumed that 15 per cent of the oxygen absorption is utilized for protein oxidation.

During the shivering which followed the second cold bath the metabolism as measured by the heat production was 63 per cent higher than during the subsequent resting period, but the respiratory quotient of 0.75 remained unchanged during both periods. It is evident from this experiment that *the influence of two successive cold baths which cause shivering during a period when the intestine is free from carbohydrate, is sufficient to change the metabolism from one maintained at the expense of carbohydrate* (*R. Q.* = 0.99) *to one maintained essentially by the combustion of fat* (*R. Q.* = 0.75). *Hence the organism of man may be quickly rid of glycogen by shivering.*

Case B. — November 4, 1910. 2 P. M., usual luncheon. 6.30, dinner of roast beef without carbohydrate, appetite unappeased. 9.30, retired.

[14] Magnus-Levy: von Noorden's Handbuch der Pathologie des Stoffwechsels, 1906, i, p. 207.

November 5. 5 A. M. Rose and took a cold bath. Studied till 6.45. 7.30, took a large cup of black coffee.

Period I. — 10.18 to 10.33 A. M. Normal resting period. Blood pressure, max. = 135 mm., min. = 90 mm.; pulse, 74.

First Bath, 10.44 to 10.53. Immersed nine minutes in bath with ice blocks; temperature, 10° C. Commenced shivering after half a minute; skin red.

Period II. — 10.59 to 11.14. Shivering continued throughout the period. Skin partly dry, partly covered with a sheet.

11.05. Blood pressure, max. systolic = 150, min. diastolic = 100; pulse, 92.

11.15. Urine is free from sugar and albumin.

Period III. — 11.56 A. M. to 12.11 P. M. Normal rest, covered with a warm blanket.

Second Bath, 12.16 to 12.26; temperature, 10°. General shivering throughout the period of immersion.

Period IV. — 12.32 to 12.47. Shivering during eight minutes of the period.

Period V. — 1.33 to 1.49. Normal resting period.

Food, 1.55. Took cup of coffee containing 50 gm. of dextrose.

Period VI. — 2.13 to 2.28. Resting period showing the influence of dextrose ingested eighteen minutes before the commencement of the experiment.

During two periods of the experiment the respiratory quotient sank to 0.67 and below, or near the diabetic quotient when even the sugar arising from metabolized protein is not oxidized. On the assumption that 250 gm. of fat and 100 gm. of protein are oxidized by the diabetic and that 60 gm. of protein-sugar appear in the urine Magnus-Levy [15] has calculated the respiratory quotient to be 0.699. Fat would here furnish 2325 calories, protein 185 calories (225 calories being eliminated as urinary dextrose). This oxidation would require 549.3 litres of oxygen. Hence 1 litre of oxygen would have a calorific value of 4.589. When fat alone is oxidized, the value is 4.686. The error in using the former figure given cannot therefore be great. On account of the loss of carbon dioxide through the skin the R. Q. of 0.67 may be raised to 0.685 in the present experiment.

No explanation of the quotient 0.62 is available, although it has been observed by other investigators, and no attempt has been made to calculate the heat value of the metabolism of the period with which it is associated. (See Table II).

[15] MAGNUS-LEVY: Archiv für Physiologie, 1904, p. 381.

TABLE I.

PERSON A. WEIGHT = 75.95 KG. AREA OF BODY SURFACE

Period.	Time.	Per minute.		R. Q.
		CO_2	O_2	
		c.c.	c.c.	
I	10.04–10.19	271	273	99
II	10.48–11.03	301	366	82
III	12.04–12.19	272	310	88
IV	12.41–12.56	332	432	75
V	1.26– 1.41	198	265	75
VI	3.30– 3.45	281	320	88

TABLE II.

PERSON B. WEIGHT = 64.71 KG. AREA OF BODY SURFACE

Period.	Time.	Per minute.		R. Q.
		CO_2	O_2	
		c.c.	c.c.	
I	10.18–10.33	212	224	95
II	10.59–11.14	551	645	85
III	11.56–12.11	205	305	67
IV	12.32–12.47	530	628	84
V	1.33– 1.49	188	302	62
VI	2.13– 2.28	240	321	75

TABLE I.

= 2.206 SQ. M. HEIGHT = 176.5 C.M. AGE, 44 YEARS.

Calories per hour.		Calories per day.		Calorific value 1 litre O_2	Remarks.
Per kgm.	Per sq. m. surface	Per kgm.	Per sq. m. surface.		
1.08	37.2	25.9	893	5.000	Normal.
1.39	47.7	33.3	1145	4.794	After Bath I.
1.20	41.0	28.6	985	4.867	Normal.
1.60	55.4	38.6	1329	4.708	After Bath II.
0.99	33.9	23.7	815	4.708	Normal.
1.23	42.4	29.6	1018	4.867	After food.

TABLE II.

= 1.982 SQ. M. HEIGHT = 170.5 CM. AGE, 26 YEARS.

Calories per hour.		Calories per day.		Calorific value 1 litre O_2	Remarks.
Per kgm.	Per sq. m. surface.	Per kgm.	Per sq. m. surface.		
1.03	33.5	24.6	804	4.954	Normal.
2.89	94.4	69.4	2265	4.831	After Bath I.
1.30	42.4	31.2	1017	4.589	Normal.
2.81	91.7	67.4	2202	4.819	After Bath II.
...	...	...	...	...	Normal.
1.40	45.8	33.7	1099	4.708	After 50 gm. dextrose.

During the shivering which followed the first cold bath the metabolism was increased 181 per cent, and the respiratory quotient was 0.85, which indicated that the oxygen distribution was divided as follows: for protein 15 per cent, for carbohydrates 44 per cent, for fat 41 per cent. During the next resting period the quotient was 0.67, that found by Zuntz [16] during complete rest in a fasting man and interpreted by him to signify a storage of glycogen derived from protein. During the period following the second bath the quotient rose to 0.84, and this can only be interpreted as due to a further oxidation of glycogen. During the subsequent period of rest the quotient sank to 0.62. Such quotients have been found after exhaustive exercise. Thus Durig [17] states that he was forced to take sugar on reaching the summit of a mountain, in order to obtain a respiratory quotient which was interpretable. In the present research it will be noticed that the administration of 50 gm. of dextrose to Case B caused the quotient to rise within half an hour from 0.62 to 0.75.

TABLE III.

PERSON C. WEIGHT = 70.8 KG. AREA OF BODY SURFACE

Period.	Time.	Per minute.		R. Q.
		CO_2	O_2	
		c.c.	c.c.	
I	10.45–11.00	266	304	88
II	11.45–12.00	694	865	80
III	12.47– 1.02	270	314	86
IV	1.46– 2.01	576	711	81
V	2.48– 3.03	276	320	86
VI	4.15– 4.30	259	297	87

It is apparent from this discussion, that the treatment of Case B by cold baths had a much more pronounced effect than on Case A. The difference lies in the relative difference in weight. If the normal

[16] ZUNTZ: *Loc. cit.*

[17] DURIG: *Loc. cit.*, p. 78.

weight of a person in kilograms is the number of centimetres of his height less one hundred, then Case A was of normal weight, whereas Case B was nearly 6 kgm. under the normal. The difference in adipose tissue represented by this difference in weight accounts for the greater readiness of heat loss in Case B when subjected to cold baths.

TABLE III.

= 2.105 SQ. M. HEIGHT = 173.2 CM. AGE, 23 YEARS.

Calories per hour.		Calories per day.		Calorific value 1 litre O_2	Remarks.
Per kgm.	Per sq. m. surface.	Per kgm.	Per sq. m. surface.		
1.26	42.4	30.2	1017	4.869	Normal.
3.50	117.9	84.0	2829	4.770	After Bath I.
1.29	43.6	31.0	1046	4.844	Normal.
2.88	97.2	69.1	2332	4.783	After Bath II.
1.31	44.3	31.4	1063	4.844	Normal.
1.22	41.3	29.3	991	4.856	After protein food.

Case C. — December 11, 1910. Noon, usual dinner. 5 P. M. to 6 P. M., hard exercise. 6 P. M., supper of meat, fat, and a single piece of toast. Sound sleep for eight hours during the night.

December 12, 7.30 A. M. Rose, drank some black coffee without sugar, and came to the laboratory.

Period I. — 10.45 to 11.00 A. M. Normal resting period; pulse, 68.

First Bath, 11.28 to 11.40 A. M. Began to shiver at 11.44. Immersion in bath with ice blocks for twelve minutes. Temperature of baths rose from 8° to 10° during the experiment.

Period II. — 11.45 to 12 noon. Subject shivered throughout the entire period.

Period III. — 12.47 to 1.02 P. M. Normal resting period. Subject does not feel as warm as before taking the bath.

Second Bath, 1.29 to 1.41 P. M. Immersed in water with ice blocks; temperature of water, 8°. Shivering.

Period IV. — 1.46 to 2.01 P. M. Violent shivering throughout the entire period. Pulse, 85; blood pressure, max. systolic = 144, min. diastolic = 82.

Intermission. Hot bath at 44° taken for fifteen minutes in order to restore the feeling of warmth.

Period V. — 2.48 to 3.03 P. M. Normal resting period. Pulse, 80; blood pressure, max. systolic = 92, min. diastolic = 60.

Food, 3.15 P. M. Large beefsteak, soup, and black coffee without sugar.

Period VI. — 4.16 to 4.30 P. M. Normal resting period.

This individual had a well-developed musculature, and was a highly trained and accomplished base-ball player. There was no surplus fat on his body. It is evident, from the respiratory quotients obtained, that in spite of intense shivering caused by the two cold baths, the treatment was not sufficient to remove the glycogen from the highly developed musculature of this individual. The results are shown in Table III.

An interesting detail is that during the first hour following the ingestion of beefsteak no increase in the heat production was noted.

In all three individuals experimented on, the skin became intensely red during the bath in ice water. This may be explained as being due to paralysis of the contractile elements of the superficial capillaries caused by the influence of cold on the protoplasm. This does not necessarily indicate an increased blood flow to the skin area, for the superficial arterioles are probably so constricted as to reduce the blood flow to the skin. The blood pressure rises considerably, but its greatest height could not be obtained because the profound shivering prevented taking the pulse.

Only these three experiments were made, for it was fully realized that the ordeal of being immersed amid cracked ice was not to be lightly undertaken. No ill effects whatever were noticed in any of the men who served as subjects. The only after effect observed was that of general muscular lassitude.

Conclusions.

1. Immersion of normal men in cold baths at a temperature of 10° when the intestine is free from carbohydrates, induces shivering which causes a rapid utilization of body glycogen as determined by a fall in the respiratory quotient to the fasting level. In one very muscular individual this result could not be obtained.

2. In one individual in whom the shivering had been severe, a quotient of 0.67 and another of 0.62 were found during subsequent periods of rest, which correspond to those observed during rest after a period of exhaustive exercise (glycogen formation from protein).

3. The greatest increase in heat production which was brought about by the cold baths was 181 per cent above the normal. The urine remained free from albumin and from sugar.

ON NUCLEIN METABOLISM IN THE DOG.

By P. A. LEVENE and F. MEDIGRECEANU.

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THE very large recent literature on nuclein metabolism abounds in controversial statements and contains conclusions apparently irreconcilable with one another. However, disregarding the personal attitude of the writers to the results of their own experiments, and viewing them in the light of impartial analysis, it is possible to formulate a very definite conception regarding certain phases of nuclein metabolism. Particularly the final phases in the long cycle of nuclein metamorphosis have been made obvious. It has been established, principally through the work of Schittenhelm and his coworkers, Wiechowski, and Mendel and his coworkers, that the end products of purin metabolism are uric acid, allantoin, and urea. The intermediate stages between allantoin and urea are not known. Whether or not the end product of purin deterioration is only one for each animal or for each species, is answered differently by individual writers. By means of chemical manipulations it is possible to dismember the complex nucleic acids into nucleotides, these further either into purins and carbohydrate phosphoric acids or into phosphoric acid and nucleosides. It is also possible to remove the purins from the nucleic acid molecule before it suffers any other alteration in its composition.

It will always remain a very difficult task to ascertain all the exact phases through which a nucleic acid passes in the organism on its way to transformation into uric acid and into still simpler bodies. The methods which can be employed for the solution of this problem are many, and perhaps alone none of them could lead to a decisive answer. It is possible, therefore, that more than one method will have to be employed before all the phases of the nuclein metamorphosis will become known. One of the ways capable of bringing a certain amount of light on this process consists in a comparison of the proportions of uric acid, allan-

toin, or urea eliminated after administration of nucleic acid on one hand, and its more or less complex components on the other hand.

It seems most probable that all organisms possess the capacity of furnishing all the three named end products of purin metabolism, and it is absolutely certain that in definite species of animals the predominating end product is uric acid, and in others allantoin, or still in others urea. The relative proportion of these substances eliminated in the urine is to a degree influenced by the condition of the animal and by the character of the diet. However, the purins compose only one component of the nucleins and even of nucleic acids. The phases through which nucleic acid has to pass in order that its purin may be liberated and further metamorphosed are not known. On the other hand, owing to the progress in the knowledge of the chemistry of nucleic acid, all the possible intermediate stages have become obvious. Through the work of Jacobs and one of us, it has become known that purins enter the molecule of nucleic acid in form of nucleotides. These are composed of phosphoric acid and a purin pentosid or a purin glucosid.

Experiments with feeding nucleic acid and its derivatives of various degrees of complexity have been performed by earlier investigators. Unfortunately they were not carried out systematically, and many of them were made at a time when the methods of analysis were imperfect and the knowledge of the constitution of nucleic acids was even less perfect.

The general impression gained from this work is that after administration of nucleic acid the output of uric acid or of allantoin is greater than after ingestion of its decomposition products. However, it is not possible to accept without further investigation the results of the majority of workers, for the reason that in the analysis of their results they failed to take into account the influence of the ingested nuclein derivatives on the general metabolism. Even the most recent investigators frequently omitted these considerations. And yet it is evident that a definite estimate of the quantitative transformation of any given purin derivative into uric acid, allantoin, or other final decomposition product, cannot be obtained if the administration of that derivative caused the output of nitrogen to rise above the intake. In such experiments it is impossible to determine the part of the increased nitrogen output which may be referred to the administered

substance. Thus it is evident that only the experiments in which the health of the animal was not affected by the administration of nuclein derivative can be taken into consideration. The records of such experiments are very few.

Another very frequent occurrence after the administration of purins is the failure to produce any impression on the total nitrogen output. This may be best illustrated by an experiment of Kruger and Schmidt.[1] It was performed on a man maintained on a purin-free diet; 3.0 gm. of hypoxanthin containing 1.236 gm. of nitrogen, were administered. This raised the average uric acid nitrogen output from 0.1533 gm. to 0.346; or for four days the increase in nitrogen output caused by the high uric acid output amounted to 4 (0.346 − 0.1533) gm. = 0.7708 gm., or 62.3 per cent of the ingested hypoxanthin. On the other hand, the total nitrogen output shows the following values: the average normal nitrogen output was 10.89 gm.; after hypoxanthin feeding, 10.94 gm. The excess in four days amounted to 4 (10.94 − 10.89) gm. = 0.2 gm. It is evident that in a similar experiment it is impossible to establish the origin of the uric acid. There is an abundance of similar records. In reality they should not be considered when an attempt is made to establish the actual process of purin metabolism.

In the present investigation an attempt was made to maintain the animals in nitrogenous equilibrium between experiments. No new experiments were performed before the animal returned to its normal condition. It was noted in course of the experiments that administration of sodium carbonate simultaneously with the nuclein derivatives averted all undesirable influences.

The substances employed in the experiments were allantoin, uric acid, hypoxanthin, inosin, and thymus gland.

The urine was analyzed for the following substances: total nitrogen, uric acid, purin bases, ammonia, amino nitrogen, and allantoin. In order to investigate the possibilities of the intermediate formation of glycocol from purin bases, an attempt was made to ascertain the output of the uric acid after the administration of sodium benzoate before and during the experiment to be described. (See H. Wiener,[2]

[1] Kruger and Schmidt: Zeitschrift für physiologische Chemie, 1902, xxxiv, p. 558.

[2] H. Wiener: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xi, p. 313.

Hirschstein,[3] Wiechowski,[4] Brugsch and Schittenhelm,[5] Abderhalden and Guggenheim.[6]) The most favorable results were obtained on administration of 4 gm. sodium benzoate and 2 gm. sodium bicarbonate. It was noticed that after administration of the substances the nitrogen output increased from 0.2 to 0.3 gm. per day. The daily quantities of urine were obtained by means of catheterization.

METHODS OF ESTIMATION.

Nitrogen . . .	After Kjehldal-Gunning.
Urea	By a modification of the method of Benedict and Gephart.[7]
Uric acid . . .	After Ludwig Salkowski.
Purin Bases . .	Obtained from the filtrate of the uric acid precipitated by means of mercuric sulphid. The nitrogen estimation was made on the precipitate.
Amino Nitrogen	Estimated by the method of Van Slyke.[8]
Ammonia . . .	By the method of Folin.
Allantoin . . .	After the new process of Wiechowski.[9]

Regarding allantoin estimation it should be noted that the estimation was made on the crystallized substance dried at 100° C. The substance was identified by the melting point, which varied between 223° and 225°. Pure allantoin obtained from uric acid was estimated at a point of 225° C. The use of charcoal for purification of the substance was avoided, since such treatment leads to a loss of the substance. Pure allantoin was obtained by repeated precipitation with a mixture of mercuric acetate and sodium acetate and by repeated crystallization. In order to test the method, analyses were made on

[3] HIRSCHSTEIN: Zeitschrift für experimentelle Pathologie und Therapie, 1907, iv, p. 119.

[4] WIECHOWSKI: HOFMEISTER'S Beiträge, 1906, vii, p. 204.

[5] BRUGSCH and SCHITTENHELM: Zeitschrift für experimentelle Pathologie und Therapie, 1907, iv, p. 540.

[6] ABDERHALDEN and GUGGENHEIM: Zeitschrift für physiologische Chemie, 1909, lix, p. 29.

[7] LEVENE and MEYER: Journal of the American Chemical Society, 1909, xxxi, p. 717.

[8] VAN SLYKE: Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, pp. 46–48; Berichte der deutschen chemischen Gesellschaft, 1910, xliii, p. 3170.

[9] WIECHOWSKI: Biochemische Zeitschrift, 1910, xxv, p. 431.

TABLE I.

Date. 1910.	Body-weight. kgm.	Urine.		Feces.		Remarks.
		c.c.	Total N gm.	gm.	N gm.	
June 15	11.040	1000	3.26	....	....	4 gm. sodium benzoate. 2 gm. sodium bicarbonate.
16	11.040	750	3.04			
17	11.060	750	3.64	5.0	0.42	3.8 gm. monosodium urate = 1.0 gm. N. 4.0 gm. sodium benzoate. 2.0 gm. sodium bicarbonate.
18	11.120	750	3.37			
19	11.160	750	2.84	14.75	0.50	.....
20	11.060	750	2.95			
23	11.060	750	2.74			
24	11.060	850	4.05	18.1	0.57	2.841 gm. allantoin = 1.0 gm. N. 4.0 gm. sodium benzoate. 2.0 gm. sodium bicarbonate.
25	11.000	750	2.99			
26	11.000	750	2.97			
28	10.980	750	2.60			
29	11.040	750	3.81			4.0 gm. inosin = 0.83 gm. N. 4.0 gm. sodium benzoate. 2.0 gm. sodium bicarbonate.
30	10.980	750	3.09	19.3	0.74	
July 1	..	....	....			The dog lost its appetite and appeared ill.
Sept. 5	10.110	750	3.08	9.0	0.30	
6	10.140	800	3.33			
7	10.180	800	2.95	8.5	0.36	4.109 gm. yeast nucleic acid = 0.6 gm. N. 4 gm. sodium carbonate.
20	9.880	750	3.34			
21	9.960	800	3.32			
22	9.920	900	2.90	10.0	0.35	92 gm. veal thymus = 1.61 gm. N instead of 14 gm. plasmon = 1.61 gm. N.
23	9.980	800	3.06	15.6	0.60	5 gms. Na_2CO_3
24	9.960	900	3.60			2.514 gm. hypoxanthin = 1.0 gm. N. 5.0 gm. Na_2CO_3.
25	10.020	800	3.04			
26	10.080	1000	2.80			5 gm. Na_2CO_3.
27	10.140	1000	3.59	11.9	0.53	4.784 gm. inosin = 1.0 gm. N. 5 gm. Na_2CO_3.
28	10.120	750	2.95			
29	10.060	825	2.91			

DAILY DIET.

June 15–July 1: Plasmon, 14 gm. = 1.66 gm. N; cracker meal, 100 gm. = 1.59 gm. N; sugar, 20 gm.; lard, 10 gm. Total N, 3.25 gm. Approximate calories, 700.

Sept. 5–Sept. 7: Plasmon, 14 gm. = 1.61 gm. N; cracker meal, 100 gm. = 1.77 gm. N; sugar, 40 gm.; lard, 10 gm. Total N, 2.38 gm. Approximate calories = 800.

Sept. 20–Sept. 29: Plasmon, 14 gm. = 1.61 gm. N; cracker meal, 100 gm. = 1.77 gm. N; sugar, 60 gm.; lard, 15 gm. Total N = 3.38 gm. Approximate calories = 900.

TABLE II.

URINARY NITROGEN PARTITION. NITROGEN IN GRAMS.

Date.	Urea.[1]	Ammonia.	Uric acid.	Purin bases.	Amino N.	Allantoin.	Undetermined.
June.							
15	2.66	0.175	Traces	Traces	0.248	0.058	0.170
16	2.64	0.092	Traces	Traces	0.101	0.088	0.200
17	3.05	0.122	0.029	0.005	0.232	0.152	0.219
18	2.95	0.130			0.111	0.120	0.162
19	2.40	0.132	0.014	0.005	0.085	0.066	0.214
20	2.48	0.144			0.114	0.081	0.203
23	2.28	0.169	Traces	Traces	0.097	0.046	0.186
24	3.45	0.192	0.009	Traces	0.206	0.384	0.190
25	2.53	0.133	0.008	Traces	0.103	0.088	0.213
26	2.52	0.170	Traces	Traces	0.097	0.059	0.175
28	2.16	0.173	0.007	0.003	0.088	0.077	0.169
29	3.09	0.195	0.043	0.017	0.232	0.388	0.233
30	2.39	0.135	0.007	0.003	0.235	0.070	0.320
July.							
1	...	...	...	...	...	...	...
Sept.							
5	2.56	0.220	0.015	0.008	0.106	0.084	0.171
6	2.84	0.224	0.005	0.008	0.102	0.266	0.151
7	2.49	0.201	0.009	0.011	0.101	0.111	0.138
20	2.90	0.199	0.008	0.007	0.090	0.092	0.136
21	2.81	0.252	0.011	0.006	0.101	0.204	0.140
22	2.38	0.260	0.010	0.007	0.099	0.111	0.144
23	2.76	0.098	0.006	0.006	0.092	0.115	0.098
24	3.22	0.094	0.017	0.007	0.100	0.568	0.162
25	2.64	0.185	0.006	0.004	0.096	0.124	0.109
26	2.38	0.126	0.009	0.012	0.110	0.149	0.163
27	2.18	0.090	0.085	0.036	0.107	0.566	0.092
28	2.48	0.182	0.006	0.004	0.097	....	0.181

[1] The values for urea nitrogen include allantoin nitrogen.

human urines to which 0.2 gm. of pure allantoin was added. The added allantoin was recovered nearly quantitatively. The loss seldom exceeded 0.02 gm.

Percentage transformation of the fed purin was calculated on the basis of nitrogen eliminated in the feeding experiments in excess over the nitrogen output in the normal periods.

Results of Experiments.

I. **Allantoin.** — One gram of nitrogen fed in form of allantoin was removed by the dog in the course of twenty-four hours; 31 per cent of it was unchanged, and the rest oxidized to urea.

II. **Sodium urate.** — In the course of two days 60 per cent of the nitrogen introduced in this form was eliminated by the urine, 15 per cent in form of allantoin, 2 per cent in form of the unchanged substance, and the rest in form of urea.

III. **Hypoxanthin.** — After the administration of 1 gm. of nitrogen in form of hypoxanthin, 0.56 gm. were removed, 80 per cent as allantoin, 2 per cent as uric acid, and the remainder as urea.

IV. **Inosin experiments.** — In the course of twenty-four hours 0.83 gm. of nitrogen ingested in form of inosin (4 gm.) were removed; 40 per cent of it in form of allantoin, 4 per cent as uric acid, 2 per cent as purin, 3 per cent as ammonia, 4 per cent as undetermined nitrogen, and the rest as urea. It should be noted that the feeding of solutions of hypoxanthin and inosin in water was frequently followed by disturbances of nitrogenous equilibrium, lasting for a considerable time. These disturbances were avoided when, simultaneously with the hypoxanthin, sodium carbonate was administered. After the administration of hypoxanthin, inosin, and yeast nucleic acid, simultaneously with sodium carbonate, there was always noted a retention of nitrogen. Thus, after the administration of the 1 gm. of nitrogen in form of inosin, 0.6 gm. were removed in course of the first twenty-four hours, 75 per cent in form of allantoin, 13 per cent as uric acid, 5 per cent in form of purin bases, and 8 per cent in form of urea.

V. After the administration of nucleic acid in a quantity containing 0.6 gm. nitrogen, of which 0.4 were in form of purin nitrogen, there reappeared in the urine in the course of the first twenty-four hours 0.3 gm. of nitrogen. Calculating on the basis of the nitrogen dis-

tribution in the nucleic acid, 0.2 gm. of the total excessive output have to be attributed to the purin nitrogen. Of this value 85 per cent were removed in form of allantoin, the remainder in form of urea.

VI. After the administration of thymus containing 0.6 gm. purin nitrogen there were removed in the course of twenty-four hours following the injection 17 per cent in form of allantoin, 5 per cent as uric acid, and the rest as urea. There was no increase in the amino nitrogen output after any one of the experiments.

From the results of these experiments it is apparent that the highest proportion of allantoin output follows the administration of nucleic acid and of hypoxanthin; the proportion is lower after the administration of inosin. Thus it seems possible that the first step in the disintegration of nucleic acid in the organism is the liberation of purins and not of inosin. Experiments of a totally different nature, which will be published later, have made a similar conclusion suggestive.

We realize that further experiments will be necessary before this conclusion can be definitely established.

THE EFFECTS OF VARIOUS FORMS OF EXERCISE ON SYSTOLIC, DIASTOLIC, AND PULSE PRESSURES AND PULSE RATE.

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Introduction.

SINCE the discovery of reliable methods of determining blood pressure in man, numerous attempts have been made to apply these methods to the study of the effects of exercise.[1] Most of these investigations have agreed in finding that there is a rise in blood pressure as well as an increase in heart rate during exercise, but that subsequently the blood pressure may sink to a subnormal level. In the recent paper by Barach, published after the results of the present study were nearly completed, it is stated that a fall below normal of about 20 per cent occurs in cases of violent exercise (Marathon racing) immediately at the end of the exercise, at a time when the pulse rate is still above normal. My results, on the contrary, indicate that even after the most exhaustive exercise a rise of pressure, systolic as well as diastolic, may still be observed at the end of the exercise, although a long lasting fall of pressure ensues shortly afterward. In the present paper advantage has been taken of an excellent opportunity for such work to study the immediate and after effects of various forms of exercise upon systolic and diastolic pressures, pulse pressure, and heart rate.

The records were secured by the use of the Erlanger sphygmomanometer.[2]

[1] McCurdy: This journal, 1901, v, p. 95; Bowen: *Ibid.*, 1904, xi, p. 60; Pembrey and Todd: Journal of physiology, 1908, xxxvii, p. lxvi; Edgecombe and Bain: *Ibid.*, 1899, xxiv, p. 48; Gordon: Edinburgh medical journal, 1907, xxii, p. 53; Krone: Münchener medicinische Wochenschrift, lv, p. 69; Potter and Harrington: Journal of the American Medical Association, 1909, liii, p. 1957; Barach: Archives of internal medicine, 1910, p. 382.

[2] This journal, 1902, vi, Proceedings of the American Physiological Society, p. xxii.

The subjects experimented upon were all healthy men who were in the habit of exercising regularly. With the exception of a few individuals experimented upon in Group I of Part II, the subjects were young athletes in the midst of a training season. Records were taken at all times of the year, varying from the middle of summer (twenty-mile race) to the dead of winter. Several practice runs (thirteen miles, one hundred yards) and one hard race (ten miles) were made in very cold weather with snow on the ground. An average of about one-half minute elapsed between the completion of all exercises considered in Part II and the determination of systolic pressure.

The records in Part I were made during exercise on the stationary bicycle. The normal pressures and heart rate for each individual were obtained by averaging a number of readings taken at different times; none of these readings were made within the twenty-four hours preceding a race, in order to avoid the stimulating effect of the excitement attendant upon such contests.

PART I.

The Systolic, Diastolic, and Pulse Pressures and Pulse Rate during Exercise.

This part of the investigation includes records taken during short fast sprints on a stationary bicycle, longer somewhat slower bicycle riding, and finally during a slow ride upon a bicycle following upon a walk of eighteen miles. In one such case there was an interval of rest of ten minutes between the walk and the ride, in a second case there was a rest of one hour.

Systolic pressure. — In all of the experiments except the last mentioned, there was an immediate and rather great rise in systolic pressure, which reached a maximum in from five minutes or less to twenty-five minutes, an average in nine cases of 14.33 minutes after the commencement of exercise. The rapidity of this rise seems to depend on the physical condition of the subject at the time of the experiment. This fact is shown clearly in the cases of O. S. L. and R. A. K. O. S. L. reached a maximum of systolic pressure in twenty minutes after the commencement of work when in a perfectly fresh condition, but after walking eighteen miles and then resting for ten minutes, allowing the

systolic pressure to return to normal, and then riding the bicycle, a record taken at the end of five minutes showed that the systolic pressure had already reached its maximum. R. A. K. when in a perfectly fresh condition reached a maximum of systolic pressure on one occasion in seventeen minutes, and on another in twenty-five minutes. After walking eighteen miles and then resting for one hour his systolic pressure, which had dropped to normal, had already reached a maximum at the end of eight minutes of work.

Following the maximum rise there usually was a slight fall which seemed to be due to fatigue, and a secondary rise was possible if the efforts of the subject were increased.

The extent of the rise in systolic pressure was greatest in the case of R. A. K., who showed an increase of 65 mm. Hg at the end of seventeen and one-half minutes of riding. The smallest increase noted was in the case of the same subject after a rest of one hour following an eighteen-mile walk. This increase amounted to 10 mm. Hg eight minutes after commencing the exercise. *The average rise of systolic pressure in sixteen observations was 32.7 mm. Hg.*

Diastolic pressure. — The diastolic pressure showed a distinct rise during exercise, although not nearly so much as the systolic. The maximum diastolic pressure was reached rather later than the systolic in some cases, but generally it occurred at the same time. The maximum diastolic pressure was reached at from five to sixty-five minutes after the beginning of exercise, the average of eight experiments being 20.5 minutes.

The rise in diastolic pressure was greatest in the cases of O. S. L., who showed a rise of 40 mm. Hg thirty minutes after commencing work, and C. R. S., who also showed a rise of 40 mm. Hg. The least rise was in the case of R. A. K., who showed no change at all. R. A. K., McL., and J. O. showed a rise of 10 mm. Hg. *The average rise of diastolic pressure in seventeen experiments was 22.9 mm. Hg.*

Pulse pressure. — The pulse pressure in over 50 per cent of the seventeen observations followed rather closely the fluctuations of the systolic pressure. In the remaining experiments there was an initial fall in pulse pressure followed by a subsequent rise to a maximum.

The greatest rise in pulse pressure occurred in the case of R. A. K., who showed an increase of 65 mm. Hg after seventeen and one-half minutes of fast riding. Four experiments showed an increase of only

5 mm. Hg. One of these was R. A. K. after the eighteen-mile walk and one hour's rest. Almost immediately after the cessation of work there resulted a subnormal pulse pressure due to the fact that the systolic pressure dropped more rapidly than the diastolic. *The average rise of pulse pressure in seventeen experiments was* 18.33 *mm. Hg.*

Pulse rate. — Immediately after work commenced there was a sudden increase in the pulse rate, which reached a maximum in from five minutes or less to seventy-five minutes after starting. *The average time before the maximum rate occurred in eight observations was* 35.4 *minutes.*

The greatest increase in pulse rate occurred in the case of McL., a young strong athlete, whose rate increased from 65 to 165, an increase of 95 per minute five minutes after commencing the ride.

The smallest increase occurred in the case of R. A. K., who had a pulse rate of 85 before walking for five hours. After resting for one hour this rate had decreased to 78, and after fifty-three minutes of slow riding it increased to 90 per minute. *The average increase of pulse rate in nine experiments was* 51 *per minute.*

It is to be noted that after the primary increase in pulse rate there was not a great deal of variation, even though the maximum was not reached for some time. Immediately after the cessation of work there was a rapid fall, which was more rapid after a short period of exertion and usually became subnormal. After longer periods of exercise, particularly running, there was a much slower fall to normal but rarely to a subnormal pulse. This interesting difference may be explained perhaps by the fact that the acceleration of the heart rate is referable to two factors: first, a reflex effect through the accelerator nerves, a factor which it may be supposed is responsible for the initial effect of exercise and which would fall away promptly upon the cessation of the exercise; second, a metabolic effect due to the accumulation of acid products in the blood which then react upon the accelerator centre. We may assume that this effect is slow in developing and also slow in disappearing. The long after acceleration noticed in prolonged exercise may be attributed to this factor.

Summary.

The conclusions to be deduced from these observations are: 1. Exercise causes an immediate rise in systolic pressure, but the maximum

attained may occur some time after the exercise is begun. As fatigue advances the systolic pressure falls, but it may be caused to rise again by a greater effort on the part of the subject. Cessation of activity causes a very rapid return to normal and in almost every case to subnormal. A maximum systolic pressure is reached more rapidly in the case of a fatigued individual but is not nearly so extensive.

2. The maximum diastolic rise during exercise is generally reached at the same time as the systolic maximum, although sometimes it occurs later. The average rise in systolic pressure exceeds the average rise in diastolic by about 10 mm. Hg. The diastolic pressure fluctuates very little as a rule after its maximum has been reached. It returns to normal rather more slowly than the systolic and invariably shows a fall to subnormal after exercise.

3. The pulse pressure curve generally follows the contour of the systolic curve, due to the fact that systolic pressure fluctuates more than diastolic pressure.

4. The pulse rate increases rapidly at first, but usually does not reach a maximum for some time. After the initial rise it does not change greatly, although it is evidently influenced by the fatigue of, and the effort expended by, the subject.

If a considerable time elapses between the completion of prolonged fatiguing exercise and renewed effort, the new exercise causes but little acceleration of the heart, while, if there is no interval or only a slight interval of rest, there is a perfectly definite effect produced on the heart (acceleration) and blood pressure (rise).

PART II.

After Effects of Various Forms of Exercise on Systolic, Diastolic, and Pulse Pressures and Pulse Rate.

The subject here considered may be conveniently arranged under five heads as follows:

I. Moderate exercise for a considerable time.
II. Rapid exercise for a short time.
III. Vigorous exercise.
IV. Fatiguing exercise.
V. Exhaustive exercise.

I. Moderate Exercise for a Considerable Time.

The six men upon whom the records for this experiment were made varied in age from nineteen to fifty-nine years, and the types of exercise used were swimming and playing in the water, tennis, baseball, shot putting, jumping, hammer and discus throwing. All of the exercises were performed for the purpose of recreation, no match games or attempts to make records entering into consideration. The physical effort thus made was continued from about half an hour to two hours.

Systolic pressure. — Immediately after such exercise there was a slight rise in systolic pressure, rarely over 10 mm. Hg, and only in one case as much as 15 mm. Hg. Following this there was invariably a drop to subnormal, the extent of which in a large number of cases was 20 mm. Hg and in one case 30 mm. Hg.

Diastolic pressure showed a more marked rise in three out of six experiments than did systolic. In all three cases the systolic pressure recorded immediately after exercise showed a rise of 10 mm. Hg, while the diastolic showed a rise of 20 mm. Hg. In one of these experiments the subject was a man of forty-nine years, and in another a lad of seventeen.

Two of the other three cases showed a diastolic rise of 10 mm. Hg, and the third showed no rise in diastolic pressure, although a systolic rise of 15 mm. Hg was present after two hours of shot putting, discus throwing, and broad jumping.

Accompanying the fall in systolic to subnormal there occurred a fall in diastolic pressure varying from 5 to 20 mm. Hg. The fall in diastolic pressure was slower than systolic. One case in which after two and one-half hours of moderate exercise, systolic pressure had dropped to normal still showed a diastolic pressure of 5 mm. Hg above normal for fifteen minutes. In another case a normal diastolic pressure was reached twenty minutes after a swim of three quarters of an hour, at which time the systolic pressure was 20 mm. Hg below normal.

Pulse pressure varied greatly after this type of exercise. Three of the cases showed a drop in pulse pressure from 10 to 15 mm. Hg below normal, two others gave no change, and another a rise of 15 mm. Hg. During the subnormal period there was invariably a fall in pulse pressure ranging from 5 to 20 mm. Hg, due to the fact that systolic pressure fell more rapidly than diastolic.

Pulse rate increased from 16 to 36 per minute above normal immediately after exercise, and during the period of subnormal pressures was still above the normal rate in all, except one case, in which the rate had dropped to normal five minutes after playing in the water for three quarters of an hour.

Two young athletes in good condition worked for thirty-five and forty minutes respectively in the gymnasium. They tossed a medicine ball for about fifteen minutes and then did combination work on the side horse and parallels very much as a regular gymnasium class would do.

Systolic pressure in both cases showed a rise of 10 and 25 mm. Hg respectively. Diastolic pressure at the end of exercise in the former case (A) was 20 mm. Hg above normal, and in the latter (B) 15 mm. Hg. Pulse pressure fell 10 mm. Hg in one case (A) and rose 10 mm. Hg in the other (B). Pulse rate increased 15 in A and 35 in B.

The subnormal stage was not particularly marked in A, but was more noticeable in B, reaching a systolic subnormal of 10 mm. Hg thirty-five minutes after exercise. Diastolic pressure did not become subnormal in either case. Pulse pressure was 10 mm. Hg subnormal in A at the end of exercise; it returned almost immediately to normal and again dropped 10 below after walking ten squares slowly. This walk had no effect on systolic pressure in either case. In B the minimum of pulse pressure (10 mm. Hg below normal) came thirty-five minutes after the completion of exercise. Pulse rate became subnormal in A one hour and twenty-two minutes after exercise and was not marked by a subnormal stage in B.

Return to normal. — Systolic pressure returned to normal in A forty-eight minutes after exercise, and in B one hour and twenty-seven minutes after. Diastolic returned to normal at eight minutes in one case and thirty-five minutes in the other. Pulse pressure was at normal in forty-eight minutes in A and only 5 below normal in fifty-five minutes in B. Pulse rate returned to normal in one hour and forty-two minutes in A and in forty-five minutes in B.

Four young athletes ran three miles to a swimming pool, swam and played in the water twenty minutes, rested, then walked two miles and finally ran the last mile to the club house. One of the four had a supranormal systolic and also diastolic pressure of 10 mm. Hg immediately after finishing and a pulse rate of 124 per minute. Forty

minutes later his systolic pressure was 5 below normal and his diastolic pressure was exactly normal, and the pulse rate had fallen to 96 per minute. Two of the others had a subnormal systolic pressure of 35 mm. Hg and 5 mm. Hg respectively, and the diastolic pressure of the former registered 5 below normal and the latter 5 above. The pulse pressure in the former dropped from 35 to 5,[3] and the pulse rate changed from 56 at normal to 84 after the test. The pulse pressure in the case of the latter dropped from 25 to 15, and the pulse rate rose from 80 to 120. The fourth member of this group, whose record was taken five minutes after finishing, showed a systolic pressure 20 mm. Hg below normal, a diastolic pressure 5 mm. Hg below normal, a pulse pressure 15 mm. Hg below normal, and his pulse rate had dropped from a normal of 84 to 76.

II. Rapid Exercise for a Short Time.

The three young men performing these experiments ran 100 yards as fast as they could, and the records, taken as quickly as possible after the exercise, showed an average rise in systolic pressure of 45 mm. Hg and in diastolic pressure of 17 mm. Hg. (One rose 5 mm. Hg, another 20, and the third 25.) The pulse pressure rose on an average of 28.3 mm. Hg, and the average pulse rate increased 45 per minute.

The subnormal phase reached its lowest ebb in ten minutes in one case, thirty in another, and fifty in the third. The amount of depression of systolic pressure in the first two cases amounted to 15 mm. Hg below normal, while in the third case the fall was 25 mm. Hg below normal. The diastolic pressure in case I fell to 15 mm. Hg subnormal twenty-seven minutes after exercise. In case II 15 mm. Hg subnormal was recorded forty-five minutes after exercise, and case III showed 10 mm. Hg subnormal thirty-eight minutes after. Pulse pressure in case I amounted to 5 mm.[4] Hg twelve minutes after the race. In case II twenty minutes after the completion of exercise the pulse pressure was 10 mm. Hg below normal. Case III showed a pulse pressure of only 5 mm.[4] Hg sixty-five minutes after exercise, which

[3] A pulse pressure of 5 mm. Hg is very low. Probably the circulatory conditions changed during the observations.

[4] There is a possibility of pressure conditions having changed during these observations, but there is no doubt that pulse-pressure was extremely low.

was 25 below normal. The pulse rate did not become subnormal in any of these experiments.

The return to normal systolic pressure occurred in case I in one hour and ten minutes. Cases II and III were 10 mm. Hg below normal one hour and ten minutes after exercise. Diastolic pressure in case I was 10 mm. Hg below normal one hour and ten minutes after exercise, and 10 mm. Hg below normal in case II one hour and twenty-five minutes after exercise. Normal was reached in case III one hour and five minutes after exercise. Pulse pressure fluctuated in cases I and II, but in III was subnormal for over one hour and thirty-five minutes after exercise. Pulse rate returned to 10 above normal one hour and ten minutes after exercise in case I, and in cases II and III returned to normal in one hour and one hour and twenty minutes respectively.

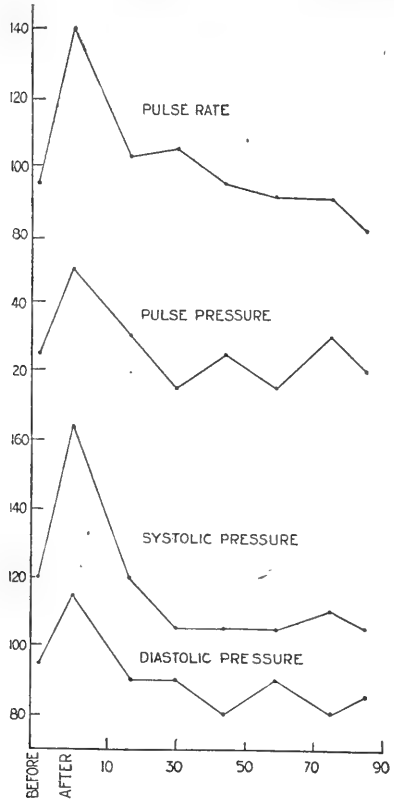

FIGURE 1 (G 100 yds).— Shows the effect of a short hard run upon the blood pressures and heart rate. Ordinates = mm. Hg pressure and rate per minute. Abscissa = time in minutes.

Records taken on sixteen other men and averaged with those just considered yield the results given in the table.

III. VIGOROUS EXERCISE.

The types of muscular activity classed in this group include a hard wrestling bout lasting eight minutes and runs varying from one and one-half miles to five miles.

The systolic pressure of both of the wrestlers rose 40 mm. Hg after exercise (No. 1 ran until the record of No. 2 was completed). Diastolic pressure rose 25 mm. Hg above normal in the case of No. 1 and 15 mm. Hg in that of No. 2. Pulse pressure accordingly rose 15 mm. Hg in the first case and 25 mm. Hg in the second case. Pulse rate increased 65 per minute in one case and 80 in the other.

The subnormal phase was marked in No. 1 by a systolic drop to 15 mm. Hg below normal thirty-six minutes after exercise. This was followed by a rise to normal twenty minutes later, and there was then a drop to about 20 mm. Hg. below normal which lasted for nearly an hour. The chart of No. 2 demonstrated a similar fall in systolic pressure of from 15 to 20 mm. Hg lasting from one hour and ten minutes after exercise to one hour and sixty-five minutes. Diastolic pressure also showed a fall which came on a little more slowly and was less extensive. In No. 1 it dropped to 15 mm. Hg below normal at one hour and twenty-eight minutes and again at two hours and twenty-eight minutes after exercise. In No. 2 its lowest drop was to 10 mm. Hg. below normal after one hour and forty-three minutes. The pulse pressure was very irregular in No. 1, but in No. 2 it remained considerably below normal for two hours and twenty minutes. The pulse rate did not show any considerable subnormal stage.

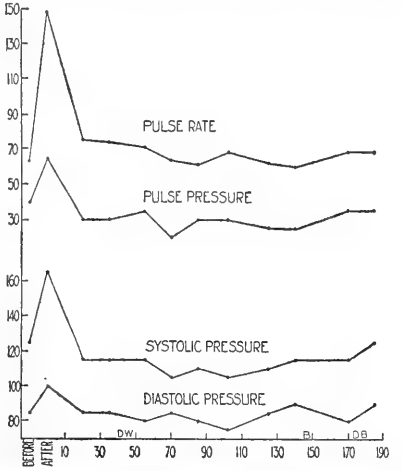

FIGURE 2. — Shows the effect of vigorous exercise sustained for several minutes (wrestling 8 minutes) upon the blood pressures. *D W*, drank four glasses of water. *B*, bath. *D B*, drank one bottle of beer. Ordinates = mm. Hg pressure and rate per minute. Abscissa = time in minutes.

The return to normal. — Systolic pressure returned to normal in about two hours and forty minutes in one case and three hours and five minutes in the other. Diastolic pressure became normal in No. 1 one hour and twelve minutes after exercise, but this was followed by an extensive subnormal stage which had disappeared two hours and forty-three minutes later. In No. 2 the diastolic pressure was irregular, but tended to remain below the normal for two hours.

Records of sixteen men who ran from 1.5 miles to 4 miles showed an average systolic rise of 35 mm. Hg immediately after exercise. The smallest rise was 10 mm. Hg and the greatest was 75 mm. Hg. The magnitude of the rise appeared to be associated directly with the effort of the final sprint. The average diastolic rise was 25 mm. Hg

and ranged from 5 mm. Hg to 40 mm. Hg. Pulse pressure in ten cases increased on an average of 17.5 mm. Hg and in six cases decreased an average of 10.8 mm. Hg. Pulse rate varied from no increase to an increase of 70 per minute and averaged for the sixteen cases an increase of 39 per minute. The case that showed no increase at the end of a three-mile run was recorded thirty minutes later and at that time an increase of 36 per minute was noted.

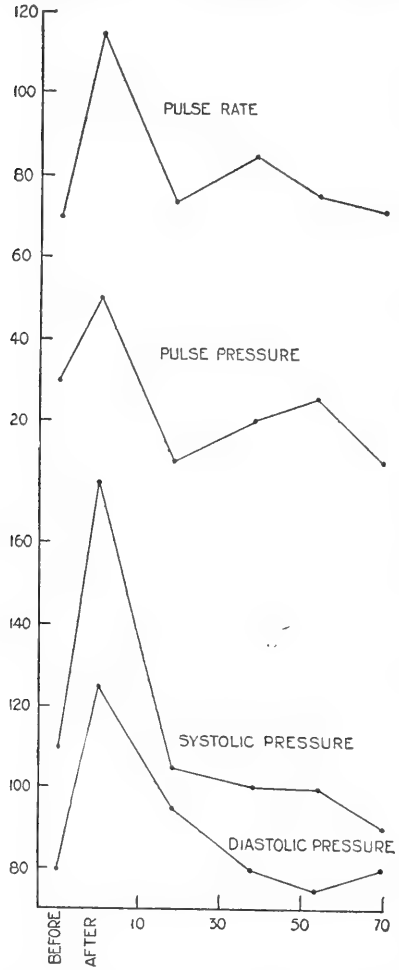

FIGURE 3.— Shows the effect of fatiguing exercise (6-mile run) upon the blood pressure and heart rate. Ordinates = mm. Hg pressure and rate per minute. Abscissa = time in minutes.

Subnormal. — Systolic pressure always showed a subnormal stage after this form of exercise, and in thirteen of the sixteen subjects the subnormal phase had developed between thirty and forty minutes after work. The average fall in these cases at this time was noted to be 18 mm. Hg, the greatest being 30 mm. Hg and the least 5 mm. Hg. From a study of six of these cases, it is noted that the minimum systolic pressure occurred in runs of this distance from thirty-five minutes to one hour and ten minutes after the completion of the exercise, averaging in the six cases 62.5 minutes. Subnormal diastolic pressure was noted in ten out of sixteen cases, but its extent was noteworthy only in two cases in which the fall was 15 mm. Hg below normal. The average for the ten cases was 8.5. mm. Hg. Pulse pressure showed a subnormal stage in fifteen cases, varying from 5 to 30 mm. Hg and averaging 15 mm. Hg. Three cases did not show a subnormal pulse

pressure during the time for which records were taken, but if the time had been extended they probably would have done so, as this period extends from ten minutes to one hour and fifteen minutes, although in most cases it seems to come between twenty and forty minutes after work. Pulse rate did not become subnormal to any noteworthy extent.

Return to normal. — Systolic pressure seemed to return to normal in about one hour and forty minutes in a well-trained man, but in persons out of condition it took much longer. Diastolic pressure was not so greatly disturbed, and although its decline was slower it returned to normal more quickly than systolic. Pulse pressure followed rather closely systolic pressure in this sort of exercise, and consequently returned to normal at about the same time. Pulse rate in well-trained men reached normal in a little over one hour, but in untrained men remained rather high for considerably over an hour. The above results are collected in the table.

IV. Fatiguing Exercise.

The eight young men experimented upon in this test ran from five to nine miles and were all training for a Marathon race of twenty miles.

Systolic pressure. — The rise in systolic pressure noted in the man who ran five miles was 25 mm. Hg; the three who ran six miles showed a rise varying from 30 to 65 mm. Hg, with an average of 43. In the seven-mile run a rise of 25 mm. Hg was noted, and the three men who ran eight miles varied from 15 to 35 mm. Hg above normal, averaging 27 mm. Hg. The average increase for all eight runners was 32.5 mm. Hg.

Diastolic pressure. — Diastolic pressure showed the following rises:

5 miles		15 mm. Hg	
6 miles	1.	10	
	2.	35	average, 30 mm. Hg
	3.	45	
7 miles		25 mm. Hg	
8 miles	1.	20	
	2.	20	average, 15 mm. Hg
	3.	5	

Average rise for 8 runners, 20.6 mm. Hg

No pulse pressure rise was noted in two of the cases, the others varied from an increase of 5 (six-mile run) to 20 (six-mile run). The average increase for the runners was 9 mm. Hg. The increase in pulse rate varied from 20 to 62 per minute, with an average increase of 44.6.

The subnormal systolic pressure varied from 5 to 25 mm. Hg below normal, with an average of about 20 mm. Hg. The time at which this stage was most pronounced occurred between thirty-five minutes and one hour and ten minutes after running, although systolic pressure was always subnormal within ten minutes after the end of a long run. Diastolic pressure dropped much more slowly than systolic and did not show so marked a subnormal phase. Pulse pressure showed an average drop of 19 mm. Hg below normal, and this might last from fifteen minutes to one hour and ten minutes after a run of this distance. Pulse rate did not become subnormal.

Return to normal. — Systolic pressure might return to normal in about an hour, but in case the sprint at the end of the race was hard one it took considerably longer. Diastolic pressure seemed to reach a normal condition in about thirty-five minutes. Pulse pressure was observed to follow in some cases the fluctuations of systolic pressure.

The above results are collected in the table.

V. Exhaustive Exercise.

This test was made on the same group of men (members of the Cross Country Club of Baltimore) who furnished records for the previous tests on runners. Records were made after a ten-mile race and two twenty-mile races and after several practice runs varying from ten to thirteen miles.

In the ten-mile handicap race the average rise in systolic pressure was 40.8 mm. Hg in six runners. The greatest rise, 60 mm. Hg, was noted in the case of the winner, and the least rise occurred in the case of the scratch man, who made the best time; it amounted to 25 mm. Hg. However, it is noteworthy that this man rarely had a rise of more than 25 mm. Hg in any run, the single exception noted being a rise of 30 mm. Hg after a hundred-yard dash, which is below the average for the cases examined, *i. e.*, 45 mm. Hg. The diastolic rise averaged 22.5 mm. Hg, varying from 15 to 30. Pulse pressure rose on an average about 18.3 mm. Hg, the extremes varying from 5 to 40.

Pulse rate increased an average of 66.3 per minute, the greatest increase being 80, a figure more than double that usually observed. In fact, nearly all of the pulse rates were about doubled at the end of the race.

The subnormal phase. — Systolic pressure was observed to reach its lowest point in from forty-two minutes to four hours and seven minutes. It is to be noted that even after dinner, which always sends systolic pressure up, as shown by Erlanger and Hooker, the pressure returned to 20 mm. Hg below normal after the immediate effects of the dinner had worn off. The average fall of systolic pressure in eight tests was 14.5 mm. Hg, varying from 0 to 25 mm. Hg. Diastolic pressure averaged a fall of 9 mm. Hg below normal, and came on much more slowly than in the case of the systolic pressure. Pulse pressure had a corresponding fall, averaging 13.3 mm. Hg, which was in most cases coincident with the systolic fall. Pulse rate did not fall below normal.

Return to normal. — Systolic pressure in the two cases least affected by other influences than the race had not returned to normal in one case until after dinner (four hours and thirty-five minutes), and in the other at one hour and thirty minutes after the race, but in this case it dropped again, and reached normal the second time in five hours and five minutes. Diastolic pressure reached normal in these experiments rather rapidly. Pulse pressures followed the fluctuations of the systolic pressure very closely. Pulse rate did not show a tendency to return to normal until a long time after the completion of the exercise (four hours in one case, six hours in another, and after the sixth hour in a third.

Thirteen-mile practice run. — This run, which was a hard one, was similar in its effects to the ten-mile race already discussed.

The rise in systolic pressure in four cases averaged 36.25 mm. Hg. Diastolic pressure showed an average rise of 15 mm. Hg. Pulse pressure rose an average of 20 mm. Hg. Pulse rate decreased in one case and in the other three showed an average increase of 29 per minute.

The subnormal phase was quite pronounced. Systolic pressure fell on the average 22 mm. Hg below normal in five cases. Diastolic pressure fell on the average to 17 mm. Hg below normal. Pulse pressure did not fall below normal in one case, but in the other four gave

an average fall below normal of 15 mm. Hg. Pulse rate showed a secondary rise in four of the curves after about twenty minutes.

Return to normal. — In the case whose record is given in Fig. 4, the systolic pressure showed a tendency to remain below normal for three and one-half hours until caused to rise by the effect of smoking, bearing out the experiments of Bruce, Miller, and Hooker,[5] who found that smoking caused vaso-constriction and rise of blood pressure.

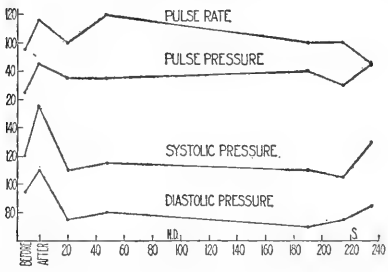

FIGURE 4.— Shows the effect of exhaustive exercise (13-mile run) upon the blood pressures and heart rate. *ND*, no dinner. *S*, smoking. Ordinates = mm. Hg pressure and rate per minute. Abscissa = time in minutes.

The diastolic pressure in this case had a general tendency downward for three hours and ten minutes, and was brought up to 10 mm. Hg below normal by smoking. Pulse pressure returned to normal or nearly to normal in three hours and thirty-five minutes. Pulse rate returned to normal more slowly than in the other experiments recorded.

Twenty-mile races. — The contestants in two races of this length were examined. It was impossible to make observations immediately after the first race, hence only the subnormal phase was studied. When the runners were brought to the examination room, they were practically in a fainting condition and one or two of them were delirious. The pulse pressures in most cases were so low that the instrument would not record the fluctuations and it was impossible to get the diastolic pressure.

Records taken from four to twenty minutes (average twelve minutes) after the completion of the race on twelve runners showed a variation in systolic pressure of from 10 mm. Hg to 30 mm. Hg below normal, averaging 24 mm. Hg for the twelve men. In 50 per cent of the cases it was impossible to record the diastolic pressure on account of the greatly diminished pulse pressure. The six diastolic pressures obtained at this time varied from 10 mm. Hg subnormal to 10 mm. Hg above normal. Two of the cases showed a normal pressure, and two

[5] BRUCE, MILLER, and HOOKER: This journal, xxiv, p. 104.

5 mm. Hg above normal. Pulse pressure in the six cases recorded varied from 15 to 35 mm. Hg below normal, averaging 27.5 mm. Hg. The average pulse pressure was 14 mm. Hg.

One and one-half hours after the completion of the race the average systolic pressure was 20.5 mm. Hg below normal. Diastolic pressure averaged 8.4 mm. Hg below normal, one case only being 5 mm. Hg

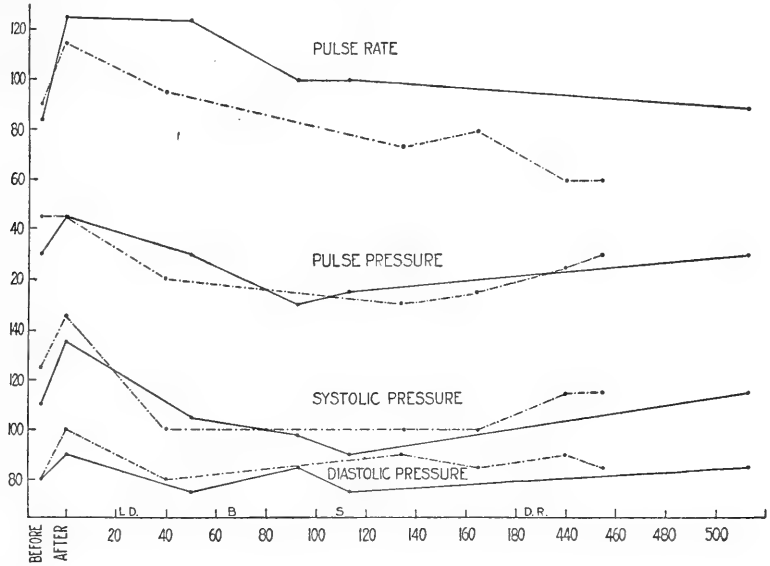

FIGURE 5.—Shows the effect of completely exhaustive exercise (20-mile run) on the blood pressure and heart rate. Note the prolonged subnormal stage exhibited in all the curves. *D*, broken lines. *G*, unbroken lines. *LD*, lying down. *B*, bath. *S*, sitting. *DR*, dinner and recreation. Ordinates = mm. Hg pressure and rate per minute. Abscissa = time in minutes.

above. Pulse pressure varied from 5 above normal in one case to 30 below, and averaged 13 mm. Hg below normal. The average pulse pressure at this time was 20.5 mm. Hg.

Four of these runners were examined eight hours after the completion of the race. Two of them recorded normal systolic pressures, and the other two recorded subnormal systolic pressures of 25 and 30 mm. Hg respectively. One of the runners who had a normal systolic pressure recorded a diastolic pressure 10 mm. Hg above normal. Pulse pressure was subnormal in three of the cases, 10, 25, and 25 mm. Hg respectively, while the fourth case was 5 mm. Hg above normal.

On account of the fact that it was impossible to learn exactly what these four men had been doing in the time between the race and the last examination, it was not deemed proper to average this record with the others, the figures of which are collected in the table.

Conditions were proper for taking observations at the finish of the second twenty-mile race, and the contestants ran to the examination chair, so that less than thirty seconds elapsed between the cessation of muscular activity and the determination of systolic pressure.

All six of the men examined had systolic pressures varying from 15 to 25 mm. Hg above normal, and averaging 17 mm. Hg. Diastolic pressure showed rises of 10 mm. Hg above normal in all cases. Pulse pressure showed an average rise of 8.33 mm. Hg. Pulse rate showed an average increase of 29.5 in the six cases.

Two hours after the completion of the race systolic pressure showed an average fall of 24 mm. Hg below normal. Diastolic pressure had become practically normal. Pulse pressure averaged 26 mm. Hg below normal. Pulse rate averaged 18 per minute above normal except one case which was 18 per minute below normal.

Tests made between seven and eight hours after the race showed two of the contestants to be still in the subnormal state (see *D*, Fig. 5), while a third individual had returned to normal.

Relation of exercise to albuminuria. — Albuminuria was first observed to follow athletic exercise by Dunhill and Patterson in 1902,[6] and has since been commonly noted. The present research gave an opportunity to test further the hypothesis advanced from this laboratory[7] that albuminuria in otherwise healthy individuals is dependent upon a relative decrease in the magnitude of the pulse pressure. Accordingly the urine was examined in seven of the cases here studied. When the period of subnormal pressure came on accompanied by low pulse pressure, protein was found in the urine even after the short runs, and the more extensive the period of low pulse pressure the greater seemed to be the amount of protein present.

For convenience in comparing the results obtained for the different forms of exercise, the following general table has been prepared summarizing the figures previously given:

[6] Dunhill and Patterson: Intercolonial medical journal of Australasia, 1902, vii, 334.

[7] Erlanger and Hooker: Johns Hopkins Hospital reports, 1904, xii, pp. 145–378.

TABLE SHOWING THE AVERAGE CHANGES IN SYSTOLIC, DIASTOLIC, AND PULSE PRESSURES AND PULSE RATE FOR ALL THE CASES UNDER OBSERVATION AFTER MODERATE, RAPID, VIGOROUS, FATIGUING, AND EXHAUSTIVE EXERCISE. THE FIGURES IN ITALIC TYPE IN PARENTHESES REFER TO THE NUMBER OF INDIVIDUALS OBSERVED IN EACH EXPERIMENT. "RETURN TO NORMAL" IS ESTIMATED FROM THE END OF EXERCISE.

Measurement.	Rise immediately after exercise.					Subsequent subnormal stage.					Minutes required for return to normal.				
	Moderate exercise.	Rapid exercise.	Vigorous exercise.	Fatiguing exercise.	Exhaustive exercise.	Moderate exercise.	Rapid exercise.	Vigorous exercise.	Fatiguing exercise.	Exhaustive exercise.	Moderate exercise.	Rapid exercise.	Vigorous exercise.	Fatiguing exercise.	Exhaustive exercise.
Systolic blood pressure in mm. Hg	11 (*10*)	36 (*19*)	33 (*23*)	32.5 (*8*)	39 (*10*)	—17 (*10*)	—16 (*4*)	—18 (*18*)	—20 (*8*)	—19 (*25*)	31 (*2*)	82 (*3*)	124 (*4*)	70+ (*2*)	232 (*8*)
Diastolic blood pressure in mm. Hg	11 (*10*)	20.3 (*19*)	21.3 (*23*)	20.6 (*8*)	39 (*10*)	—6.5 (*10*)	—9 (*4*)	—9.2 (*18*)	—7 (*7*)	—10.3 (*25*)	21 (*2*)	65 (*3*)	34 (*4*)	35 (*1*)	160 (*9*)
Pulse pressure in mm. Hg	9 (*4*)	16.3 (*19*)	17 (*18*)	9 (*8*)	18 (*10*)	—11.5 (*10*)	—18 (*4*)	—16 (*17*)	—19 (*8*)	—13.4 (*24*)	31 (*2*)	50 (*3*)	88 (*3*)	70 (*8*)	152 (*8*)
	10[1] (*3*)	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Pulse rate per minute	26 (*8*)	33.5 (*19*)	44 (*22*)	44.6 (*8*)	54 (*9*)	—9 (*2*)	—7 (*2*)	+ (*18*)	+ (*8*)	+ (*24*)	31 (*2*)	60 (*3*)	65 (*6*)	70 (*2*)	213 (*7*)
	...	...	...	...	...	+20 (*4*)	+ (*2*)	...	...	...	...	...	...	...	...
	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[1] Fall after exercise.

Summary of Results.

A. **Rise above normal after exercise.** — 1. Systolic pressure rises more in rapid and exhaustive than in other forms of exercise. The extent of this seems to depend partly on individual characteristics and partly on the amount of energy put into the sprint at the end of exercise.

2. Diastolic pressure rises about the same for all types of exercise except the moderate, in which it shows about one-half the rise noted in other types of exercise.

3. The pulse pressure rise is greatest after rapid and exhaustive exercises.

4. Pulse rate increases in all exercises, and the more vigorous the exercise the greater the increase.

B. **Fall below normal due to the effects of exercise.** — 1. Systolic pressure falls about equally below normal for all kinds of exercise, although in the more exhaustive types there is a slightly greater fall.

2. The diastolic pressure shows a similar fall below normal following all types of exercise.

3. Pulse pressure falls below normal in all cases almost equally.

4. Pulse rate fell slightly below normal in only four cases out of sixty.

C. **Return to normal.** — 1. Systolic pressure returns to normal after the subnormal phase more slowly the more exhaustive the exercise.

2. Diastolic pressure returns to normal later in the cases of rapid and exhaustive exercise than in the others.

3. Pulse pressure returns to normal more slowly the more exhaustive the exercise.

4. The pulse rate returns to normal more slowly the more exhaustive the nature of the exercise.

Conclusions.

The conclusions to be drawn from these experiments are as follows:

1. All types of prolonged exercise which cause an increase in pulse rate cause also a rise in systolic and diastolic pressures. The systolic pressure shows the greater rise; hence there is an increase in pulse

pressure, which may be interpreted to mean that the heart beats are augmented as well as accelerated.

2. After all types of exercise here studied the systolic, diastolic, and pulse pressures invariably fall below normal and remain in this subnormal condition for a considerable time. The more exhaustive the nature of the exercise the longer will be the subnormal period which follows. Systolic pressure invariably falls more rapidly than diastolic, and hence the pulse pressure becomes weaker. The presence of albumin in the urine coincident with low pulse pressure was observed in accordance with the results reported by Erlanger and Hooker.[8] It seems to add another factor to the possible injurious results of long-continued exhaustive exercise.

3. Pulse rate, which always increases during exercise, decreases rapidly after its completion. This drop in the curve of the pulse rate is frequently followed by a secondary rise which is possibly a reflex effect due to the low blood pressure of the subnormal stage. In no case was it observed that this secondary rise was accompanied by a rise in blood pressure.

4. Rapid exercises (vigorous, fatiguing, and exhausting) are followed by a fall of pressure below normal which lasts longer than after moderate exercise, even if the former is continued for a very short period and the latter for quite a long period of time. If we consider the subnormal phase as indicative of an overstrain following upon the great reflex excitation of the heart and vaso-motor centre, then it would seem that after these so-called rapid exercises the strain is more serious, as is shown by the much longer time required before the conditions return to their normal level.

5. If our interpretation of the subnormal phase is correct, it would follow that the so-called field events, consisting of jumping, shot putting, discus and hammer throwing and baseball, gymnasium apparatus work, and exercises of a similar nature, are preferable to rapid exercises such as basket ball, football, and running races. This is particularly true in the case of the rapidly growing youth, whose heart is under the additional demand of keeping pace with an increase in the tissue mass of the body.

6. There is less strain put upon the circulatory system by walking a number of miles at a moderate rate than by sprinting 100 yards at

[8] ERLANGER and HOOKER: *Loc. cit.*

top speed. This conclusion follows from the fact that blood pressure returns to normal after moderate exercise in about thirty minutes, while after short sprints the subnormal stage continues about three times as long.

7. Long-distance running races and similar forms of exhaustive exercise give rise to a serious strain on the heart, as is indicated by the long period of subnormal blood pressure.

8. It would seem probable that in individual cases the beneficial or injurious effect of any given form or amount of exercise might be determined by observations upon the subnormal phase following the exercise. When the subnormal phase returns to normal within sixty minutes, the exercise may be considered as lying well within hygienic limits for that individual, while a return that is delayed beyond one hundred and twenty minutes may be regarded as exceeding these limits.

This investigation was suggested by Dr. T. A. Storey and directed by Dr. W. H. Howell, to whom the author is very greatly indebted. Great appreciation is expressed to R. A. Kocher for his valuable assistance in the experiments concerned with Part I, and to Dr. P. M. Dawson, who directed that part of the work. The members of the Maryland Swimming Club, the Baltimore Athletic Club, and the Baltimore Cross Country Club were the subjects of these experiments, and particular gratitude is felt towards the members of the last-mentioned organization, who were most generous in submitting to all the demands made upon them by the author.

ON THE QUESTION WHETHER DEXTROSE ARISES FROM CELLULOSE IN DIGESTION.

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A PUBLICATION by Hoffmann,[1] which shows that although the hemicellulose of agar agar and cellulose of cabbage is digested and absorbed by the rabbit, it does not increase the sugar in the urine if the rabbit be phlorhizinized, recalled to the writer two experiments briefly reported by him [2] at a meeting of the American Physiological Society in 1901 and never fully published.

The great sugar elimination which followed the ingestion of fat and cauliflower in the phlorhizinized dogs of Hartogh and Schumm,[3] suggested that the sugar might in part have been derived from the cauliflower ingested. Twenty grams of cauliflower were therefore cooked and given to a fasting dog phlorhizinized according to the author's method.[4] The results of the urinary analyses were as follows:

1901.	Condition.	Dextrose.	Nitrogen.	D. N.
Nov. 8 . . .	Fasting	33.34	9.69	3.34
Nov. 9 . . .	20 g. cauliflower	29.18	8.82	3.30
Nov. 10 . . .	Fasting	28.20	8.30	3.40

It is evident from this, that the cauliflower did not increase the sugar output as it would have done had dextrose arisen from it in the intestine.

Another experiment was performed upon a phlorhizinized goat weighing 34.2 kgm. The cellulose ingested consisted of 4 gm. of

[1] Hoffmann: Inaugural-Dissertation, Halle-Wittenberg, 1910.

[2] Lusk: This journal, 1902, vi, p. xiii.

[3] Hartogh and Schumm: Archiv für experimentelle Pathologie, 1900, xlv, p. 2.

[4] Stiles and Lusk: This journal, 1903, x, p. 67, and other papers.

filter paper soaked in sodium chloride, given at the beginning of the twenty-four-hour period; six hours later 6 gm. of thick brown wrapping paper were given. This was all taken voluntarily. Two grams of phlorhizin were administered to the goat subcutaneously three times daily, beginning the day before the experiment and continuing throughout. The urinary analyses showed the following results:

1901.	Condition.	Dextrose.	Nitrogen.	D. N.
Dec. 17	Fasting	16.56	7.27	2.28
Dec. 18 . . .	10 g. paper	11.62	5.04	2.30

Fragments of cellulose fibres were found in the stools indicating their distribution throughout the intestinal tract.

Tappeiner[5] was the first to point out that fatty acids arose from cellulose in the intestinal canal of herbivora, and it is probable that the nutritive value of cellulose has its origin in these fatty acids.[6]

These isolated experiments are now published because they support the careful work of Hoffmann in showing that sugar does not arise from the digestion of cellulose.

[5] TAPPEINER: Zeitschrift für Biologie, 1888, xxiv, p. 105.

[6] Consult the criticisms of LOHRISCH: Zentralblatt für die gesamte Physiologie und Pathologie des Stoffwechsels, 1907, N. F. ii, p. 301.

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