THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME XLV

BALTIMORE, MD.
1918

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 45 DECEMBER 1, 1917 No. 1

GASTRIC RESPONSE TO FOODS

I. The Determination and Significance of Intragastric Conductance[1]

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Received for publication September 29, 1917

In connection with the series of studies being carried out in this laboratory on the response of the human stomach to various stimuli, it appeared that the determination of the electrolytic conductance of the gastric contents would be of interest in supplementing or, possibly, in part replacing other methods of study. A retention stomach tube carrying an electrolytic cell was therefore devised and an attempt made to determine the significance of variations in intragastric conductance as determined with the aid of this instrument.

The specific conductance of any solution depends only upon the number of ions present and the mobility of those ions being thus more specific than the cryoscopic index which is influenced also by the molecules of undissociated substances. Strong acids and bases or the salts of strong acids and bases are very completely dissociated in solution in the concentrations commonly met with in physiological fluids. Weak acids and bases and their salts are but slightly dissociated and neutral organic substances not salts do not ionize.

It follows that the conductance of most biological fluids is dependent upon their content of salts of strong acids and bases, especially sodium

[1] This is the first of a series of researches made possible by a grant from Mrs. M. H. Henderson.

and potassium chlorides. This is particularly true because of the relative abundance of chlorides of sodium and potassium in body fluids. Thus the blood and bile of most mammals are high in chlorides and possess considerably higher conductances than milk and saliva which are lower in chlorides.

When we study the gastric juice we at once note that while the ash of this fluid is very low as compared with blood, bile, pancreatic juice, etc., its conductance is much higher than that of these fluids. Thus while the specific conductance of bile at 18°C. is 130×10^{-4} reciprocal ohms (1), that of blood serum (2) about 110×10^{-4}, and that of saliva about 50×10^{-4}, the conductance of pure gastric juice (3) is over 400×10^{-4}. The conductance of pure gastric juice is thus due very largely to the hydrochloric acid which it contains. The total chlorine of gastric juice is not notably higher than in blood, bile or pancreatic juice but the gastric secretion conducts a current much more readily than either of the other fluids because of the great mobility of the hydrogen ion. Thus the ionic speed at 18°C. of the sodium ion is 43.5, of the potassium ion 64.6, of the chlorine ion 65.5, while that of the hydrogen ion is 318. A tenth normal solution of hydrochloric acid at 18°C. has a specific conductance of 351×10^{-4} as, compared with 92.5 for sodium chloride and 111.9 for potassium chloride.

From these considerations it appeared that the determination of intragastric conductance might be used in many cases as an index of the concentration of hydrochloric acid in the stomach. A determination of this character would possess the advantage over titration methods of obtaining the desired data at as frequent intervals as desired, without any disturbance or removal of gastric contents. Titration methods for free hydrochloric acid in the presence of protein are especially unsatisfactory because of the further dissociation of the protein salt during the course of analysis. Phosphates from the saliva or other sources produce a similar effect while the coloring matter of certain foods, especially fruits, may make titration almost impossible. These difficulties are avoided by the electrometric method. The values obtained with its aid will be more accurate indexes of the hydrogen ion concentration (4) the higher the concentration of hydrochloric acid and the lower that of other electrolytes.

APPARATUS AND METHOD

The cell used in this work is illustrated in figure 1. It consists essentially of a hard rubber tip of the size used in the Rehfuss stomach tube

(about 1 cm. in diameter), slotted however in only one direction and made in four pieces for convenience in wiring. Into this tip are fitted opposite to each other the two platinum electrodes, about 10.0 × 6.0 mm. in size. All edges are of course rounded off smoothly so as to cause no irritation in swallowing. The leads consist of double or triple copper wire no. 36 about 3 feet long or longer if it is desired to pass the tube into the intestine. The conductance of the wire is appreciable and must be balanced in the other arm of the bridge. The use of heavier wire decreases the flexibility of the tube and it is desirable to maintain this. The electrodes must of course be properly platinized by the electrolysis of platinic chloride solution and must be kept clean. The cell constant is readily determined by means of $\frac{N}{10}$ potassium chloride solution whose specific conductance at 18°C. is 0.01119.

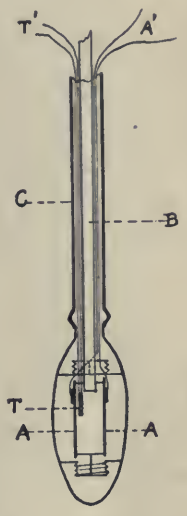

Fig. 1. Conductance cell for intragastric work. Diagrammatic cross section showing platinum electrodes *A*, *A*, with leads *A'*; thermocouple *T* with leads, *T'*; tube for aspiration *B*; and outer protecting tube *C*.

The tip with tubes attached is swallowed until aspiration shows it to be resting in the stomach. The conductance is determined in the ordinary manner using a good Wheatstone bridge, buzzer and telephone receiver. More expensive types of apparatus are not essential for intragastric work. Two or three dry cells will furnish current. A switch convenient to the bridge is desirable so that the circuit may be kept closed as much as possible. The buzzer should be enclosed in a soundproof box and placed at some distance from the bridge.

The thermocouple used is a base metal couple (copper and iron-constantan). Fine wire (0.1 mm.) is used for reasons previously mentioned and also that the current through the cell may be interfered with as little as possible.

The couple must be coated with paraffin and gastric juice must not be allowed to come into contact with any of the connecting wires. The couple is placed in circuit with a potentiometer indicator and high sensitivity mirror galvanometer.[2] A similar arrangement has been used by Stengel

[2] The tips used were made for us by Chas. Lentz & Sons, Philadelphia, and all other apparatus for conductance and temperature measurements by the Leeds & Northup Company of Philadelphia.

and Hopkins (5) in the study of intragastric temperature. By this means it is possible to observe at any moment the temperature of the stomach contents by merely adjusting the indicator until the galvanometer stands at zero and then reading off the temperature directly upon the indicator scale.

The fact that conductance increases about 2 per cent for each degree rise of temperature makes accurate observations of the latter necessary. The stomach is a fairly good thermostat if given time enough to adjust itself, but after the ingestion of cold foods or liquids of any kind, a half hour or more may be required to reach body temperature. By the aid of the thermocouple it is possible to study the conductance in this early period after the ingestion of food with a fair degree of accuracy by applying a temperature correction. The tip may also be used simply to replace dip electrodes for determination of conductances of small amounts of fluids in test tubes.

In studying the significance of intragastric conductance as determined with this apparatus the gastric contents obtained by aspiration at fifteen minute intervals were analyzed (6) and values obtained compared with conductances determined at the same intervals. Total acidity was titrated using phenolphthalein as an indicator, and free acid in most cases using Toepfer's reagent although in a few cases the Sahli iodine method was employed. Pepsin was determined by a modified Mett method. Trypsin was determined by Spencer's method (7) slightly modified. The presence or absence of bile, food residues, etc., was also noted.

EXPERIMENTAL AND DISCUSSION

A series of tests was first made using water and sugar solutions. These were practically non-conducting so that changes in conductance after introduction into the stomach would be expected, unless other diluting fluids were involved, to be due mainly to the hydrochloric acid of the gastric juice secreted in response to the stimulus. The four experiments charted in figures 2, 3 and 4 show that under these conditions the conductances of the gastric contents ran very closely parallel to the acidities (total acidity and free hydrochloric acid) and were mainly dependent upon the free hydrochloric acid present.

Conductances for convenience are plotted in whole numbers, a value of 250 signifying a specific conductance of 250×10^{-4}. All values for conductance were obtained at body temperature (37°) or corrected for deviations from this temperature. In the charts the curves for

conductance and titration values for free hydrochloric acid nearly overlap because the conductance of $\frac{N}{10}$ hydrochloric acid solution at 37°, expressed as above indicated, gives values very close to five times the titration values expressed in cubic centimeters of $\frac{N}{10}$ alkali.

These experiments show that ordinarily after the introduction of a 5 per cent sugar solution the concentration lowering is brought about

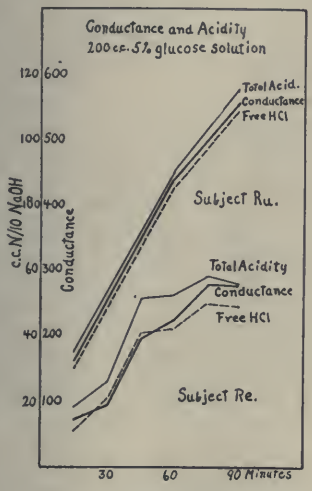

Fig. 2

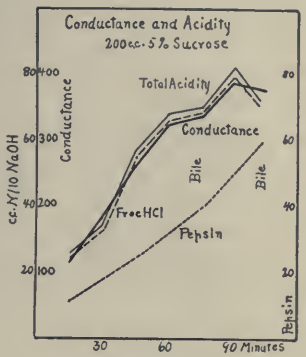

Fig. 3

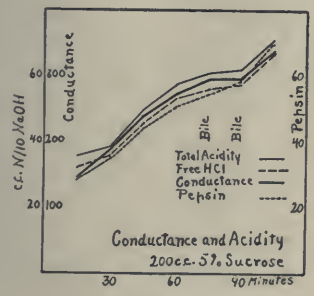

Fig. 4

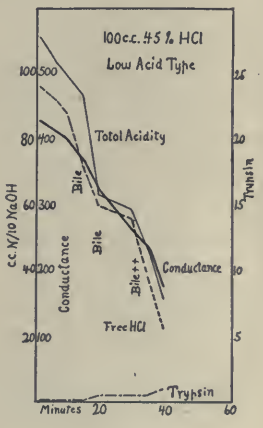

Fig. 5

by the secretion of gastric juice of normal hydrochloric acid content, and the conductance throughout the intragastric phase studied is almost entirely dependent upon the free hydrochloric acid of this secretion. A rather unusual degree of parallelism of pepsin with conductance and acidity is noted in figures 3 and 4, particularly the latter.

The effect of the introduction of 100 cc. of 0.45 per cent hydrochloric acid solution into the stomach of an individual of low acid type was studied. The results are given in figure 5. Here again we note a parallelism of conductance with free acidity, both values falling off rapidly. That the fall is due mainly to regurgitation of pancreatic juice and bile was shown by the color and general appearance of the later samples. The high concentration of hydrochloric acid has however destroyed most of the trypsin. Note the relative rise of the conductance as compared with free acid when the latter falls off toward the end of digestion.

The effect of "combined" acidity upon conductance is well shown after the administration of a protein food such as bread (see fig. 6). The values for conductance closely parallel those for free acid. They lie a little lower, however, indicating that, as would be expected, titration values for free hydrochloric acid are too high in these circumstances. The regurgitation of bile reduces both acidity and conductance but not in direct proportion. The conductance rises relatively, due to the chlorides of the bile. The "combined" acid is shown to have relatively little influence on conductance. The rise in pepsin with fall in acidity should also be noted.

The experiment charted in figure 7 illustrates the same facts as the preceding one, the parallelism of free hydrochloric acid with conductance, the high values obtained for the former by titration and the relative rise of conductance toward the end of digestion when regurgitation begins. It will be noted also that while the free acidity starts at little above zero, the conductance is already 100, so that obviously care is necessary in ascribing conductances at so low a level to hydrochloric acid.

The preceding experiments were carried out on normal individuals and indicate the usual response under the conditions of study. Figures 8 and 9 illustrate experiments on an individual with near-achylia. They illustrate clearly the point just mentioned that great care is necessary in interpreting changes in conductance when the values lie below 150.

In the experiment illustrated in figure 8, 500 cc. of distilled water were given. The conductance rises slowly at first and then very rapidly to 150, although the free hydrochloric and even the total acidity remain nearly negligible throughout. The reason for this is clearly brought out by a study of the tryptic values. Marked regurgitation takes place so that the final sample is nearly pure pancreatic juice.

In the second experiment (fig. 9) 250 cc. of 10 per cent sucrose solution were given. The result was similar to that of the preceding experiment except that evacuation was somewhat delayed by the sugar present. In both of these cases then there was noted a parallelism

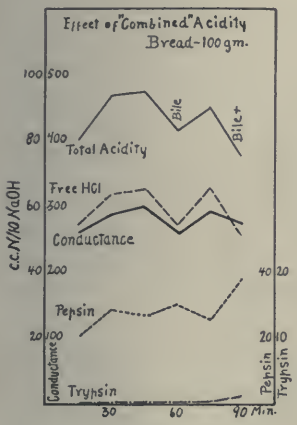

Fig. 6

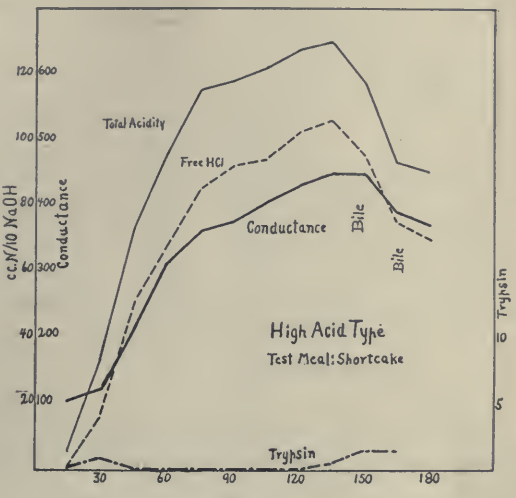

Fig. 7

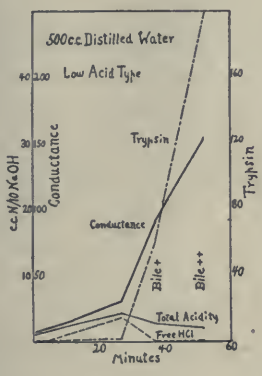

Fig. 8

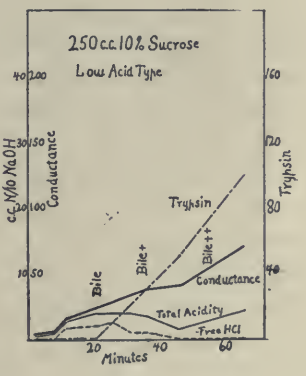

Fig. 9

of conductance with trypsin values (that is, with the concentration of pancreatic juice), gastric secretion being entirely secondary in its influence. Such findings could of course only be expected in achylias.

More typical of normal gastric digestion are the curves given in figure 10. Here the total acidity begins high due to organic acid

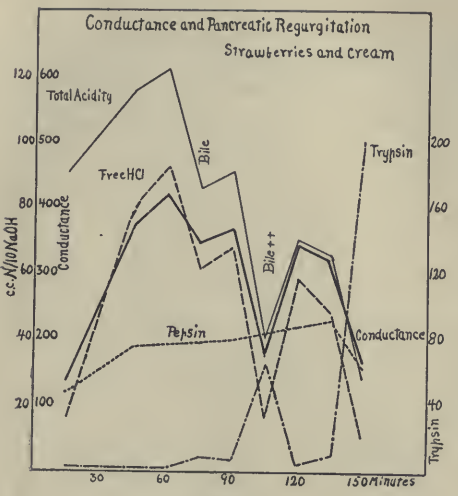

Fig. 10

which has but little influence on conductance. After about an hour regurgitation of pancreatic juice and bile begins as indicated by the tryptic values, also by the color of samples withdrawn. Both acidity and conductance fall sharply, rise due to secondary gastric secretion and again fall due to further regurgitation. The last specimen is very high in trypsin and is nearly pure pancreatic juice. Here again may be noted the rise of conductance relative to free acidity, so that the final sample has hardly any free acid, but has a conductance of 165.

Figure 11 shows somewhat similar conditions. Here, however, the tryptic index remains very low and the intense green color which the samples developed on standing showed the regurgitated fluid to be almost entirely bile. The reduction of acidity is more gradual than in the preceding case and the conductance falls off less rapidly. Pepsin, as in the preceding case, remains nearly constant in spite of the dilution of the gastric juice by regurgitation. The reason for this is not entirely clear. Certain results of this type indicate that the acidity lowering may be due in part to the slightly alkaline duodenal and pyloric secretions which contain pepsin. It has not been shown however that the pepsin contents of these fluids are sufficiently high to explain some of the results obtained. In the absence of regurgitation the pep-

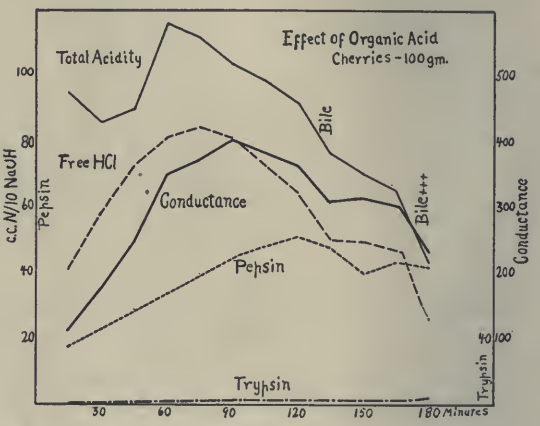

Fig. 11

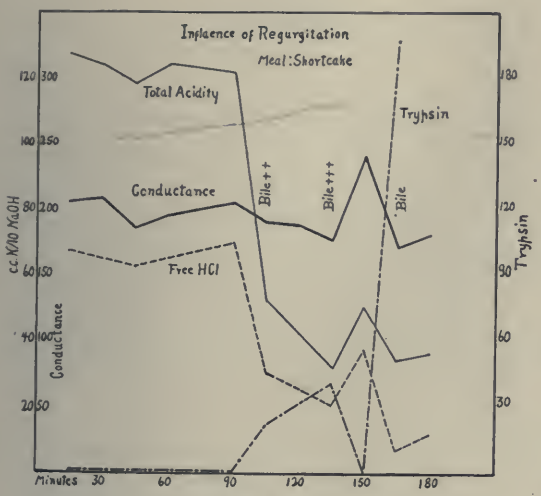

Fig. 12

sin rises with the acid, showing a certain parallelism. Regurgitaion brings about a neutralization of acid, while pepsin is not destroyed. Hence the latter will be expected to rise relatively.

The organic acid of the fruit ingested in this case causes titration values for acidity to lie considerably above the true curve for free hydrochloric acid which would more nearly follow the curve for conductance. This illustrates the value of conductance determinations as a check on free acid where organic acid is present.

The experiment illustrated in figure 12 is unusual in certain respects. Here we have very marked regurgitation of pancreatic juice and extreme changes in acidity with but little influence on conductance. In this case during the first hour and a half the acidity (even a part of that titrating as free hydrochloric acid) is due to organic and "combined" acids which are low in conductance. The conductance is so low in fact that the values obtained are but slightly above those for pure pancreatic juice and bile. Hence admixture with these secretions produces only slight lowering of conductance.

In figure 13 we have represented a case of almost perfect regulation of gastric acidity by means of regurgitation. The acidity remains nearly constant for a period of two and a half hours. As long as the acidity thus remains constant the rise in conductance parallels that of trypsin. When the acidities

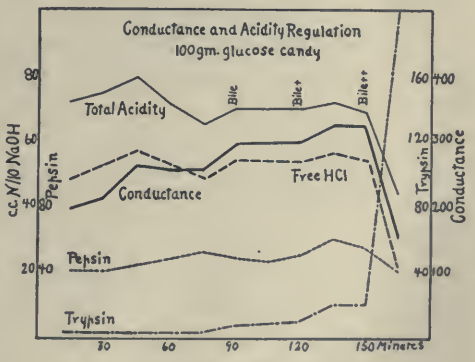

Fig. 13

become markedly reduced, however, the relation of trypsin to conductance becomes inverse because of the lower conductance of pancreatic juice.

In this case no water was taken and the solution entering the stomach was salivary in character and hence relatively high in phosphate. This accounts for the marked difference between free and total acid which we regularly find in such cases. The conductance of the alkali phosphates being low, the conductance curve more nearly follows that for free hydrochloric acid. Several samples of saliva tested by us have shown a conductance of 50–60 $\times$ 10^{-4} at 37°. This is appreciably lower than values given in the literature although filtered saliva may have been employed in earlier determinations.

SUMMARY AND CONCLUSIONS

A retention stomach tube in the form of an electrolytic cell has been devised which makes possible the determination of intragastric conductances at any desired interval of time without disturbance or removal of gastric contents. It may also be used in place of dip electrodes. The tip contains a thermocouple which makes possible intragastric temperature determinations and corrections, and an aspiration tube by means of which samples of gastric contents may, if desired, be collected for analysis.

By means of this apparatus intragastric conductance variations were studied in connection with determinations of total acidity, free hydrochloric acid, pepsin and trypsin. The conductance of gastric juice is mainly due to the free hydrochloric acid which it contains and the same is usually true of the gastric contents.

After the introduction of water or solutions (as sugar solutions) of very low conductance, the curve for conductance very closely follows the curve for free and total acid. This indicates that the equalization of osmotic concentration is brought about primarily by secretion of normal gastric juice.

After the ingestion of food containing protein the conductance curve usually lies below that for free hydrochloric acid as determined by titration because the latter values are high due to gradual dissociation of the protein salt. In the presence of weak organic acid as after fruit ingestion or of phosphate as where much saliva is swallowed, the conductance falls below titration values and is a better measure of free hydrochloric acid.

Aside from the swallowing of saliva, the conductance of which is low, intragastric conductance is, after the first hour or so of digestion, almost always considerably modified by the regurgitation of pancreatic juice or bile or both and possibly to a lesser extent by pyloric and duodenal secretions. The conductance of pancreatic juice and bile being usually very low as compared with that of the gastric contents at maximum acidity, regurgitation tends to markedly lower intragastric conductance as well as acidity. Conductance, however, rises relative to free hydrochloric acid on account of the higher salt content of these regurgitated secretions. After the ingestion of mineral acid, neutralization is brought about in the same manner as during digestion.

In achylias where intragastric digestion is mainly pancreatic in character the conductance was found to parallel the concentration of pancreatic juice as measured by the tryptic index.

Studies of intragastric digestion by this method as well as of the influence of salt solutions in the stomach and upper intestine, are being continued.

The author is indebted to Dr. P. B. Hawk, Dr. C. A. Smith and Mr. R. J. Miller for the privilege of using certain of these cases and data. He desires also to thank Messrs. H. S. Sargent and E. L. Small for assistance in enzyme determinations.

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AN AIR-TIGHT PLEURA CANNULA

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Received for publication October 1, 1917

In the beginning of the nineties I devised an air-tight pleura cannula. The late Prof. Hugo Kronecker who in 1893 saw the efficient working of the cannula in my private laboratory asked me to give him a brief description of it; this sketch he published later in two journals (1). At the tenth annual meeting of the American Physiological Society (2) I showed the cannula in connection with another demonstration, but published no description of the cannula itself.

About fifteen years ago, I made an essential change in the construction of the cannula. It is the latter construction which we have been using in our laboratory since 1904. The cannula was often mentioned in papers which emanated from our department, but we never published a description of its construction. In connection with the following paper of Dr. A. L. Meyer, in whose investigation the cannulas played an essential part, I decided to publish the following brief description of our pleura cannula in its present form.

Figure 1 presents the later type of pleura cannula when all its parts are connected; the rubber gasket and two leather washers are here omitted.

Figure 2 shows the four parts composing the pleura cannula.

Part 1. When the larger branches are put together, the entire parts presents a T-shaped hollow cylinder terminating in two flat plates (feet). The vertical cylinder carries on about three-fourths of its length a spiral ridge (thread). The feet which taper toward the beveled end are flat on the lower surface and slightly rounded on the upper side. Both halves of the cylinder hinge at the heel. When they are separated to 90 degrees the entire part assumes again a T-shape in which both feet form one branch that stands perpendicular to the horizontal lines formed by the halves of the cylinder.

Part 2 is a rosette shaped plate with an opening in the middle which is slightly larger than the diameter of the cylinder of part 1. Around

the opening are arranged about twenty or more sectors of a flat S shape. The sectors are elastic and when the plate is pressed down it acts like a spring and adapts itself to the elevations and grooves of the thoracic wall.

Part 3. This is simply an external (female) screw adapted to the thread of the cylinder in part 1.

Part 4 presents a cylinder the lower part of which is again an external (female) screw of the same bore as the previously mentioned screw (part 3). At the end of the screw the cylinder carries a stopcock and

Fig. 1

Fig. 1. The newer type of the pleura cannula; the rubber gasket and two leather washers are here omitted.

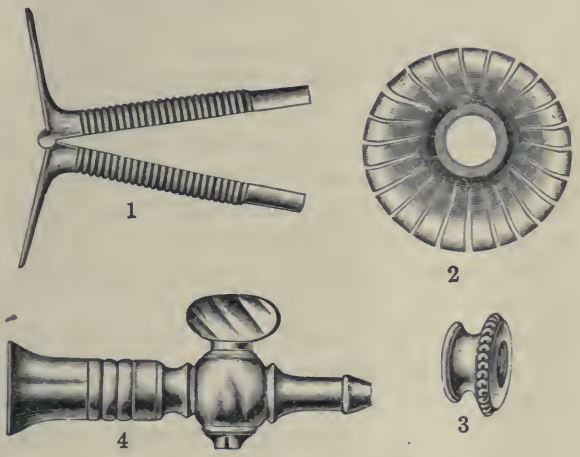

Fig. 2

Fig. 2. The four essential parts composing the cannula. The description is given in the text.

terminates with a conical thickening for the making of an air-tight connection with rubber tubing.

For the introduction and fastening of the cannula the procedure is to be as follows: In the fourth intercostal space, at about the junction between the anterior and middle part of the ribs, a small incision is made with a knife through the skin and the muscles and the pleura is broken with a blunt instrument of a small diameter. The feet of part 1 are now put together and pushed through the small opening into the pleural cavity. Now each half of the cylinder is raised to 90 de-

grees until they form an externally protruding tube while the separated feet are now resting on the opposing ribs and at right angles to them. Over the cylinder a round gasket of soft rubber, with an opening in the middle, is now pushed down to the skin and is followed by the rosette shaped plate (part 2); a leather washer is pushed down to the plate and then the external small screw (part 3) is screwed down very tightly so as to keep the cylinder in place and adapt the sectors of the rosette shaped plate to the uneven parts of the thoracic wall. While this is done the cylinder should be grasped firmly so that the position of the feet in relation to the ribs should remain unchanged. Now follows another leather washer, upon which part 4 is tightly screwed down. The collapsed lung is now being distended and the stopcock so turned that no air can enter again into the pleural cavity.

In dogs the distention of the lung can be done either by blowing through a tracheotomy tube or still better, through an intratracheal tube introduced through the mouth and larynx; in the latter case it is well to exert briefly a slight pressure just above the larynx. For rabbits (and cats) there is a simpler method of distention. The trachea should be compressed while the abdomen and the liver are pressed upwards; the air of the uncollapsed lung has no other place for escape than to enter into the collapsed lung.

The protruding end of the pleura cannula is now to be connected by means of tubing with a Marey tambour. The tubing has to have a T-tube bearing a pinch cock. While the stopcock of the cannula is still closed the pinch cock is opened and an atmospheric line is drawn on a revolving smoked drum. After closing the pinch cock and opening the stopcock of the pleura cannula, the respiration is marking its undulations upon the revolving drum. If everything is done properly, the tops of the expiratory elevations remain below the atmospheric line —an evidence of the existence of the negative pressure in the pleural cavity. It is essential that the rosette plate be tightly screwed down. When this is the case, no change in the relations of the respiratory movements to the atmospheric line is taking place except, of course, under special conditions (pressure upon the abdomen, strong active expirations, stimulations of the vagus, inferior or superior laryngeal nerves, etc.). It may happen occasionally that by over-distending the lung the pleural opening into the cannula may become closed up by a slip of the lung. This can be remedied by compressing the thorax in the dorso-abdominal direction. But it is altogether not necessary to overdistend the lung; the presence of a very small amount of air in the

pleural cavity will not interfere with most of the conditions which we wish to study. I wish to mention the further fact that in pushing in the feet of the cylinder it may happen that no sufficient opening in the pleura is made and that the feet may even remain outside of the parietal pleura. It is therefore advisable that the feet, before permitting them to separate, should be pressed well down into the pleural cavity, and furthermore, before screwing on part 4 a probe should be pushed into the cylinder in order to obtain evidence that the pleural cavity is freely connected with the cannula.[1]

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[1] The cannula may be obtained from Georg Tieman & Company, 107 Park Row, New York City.

A NEW METHOD OF OBTAINING THE RESPIRATORY GASES IN ANIMALS, AND SOME OF THE RESULTS

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Received for publication October 1, 1917

INTRODUCTION

The purpose of this paper is to present a new method of obtaining a sample of air from the lungs of animals. The method, in essence, consists in the introduction of air under pressure into both pleural spaces, the lungs being thereby completely compressed and their contents forced into a rubber bag attached to a tracheal cannula. In this manner all the air is collected with the exception of a small quantity which remains in the uncollapsible portion of the system, namely the bronchi, trachea and cannula (dead space). The bag therefore contains a mixture of *supplementary* and *residual* air.

The procedures hitherto employed fall roughly within two groups. During the latter third of the nineteenth century samples of pulmonary air were obtained after either the entire air within the lungs or only a portion of the air had been brought into equilibrium with the gases of the venous blood. This is essentially the principle of the methods adopted by Pflüger and his school. To mention only a few names of this school, Wolffberg (1) and Nussbaum (2), experimenting on dogs, used Pflüger's lung catheter which enabled them to occlude a portion of the pulmonary air until equilibrium had been established. The percentages of carbon dioxid which they found were distinctly lower than those obtained later by Loewy and von Schrötter (3). These investigators applied the same principle in human beings and succeeded in giving the technique greater refinement with the aid of bronchoscopy. Plesch, (4) dispensing with the use of the catheter but still adhering to the same principle, had his subjects rebreathe air from a rubber bag until the mixture of gases within the system had become homogeneous and had reached the same tension as the carbon dioxid and

oxygen of the venous blood. This method in modified form has enjoyed fairly extensive use in clinical investigation (5).

In all of these procedures the air within the lungs is modified and its analysis, consequently, does not represent the composition of the air normally present in the lungs at any single moment. In the group next to be considered samples of pulmonary air are obtained without first subjecting it to conditions which materially modify its composition. While these methods are pretty generally understood, I shall nevertheless mention them in some detail for purposes of comparison with my own method.

In 1905 Haldane and Priestly (6) introduced a method of obtaining a sample of alveolar air in human beings. The subject takes a comfortable position and waits until his respirations have become regular. At the end of a normal inspiration he expires suddenly and very deeply through a rubber tube 1 inch in diameter and about 4 feet long, provided with a mouthpiece, and instantly closes the tube by applying his tongue to the mouthpiece. From the portion of the tube nearest the mouth a sample is then withdrawn. In a second experiment, a sample is taken at the close of deep expiration, following a normal expiration. The mean result of the percentages thus obtained represents the mean composition of the alveolar air. In the case of J. S. H. the average percentage of alveolar carbon dioxid was 5.62, while the highest percentage was 5.87 and the lowest 5.40. In the case of J. G. P. the average percentage was 6.28; the highest 6.85 and the lowest 5.98.

Boycott and Haldane (7) made five determinations during a period of one and a half hours at a room temperature of 18.4° to 19.0°C. The actual percentages of carbon dioxid were 5.42, 5.66, 5.62, 5.34, 5.52. Fitzgerald and Haldane (8) studied the variations in carbon dioxid percentages of the alveolar air during two successive nights and days. There were twenty-five determinations. They state that the figures of the last twenty-four hours were probably influenced by exhaustion. If then we take the fourteen percentages from 5.45 p.m. to 6.15 p.m. which are means of two or three determinations, we obtain an average of 4.93; the percentages showing maximum deviation from the mean were 4.73 and 5.21; those showing a minimum deviation, 4.91 and 4.96. In another experiment carried out from 8 a.m. to 7.30 p.m., there were seven determinations with an average of 4.81. In this case the percentages showing the widest deviation from the mean were 4.62 and 4.95, while those with the smallest deviations were 4.80 and 4.86.

The differences between individual determinations carried out according to the method of Haldane and Priestley are easily understood when one remembers that the depth of successive respirations nearly always varies in most persons. Not only is its use difficult in certain pathological cases owing to the deep expiratory effort required, but experience shows that even in normal individuals its successful appli-

cation depends upon an intelligent and willing coöperation on the part of the subject.

Lindhard (9) has described what may be considered a modification of the Haldane-Priestley method. A sample of air is obtained at the close of several expirations. The respirations take place through a valved apparatus and an air-tight mask covering nose and mouth. A narrow lead tube ending immediately below the expiratory valve and opposite the mouth leads to a sampling tube filled with mercury. The top of the receiver is turned at the end of an expiration. A small sample is taken. This is repeated until the fractional samples total 50 cc. The experience of Boothby and Peabody (10) has shown that the essential point in Lindhard's method is the exact time at which the samples are collected. Furthermore it was found difficult to obtain accurate samples with a tidal air of but 300 cc. They proceeded to modify the method to the extent of not only omitting the valve and placing the lead tube as far back in the mouth as was consistent with comfort but of requesting the subject to expire 600 to 800 cc. upon a given signal, whereupon the tap was turned and 3 to 5 cc. were withdrawn. This was repeated until a sufficiently large sample had been obtained. Successive samples in one subject gave partial pressures of 38.8; 40.9; 39.1; 38.8 mm. Hg. The results are in agreement with those found by the Haldane-Priestley method.

The only effort to obtain a sample of the unmodified pulmonary air in animals by a method conforming in principle to my method is that of Scott (11). He experimented on cats and analyzed the last portion of the air obtained by compression of the thorax and abdomen.

Scott finds that great variations occur in the same animal during the course of an experiment. Ether and urethane were used as the anesthetic agents. During the period between 10.23 and 1.30, seven analyses were made. The body temperature fell 3.5°C. Two samples taken at an interval of seventeen minutes showed the percentages of carbon dioxid to be 4.68 and 4.23; two other samples twenty minutes apart contained 4.25 and 4.04 per cent of carbon dioxid. Two successive samples taken at an interval of one hour showed no difference. From 1.44 to 5.30 in the same experiment eight determinations were made. The average percentage of carbon dioxid was 4.39, while the percentages showing maximum deviation were 4.21 and 4.53; those showing minimum deviation were 4.34 and 4.44. During the first six determinations of this period the body temperature rose about 3°C; during the last two it fell.

In the Haldane-Priestley method and its modifications, as well as in the method used by Scott the sample for analysis consists of only a small part of the air within the lungs, either the last portion of a forced expiration, or an expiration somewhat deeper than the normal tidal air of the subject, or else the last part of the air obtained by forcible compression of the thorax and abdomen. In each case a considerable volume of air remains in the lungs. When manual compression of the

thorax is practiced in young cats one may possibly greatly diminish the residual air remaining in the lungs, but I believe it is quite unlikely that one could thereby force out all the residual air.

METHOD

Referring to the foregoing remark I wish to state that, while it is not the purpose of the method presently to be set forth to analyze especially the residual air, nevertheless the residual air is in large measure recovered by this method and together with the entire supplementary air constitutes the sample for analysis.

In the present series the experiments were made on dogs that had been without food for about twenty-four hours. Chloretone, dissolved in olive oil, was used as the anesthetic. It was administered intraperitoneally, in the dose of 0.24 to 0.30 gram per kilogram of body weight Fifteen to thirty minutes later tracheotomy done in the usual manner was followed by the insertion and firm fixation of a T-shaped glass cannula. Next a special cannula,[1] devised by Meltzer (12) and used in this laboratory for some years, was placed in each pleural cavity, in the fourth right and fifth left intercostal spaces, sufficiently near the lateral line to avoid the thick sternal and pectoral muscles. Once the pleural cannulas had been placed it became necessary to redistend the lungs and reëstablish the intrathoracic negative pressure. This was readily accomplished by the introduction into the tracheal cannula of air under a pressure of 40 mm. Hg. maintained for fifteen seconds. This pressure was found to be most effective if the abdomen was coincidently compressed. It is also well in order to insure free exit of air from the pleural spaces during inflation to compress alternately the anterior and posterior regions of the thorax. The stopcocks of the cannulas were closed before the inflation was discontinued. As a matter of fact the maximum negative pressures are rather to be avoided owing to the frequency with which the lungs block the internal openings of the cannulas. Such an accident would obviously interfere with the kymographic record and be apt to give an erroneous impression both of the amount of negative pressure and the character of the respirations. For this reason I have thus far used negative pressure somewhat below the maximum. How successfully one might omit the kymographic record and work with the maximum negative pressure, has not been established.

[1] The use of this cannula for the present purpose is due to the suggestion of Doctor Meltzer.

It is always important to test the pleural spaces for air-tightness. It should be possible to maintain a negative pressure within the thorax of 70 mm. H_2O for an indefinite period. If any change occurs it ought to be in the direction of an increase rather than a decrease because of the gradual absorption of gases from the pleural cavities. The pleural cannulas were therefore connected by means of a Y-piece and suitable rubber tubing with a Marey tambour and a water manometer. A record of the negative pressure and respiration could then be made upon smoked paper.

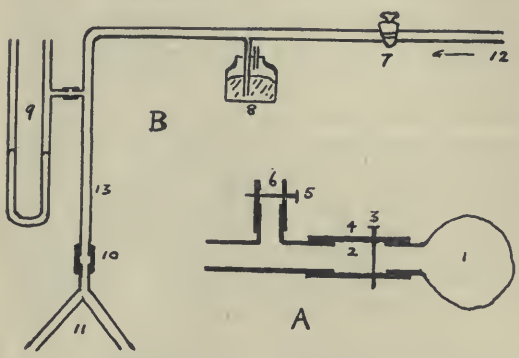

Fig. 1. Diagram *A* shows rubber bag attached to a T-shaped glass cannula. Bag may be closed by means of screw-clamp *3*. Rubber tubing *6* may be closed with a pair of hemostatic forceps *5*. *B* represents the pressure connections. Air entering at *12* passes into pleural spaces through Y-piece *11* attached to pleural cannulas, when stop-cock *7* is open. Tubing *13* can be disconnected at *10* and attached to the tracheal cannula for inflation of the lungs.

Now as to the procedure immediately preceding the sample: A small sized rubber bag of approximately 100 cc. having been attached to the tracheal cannula as indicated in figure 1, the pleural cannulas were connected by means of a Y-piece with a source of air pressure. A mercury valve introduced at *8* allowed the escape of air in excess of the desired pressure (40 mm. Hg). The next steps followed in quick succession. While compressing the rubber tube *2* of the bag with the forefinger and thumb of the left hand, the screw-clamp *3* and stopcocks of the cannulas were opened; closure of the cannula at *6* immediately at the end of the next expiration was followed by the opening of the pressure valve *7* and release of forefinger and thumb at *2*. The entrance of air into the pleural sacs compressed the lungs and forced their contents into the rubber bag. The filling of the bag was completed in about the time it requires to make a forced expiration. The remaining part of the procedure consisted in the opening of the tracheal cannula at *6*, the disconnection of the air pressure and the reëstablishment of the negative pressure. While apparently complicated, with a little practice the steps involved need not consume one minute.

In analyzing the air in the bag I followed the technique described by Haldane in connection with his smaller apparatus (13) It is of great importance that the rubber bag should not be too large. An ordinary nitrous oxid bag is much too large for the volume of air obtained from a dog of 6 to 10 kgm. It was found that the analysis of two samples taken from a large bag differed widely. The size of the bag must be such as to permit rapid and complete diffusion. On the other hand, the bag must not be so small as to offer resistance and prevent the entrance of the full amount of air.

SOME RESULTS

It was important to determine at the outset whether analyses of samples collected in this manner would yield uniform results in one and the same experiment. In the present series of experiments (table 1) no precautions were taken to maintain the body temperature of the animal. Heat was applied in certain experiments of another series but the results will be reserved for a subsequent paper. Furthermore only the carbon dioxid was studied; no analyses were made of the oxygen.

In all of the experiments uniformity in the percentages was exhibited either immediately or after a variable preliminary period. In table 2 the experiments appear in condensed form. The second column of this table shows the time that elapsed between the first and last samples of each experiment, while the third column indicates the time during which the percentages remained uniform.

In the course of three and a half hours seven samples were obtained and analyzed in the first experiment. It is evident that constancy was present from the beginning. The average percentage of the period was 5.37; the percentages showing maximum deviation from the average were 5.23 and 5.51.

The first three samples of the second experiment showed fluctuation in the percentages, whereupon the carbon dioxid became uniform and continued so for a period of over three hours, during which eight samples were obtained. The percentages showing maximum deviation from the average of 6.03 were 5.85 and 6.24.

In the third experiment the first four analyses again presented marked differences. Uniformity in the carbon dioxid was observed however for a period of two hours beginning at 2.32 p.m. During this time eight samples were taken. The average figure for the period was 5.82 while those exhibiting maximum divergence from the mean were 5.65 and 6.01.

TABLE 1

DATE AND NUMBER OF EXPERIMENT	TIME OF SAMPLE	INTERVAL BETWEEN SAMPLES	BODY TEMPERATURE	CARBON DIOXID	DATE AND NUMBER OF EXPERIMENT	TIME OF SAMPLE	INTERVAL BETWEEN SAMPLES	BODY TEMPERATURE	CARBON DIOXID
				per cent					*per cent*
Feb. 26	12.03		37.23	5.31	Mar. 8	12.34		36.22	4.87
1	12.26	23	36.92	5.35	4	12.59	25	35.98	4.80
	1.35	50	36.56	5.48		1.22	23	35.70	4.83
	2.01	25	36.35	5.51		2.22	60	35.00	4.76
	2.42	40	36.13	5.35		2.48	26	34.80	4.79
	3.09	27	36.05	5.23		3.06	18	34.70	4.95
	3.37	24	35.95	5.34		3.30	24	34.55	4.64
						3.48	18	34.42	4.87
Mar. 1	11.32		36.39	5.66					
2	11.52	20	35.95	6.05	Mar. 12	2.16		31.50	5.64
	12.07	15	35.70	5.75	5	2.45	29	30.90	5.53
						3.07	22	30.50	5.59
	12.20	13	35.45	6.15		3.35	25	30.05	5.63
	12.37	17	35.20	5.94		3.54	19	29.60	5.64
	12.53	16	34.98	6.09					
	1.52	60	34.25	6.10	Apr. 12	1.53			5.43
	2.10	18	34.05	5.95	6	2.15	22		5.66
	2.30	20	33.85	5.85		2.28	13		5.41
	3.06	36	33.60	5.95		2.41	13		5.49
	3.45	40	33.40	6.24		2.56	15		5.35
						3.26	30	34.15	5.20
Mar. 7	12.16		36.50	6.15					
3	1.00	44	36.30	5.58	Apr. 25	11.47			6.34
	1.12	12	35.85	5.78	7	12.08	21		6.26
	1.23	11	35.88	6.08		12.18	10		5.90
	2.32	50	35.60	5.69		12.30	12		5.56
	2.53	20		5.65		1.16	46		5.48
	2.56	3	35.25	6.01		1.28	12	33.95	5.53
	3.20	24	35.02	5.94		1.41	13		5.45
	3.42	22	34.85	5.88		1.53	12	33.80	5.42
	3.59	17	34.72	5.70		2.12	19		5.52
	4.18	19	34.53	5.87		2.25	13	33.65	
	4.32	14	34.45	5.79					

The carbon dioxid in the fourth experiment was constant for a period of over three hours. Eight analyses were made in this time. The percentages with the maximum deviation from the mean of 4.81 were 4.64 and 4.95. One of the percentages was comparatively low owing to a marked increase in the rate and depth of the respirations.

TABLE 2

DATE OF EXPERIMENT	DURATION OF EXPERIMENT	PERIOD OF UNIFORMITY	CHANGE IN RECTAL TEMPERATURE DURING EXPERIMENT	AVERAGE PERCENTAGE CARBON DIOXID	MAXIMUM AND MINIMUM PERCENTAGES	NUMBER OF UNIFORM DETERMINATION
1917			°C.			
February 26	12.03–3.37	12.03–3.37	−1.3	5.37	5.23; 5.51	7
March 1	11.32–3.45	12.20–3.45	−3.0	6.03	5.85; 6.24	8
March 7	12.16–4.32	2.32–4.32	−2.0	5.82	5.65; 6.01	8
March 8	12.34–3.48	12.34–3.48	−2.0	4.81	4.64; 4.95	8
March 12	2.16–3.54	2.16–3.54	−2.0	5.61	5.53; 5.64	5
April 12	1.53–3.26	1.53–3.26		5.42	5.20; 5.66	6
April 25	11.47–2.25	12.30–2.25		5.49	5.42; 5.56	

Uniformity in the percentages of carbon dioxid was immediate in the fifth experiment and continued for more than one and a half hours. During this period five samples were analyzed. The average was 5.61 while 5.53 and 5.64 were the figures showing the greatest deviation from the mean.

Again, in the sixth experiment the carbon dioxid exhibited uniform behavior with a period of preliminary fluctuation. The period of observation covered one and a half hours and during this time six determinations were made with an average of 5.42, the highest and lowest percentages being respectively 5.66 and 5.20. The respirations in this animal were quite irregular in depth.

In the last experiment three determinations in the first half hour fluctuated; these, however, were followed by a period of uniformity lasting two hours during which six samples were obtained averaging 5.49, with a minimum percentage of 5.42 and a maximum of 5.56.

There was no persistent upward or downward tendency in the percentages of any one experiment. In nearly all cases the last percentage of the period of uniformity was identical with the first percentage of the period. This is rather interesting in view of the steady fall in the body temperature. A fall in body temperature seemed rather to occasion a fall in the percentages of carbon dioxid in Scott's (14) experiments.

I am unable to state definitely the cause of the preliminary fluctuations sometimes observed. It is significant however that in all those experiments in which analyses were not begun until 12.00 m. or later this phenomenon did not appear, except in the experiment of March 7 in which the breathing in the beginning was of the periodic type.

Chloretone was always given between 9.30 and 10.00. It is possible that the volatile nature of this substance may have interfered with the analysis of the air.[2]

The method is clearly capable of a variety of modifications and ought to be useful in the solution of a number of problems bearing on the physiology of the respiration. The percentages in the above experiments are not absolute values and hence the results of one animal must not be compared with the results obtained in another animal. This is partly to be accounted for by the fact that the negative pressures were not the same in the different dogs; in some more air was allowed to remain in the pleural sacs than in others. Neverthless it is evident that the percentages of carbon dioxid in samples obtained by this method may exhibit uniformity for periods as long as three and one-half hours and that the variations are no greater than one obtains by the use of other methods.

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[2] Among other factors that may play a rôle is the possible alteration in the volume of the dead space.

THE VISCOSITY OF LYMPH

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Received for publication October 9, 1917

As the method which has been made use of by Burton-Opitz (1) in determining the viscosity of different body-fluids has been described in an earlier paper, it need only be mentioned at this time that the experiments now under discussion purpose to ascertain the factors required to calculate the coefficient of the viscosity of lymph. The apparatus is arranged in such a way that it is possible to measure how large a quantity of this fluid escapes through a capillary tube of known length and diameter in a given period of time and under a certain pressure. The coefficient derived from these factors is then compared with the coefficient for distilled water at 37°C. which, in accordance with Poiseuille (2) equals the value 4700.

The present experiments were performed upon dogs which had received a moderate amount of fatty meat about four hours previously. Light ether narcosis was employed. The lymph was gathered from the central orifice of the thoracic duct. It became necessary at times to hasten its flow by exerting gentle pressure upon the abdominal wall, because as lymph clots very rapidly, its passage through the viscosimeter could not be permitted to consume a longer time than about one minute. From 2 to 3 cc. of lymph were used for each determination. The specific gravity was ascertained with the help of small pycnometers possessing a capacity of about 4 cc.

The results of these determinations are compiled in table 1. It will be seen from this that the viscosity of lymph varies considerably. The coefficients here recorded lie between the figures 2406 and 3029 and present the average value 2682.5. If this coefficient is compared with the coefficient for distilled water of 37°C., it will be found that lymph possesses a viscosity which is 1.7 greater than that of water. It must be emphasized, however, that these determinations have been made during the absorption of fat and that, therefore, these values are some-

what above those obtainable with perfectly clear lymph. The nearest approach to the watery type of lymph was yielded by dog 5, this experiment having been performed almost six hours after the ingestion of food. The relatively high numerical value of this coefficient (3029)

TABLE I

EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE VALUE
		mgm.	*seconds*	*mm. Hg.*			
1	1.0170	1.9613	72.07	103.2	2661.3	40.7	2681.6
	1.0170	1.2592	47.70	98.6	2702.0		
2	1.0168	1.8990	74.80	91.5	2800.5	17.5	2818.0
	1.0168	1.6494	49.60	118.4	2835.6		
3	1.0141	2.4285	68.98	138.9	2565.1	119.4	2624.8
	1.0141	1.6201	50.10	121.6	2684.5		
4	1.0140	2.1238	59.08	132.7	2754.0	43.6	2732.2
	1.0140	1.9332	58.08	124.3	2710.4		
5	1.0119	2.8210	64.10	145.4	3069.9	80.5	3029.6
	1.0119	2.7177	66.05	139.6	2989.4		
6	1.0230	2.2998	59.50	149.8	2588.7	72.3	2624.8
	1.0230	2.4687	66.20	140.6	2661.0		
7	1.0194	2.5081	64.13	133.3	2976.8	35.9	2994.7
	1.0194	2.0758	53.20	131.4	3012.7		
8	1.0171	1.7019	64.20	110.0	2432.1	50.9	2406.6
	1.0171	1.2840	57.16	95.2	2381.2		
9	1.0119	1.8746	61.93	127.4	2410.1	26.1	2423.1
	1.0119	1.4588	52.63	115.4	2436.2		
10	1.0215	1.5828	49.22	130.7	2472.1	36.2	2490.2
	1.0215	1.6215	51.60	125.9	2508.3		

indicates that this lymph possesses a viscosity only 1.5 times greater than that of distilled water of 37°C.

In a similar way, it may be gathered that increasing absorption heightens the viscous resistance of the lymph. Thus, the coefficient 2406 obtained by experiment 8 shows that this lymph is almost 2.0

times more viscous than distilled water of 37°C. In this connection, brief reference might be made to the fact that the viscosity of blood is almost 5 times greater than that of distilled water (3) while that of ox bile (4) is only 1.8 and that of saliva only 1.4 times greater (5).

The specific gravity follows a course parallel to that of the viscosity. The values recorded above vary between 1.0119 and 1.0230. The average value is 1.0165. The coagulation-time which was determined by the method of inversion of a small test tube filled with a small quantity of fresh lymph, varied between one and seven minutes. The average time was two minutes and a half. It is evident, therefore, that

TABLE 2

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0230	2.2998	59.50	149.8	2588.7	2624.8	524.1
		1.0230	2.4687	66.20	140.6	2661.0		
	During stimulation	1.0285	1.3729	54.12	119.4	2120.5	2100.7	
		1.0285	1.2661	52.61	115.4	2081.0		
2	Normal	1.0194	2.5081	64.13	133.3	2976.8	2994.7	421.6
		1.0194	2.0758	53.20	131.4	3012.7		
	During stimulation	1.0228	1.5643	49.81	120.5	2695.2	2573.1	
		1.0228	1.4805	51.63	117.4	2451.0		

viscosity experiments upon lymph necessitate the same precautionary measures as those made upon the circulating blood. The time required for the completion of each test should not be longer than one minute.

Table 2 is intended to illustrate the changes which the lymph of the thoracic duct undergoes in consequence of the stimulation of the greater splanchnic nerve. The experiments with which we are concerned at this time, are those designated in table 1 as 6 and 7. In both cases the left greater splanchnic nerve was used, shielded electrodes having been applied to it through a small opening in the abdominal wall. Having collected a sufficient quantity of lymph for the determination of the normal viscosity and specific gravity, the two subsequent collections were made while the aforesaid nerve was being stimulated with a tetanic current of medium strength.

TABLE 3

	EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0141	2.4285	68.98	138.9	2565.1	2624.8	+133.9
		1.0141	1.6201	50.10	121.6	2684.5		
	After injection: 100 cc.	1.0175	1.3934	48.50	115.0	2520.0	2490.9	
		1.0175	1.4847	53.10	114.6	2461.0		
2	Normal	1.0140	2.1238	59.08	132.7	2754.0	2732.2	−38.9
		1.0140	1.9332	58.08	124.3	2710.4		
	After injection: 200 cc.	1.0145	2.2431	68.02	120.0	2780.2	2772.1	
		1.0145	1.7745	58.40	116.4	2764.1		
3	Normal	1.0119	2.8210	64.10	145.4	3069.9	3029.6	−48.9
		1.0119	2.7177	66.05	139.6	2989.4		
	After injection: 300 cc.	1.0110	2.2705	61.10	121.6	3102.4	3078.5	
		1.0110	1.9056	52.60	120.4	3054.7		

TABLE 4

	EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0119	1.8746	61.93	127.4	2410.1	2423.1	−637.0
		1.0119	1.4588	52.63	115.4	2436.2		
	After hemorrhage and injection	1.0102	1.8196	53.35	114.6	3024.0	3060.1	
		1.0102	1.8756	54.61	112.7	3096.2		
2	Normal	1.0215	1.5828	49.22	130.7	2472.1	2490.2	−325.4
		1.0215	1.6215	51.60	125.9	2508.3		
	After hemorrhage and injection	1.0180	1.8855	61.70	110.6	2785.7	2815.6	
		1.0180	1.4697	50.80	102.5	2845.6		

The stimulations resulted in each case in reductions in the discharge of lymph, each reduction being ushered in by a brief period of increased flow. The lymph collected during these periods presented a much greater viscous resistance than the normal lymph and showed, moreover, a much greater specific gravity. Thus, a comparison of the coefficients of experiment 6 will show that this procedure has increased the viscosity by 524 points. In other words, the normal relationship of 1:1.7 has been changed in this case to a relationship of 1:2.2. This alteration is also indicated by the specific gravity, because the normal value of 1.0230 has given way to the value 1.0285. While, therefore, the stimulation of the splanchnic nerve leads to a lessened production of lymph, the lymph transferred at this time into the duct is not only more concentrated but also more viscous.

Table 3 illustrates the changes in the viscosity of the lymph resulting in consequence of the introduction into the venous circulation of varying quantities of normal saline solution. The injections were made shortly after the completion of the determinations of the normal viscosity. From 100 to 300 cc. of saline were injected at a time and about fifteen minutes were allowed to elapse before the viscosity was again tested.

The results show that large doses of normal saline solution tend to lessen the viscosity in a slight measure. Smaller doses, on the other hand, may increase it, presumably on account of the fact that the more favorable dynamic conditions subsequent to the injection tend to augment the absorption and the transfer of the lymph to central channels. As is illustrated by table 4, a very rapid reduction of the viscosity results, however, if the injections of saline solution are preceded by a moderate hemorrhage. Under this condition, very naturally, the blood and lymph pressures must suffer, allowing a more prompt "thinning" of the latter to take place. In the experiments here cited 200 cc. of blood were displaced by an equal quantity of saline solution. The tests were made fifteen minutes after the injection.

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STUDIES ON ANIMAL DIASTASES

II. The Effect of the Administration of Various Substances on the Blood Diastase of Rabbits

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Received for publication October 13, 1917

In 1846 Magendie (1) demonstrated the existence of starch-splitting enzymes in drawn blood and sought to demonstrate their existence in the living blood by the injection of boiled starch. Claude Bernard, Schiff, Tiegel, Pavy, Tieffenbach, Böhm and Hoffman found sugar in the urine after the intravenous injection of starch or glycogen. Bial (2) was one of the first investigators who studied the question of blood and lymph diastase with improved methods but Röhman (3) is credited with having first proved the existence of diastase in the living body. He injected glycogen into a lymph vessel of the dog's leg and found an excess of sugar in the lymph drawn from a thoracic duct fistula. Fischer and Niebel (4) proved conclusively the presence of diastatic enzymes in blood and lymph.

Claude Bernard (5) discovered that a puncture of the floor of the fourth ventricle was followed by a transitory form of diabetes. Later Cavazzani (6) found that stimulation of the coeliac plexus caused an increased sugar production in the liver and Levene (7) obtained the same results by stimulation of the vagus. Carlson and Luckhardt (8) concluded that if the liver was the chief source of blood and lymph diastase, the increased glycolytic activity of the liver caused by nervous stimulation might involve an increased output of the liver diastase into the blood and lymph. They found this to be the case.

In cases of definite anatomical lesions the diastatic activity increased in both serum and urine. Schlesinger (9) observed an increase of blood diastase after ligating the duct of Wirsung. Clerc and Loeper (10) also found an increase in blood diastase after ligating the pancreatic duct in rabbits. Gould and Carlson (11), working with dogs,

reported that the ligation of the pancreatic ducts is followed by a great increase in the diastatic power of the serum within twenty-four hours and assumed this to be due to absorbed amylopsin. King (12) states that there is an increased diastatic activity in both serum and urine after ligation of the pancreatic ducts. Wohlgemuth and Noguchi (13) have reported that in cases of abdominal trauma where the pancreas was injured, there is a quantitative increase of amylase in the urine; this was confirmed by King (12).

Bainbridge and Beddard (14) and Carlson and Luckhardt (8) recorded that the removal of the pancreas from cats did not greatly affect the concentration of blood diastase. Schlesinger (9) reports, on the other hand, that pancreatectomy leads to complete disappearance of amylase from the blood. Otten and Galloway (15) found after complete removal of the pancreas in dogs that the blood diastase sinks rapidly, then rises somewhat, remaining at a constant level but never returning to its normal height. King (12) also claims a reduction but not a complete disappearance. Later Milne and Peters (16) obtained a decided increase of blood diastase after complete pancreatectomy.

Van de Erve (17) found that the removal of the kidney has no appreciable effect on the diastase in serum.

King (12) reported that after profound depression, for example, in parathyroid tetany, there is a high uniform content of amylase in the urine.

Many investigators have found that in some types of experimental hyperglycemia and glycosuria there is an increase of diastase at the same time in the blood and the urine.

In cases of human diabetes, Loewi, Moeckel and Rost, and more recently Myers and Killian (18) have reported an increased diastase in the blood. In the impairment of renal function, Loeper, Ficai, Hirata, Stocks, Myers and Killian have found an increase of diastatic activity in the blood and a decrease in the urine. In nephritis, Wohlgemuth, Corbett and King noted a decrease in the amylase content as compared with that of normal urine.

The experiments detailed in the present paper were undertaken to determine the influence of the administration of certain drugs and human saliva on the diastatic activity in the blood and urine.

METHODS

A roughly quantitative method for the estimation of diastase was devised by Wohlgemuth in 1908 (19). He based his method on the hydrolysis of starch into erythro-dextrin by amylase. Recently Myers and Killian (20) have suggested a more accurate method for the determination of the diastatic activity in blood and urine, based upon the hydrolysis of starch to glucose. This method was employed in the present investigations. Since the diastatic activity of rabbit blood is more than double that of human blood, one cc. of blood was employed for the test instead of the 2 cc. used by Myers and Killian for human blood. The calculations are for the one cc. For the determination of sugar in the blood and urine, the Myers and Bailey (21) modification of the Lewis-Benedict method was used. While this method does not admit of the accurate determination of the absolute amount of sugar in the urine, comparative results are very easily obtained and it is with these that we have to do in this investigation.

In all the following tables the percentage of blood sugar is higher than in normal rabbits, this being probably due to the feeding of bread, milk and barley before the experiments. The rabbits used in these experiments were injected with several types of drugs at different periods but no single drug was used twice on the same rabbit. A sufficient period of time was allowed to elapse after the first injection to permit all effects of the drug to pass away before a second injection. The rabbits used were all full grown. The blood drawn from the ear vein was treated with potassium oxalate.

Intravenous injection of human saliva. The technic of the collection of saliva. To collect saliva, the mouth was washed several times with distilled water and the saliva was then collected in sterilized centrifuge tubes. The flow of saliva was stimulated by chewing paraffin. By centrifuging the saliva in an electric centrifuge at full speed for over one-half hour, most of the bacteria and solid material were removed and the supernatant fluid was then used for injection into the ear vein.

King (12) injected saliva intravenously into dogs and stated that more than 2.5 cc. of human saliva per kilo body weight produced signs of collapse resembling an anaphylactic shock. In our experiments we found that rabbits tolerated more than double the dose given by King.

Two rabbits weighing 1750 grams and 1900 grams, respectively, were each injected with 5 cc. saliva and showed no symptoms whatso-

ever. A second injection of 10 cc. of saliva was made and rabbit 2 died with symptoms of septicemia. Rabbit 1 showed no symptoms and was again injected with 10 cc. saliva, but without deleterious effect.

In table 1 we see that after the injection of 5 cc. saliva there is an increased diastatic activity in the blood, which ceases after forty-eight hours. This fact is in harmony with King's experiments. When 10

TABLE 1

The sugar and the diastatic activity of the blood after injection of human saliva

RABBIT 1				RABBIT 2			
Date	Blood sugar	Diastatic activity	Amount of saliva injected	Date	Blood sugar	Diastatic activity	Amount of saliva injected
1916	*per cent*			*1916*	*per cent*		
12/20	0.16			12/20	0.13		
12/21	0.13	25	5 cc. at 11.20 a.m.	12/21	0.13	31	5 cc. at 11.30 a.m.
12/22	0.15	33		12/22	0.12	36	
12/23	0.11	20		12/23	0.10	20	
12/24	0.15	24		12/24	0.14	26	
12/25	0.15	29	10 cc. at 12.00 m.	12/25	0.12	29	10 cc. at 12.00 m.
12/26	0.15	47		12/26	0.15	45	
12/27	0.15	34		12/27	0.12	40	
12/28	0.18	32		12/28	0.10	36	
12/29	0.14	36		12/29		33	
12/30	0.16	30					
12/31	0.19	35					
1/1/1917	0.18	34	10 cc. at 2.30 p.m.				
1/2	0.16	54					
1/3	0.16	30					
1/4	0.16	34					

cc. saliva are injected, the increased diastatic activity does not stop after forty-eight hours but continues for several days.

Parenteral administration of soluble starch. King (12) gave 100 cc. of 2 per cent starch solution daily to a cat and 300 cc. to a dog by stomach tube and stated that there was no appreciable change in the amylolytic activity of the urine in these animals. However, after an intravenous injection of starch into two dogs, he obtained an increase of amylase in the urine, which reached its maximum on the second day after the injection and lasted about four days.

We administered soluble starch solution several times intraperitoneally to two rabbits, with a lapse of some days between each administration. Blood was drawn daily at 9.00 a.m. and urine collected from 9.00 a.m. to 9.00 a.m. the following day, toluene being used as a preservative. The amylolytic activity of the blood increased on the

TABLE 2

The sugar and the diastatic activity of the blood after the injection of starch solution

RABBIT 3				RABBIT 4			
Date	Blood sugar	Diastatic activity	Amount of starch injected into peritoneal cavity	Date	Blood sugar	Diastatic activity	Amount of starch injected into peritoneal cavity
1917	*per cent*			*1917*	*per cent*		
1/8	0.16	32	1 gram starch in 50 cc.	1/8	0.14	32	1 gram starch in 50 cc.
1/9	0.14	36	H_2O at 2.30 p.m.	1/9	0.15	31	H_2O at 2.30 p.m.
1/10	0.17	24		1/10	0.22	25	
1/11	0.13	35		1/11	0.13	37	
1/12	0.14	32		1/12	0.13	37	
1/13	0.14	32	0.5 gram starch in 25 cc.	1/13	0.13	35	0.5 gram starch in 25 cc.
1/14	0.14	36	H_2O at 2.30 p.m.	1/13	0.16	40	H_2O at 2.30 p.m.
1/15	0.15	35		1/15	0.15	37	
1/16	0.15	39		1/16	0.14	36	
1/17	0.14	32	3 grams starch in 100	1/17	0.14	32	3 grams starch in 100
1/18	0.13	35	cc. H_2O at 3 p.m.	1/18	0.14	32	cc. H_2O at 3 p.m.
1/19	0.14	36		1/19	0.14	32	
1/20	0.13	33		1/20	0.14	38	
1/21	0.12	34		1/21	0.13	39	
1/22	0.14	36	7 grams starch in 150	1/22	0.15	39	7 grams starch in 150
1/23	0.12	36	cc. H_2O at 3.30 p.m.	1/23	0.19	33	cc. H_2O at 3.30 p.m.
1/24	0.12	37		1/24	0.11	39	
1/25	0.13	35		1/25	0.13	39	
1/26	0.16	40		1/26	0.18	42	
1/27	0.14	38		1/27	0.14	42	
1/28	0.12	38		1/28	0.16	48	
1/29	0.13	37		1/29	0.13	41	

day following the injection of starch with the exception of the first injection of rabbit 4. The dose in this case, however, was small. In the last two injections large doses were given and we found that the activity of amylase failed to increase and in some cases decreased. However, in cases where large doses were given the amylolytic activity

was elevated on successive days, especially in rabbit 4. The variation in the amount of diastase in normal rabbits is very large from day to day. It can be said with certainty, however, that the amylase content of the blood increases quantitatively after the injection of starch and also that the diastase is used to a greater extent than usual after the injection of large doses of starch. This explains the fact that following large doses of starch, there is no immediate increase of blood diastase. The urine was collected only after large doses of starch and the results

TABLE 3

The sugar and the diastatic activity of the blood before and after administration of $NaHCO_3$

RABBIT 11				RABBIT 12			
Date	Blood sugar	Diastatic activity	Remarks	Date	Blood sugar	Diastatic activity	Remarks
1917	*per cent*			*1917*	*per cent*		
2/27	0.10	32	Blood usually taken at	2/27	0.09	31	Blood usually taken at
2/28	0.12	30	9 a.m.	2/27	0.09	29	9 a.m.
3/1	0.14	37	5 grams $NaHCO_3$ in 25	3/1	0.12	32	5 grams $NaHCO_3$ in 25
3/2	0.12	36	cc. H_2O at 10 a.m.	3/2	0.13	48	cc. H_2O at 10.30 a.m.
3/3	0.17	35	Blood taken at 2 p.m.				Blood taken at 2.30
3/4	0.21	35					p.m.
3/5	0.13	31					
3/6	0.14	34	10 grams $NaHCO_3$ in 50				
3/7	0.21	32	cc. H_2O at 3.30 p.m.				
3/8	0.16	34	Blood taken at 1 p.m.				
3/9	0.14	36					
3/10	0.14	32					
3/11	0.19	29					
3/12	0.15	25					

were identical with King's findings. It is certain that an increased diastase in the blood is followed by an increased elimination in the urine.

The effect of $NaHCO_3$ *administered parenterally upon the blood and urine diastase.* For this experiment two rabbits were each injected intravenously with 5 grams $NaHCO_3$ dissolved in 25 cc. H_2O. Both the blood sugar and amylase rather increased. After an interval of five days, one of the rabbits was again injected intravenously with 10 grams $NaHCO_3$ dissolved in 50 cc. H_2O and it was noted that the blood sugar and amylase increased more than usual.

King pointed out that after he fed a cat with 5 grams $NaHCO_3$, the urine was approximately neutral and the diastase content of the urine was decreased. In our case after the injection of 5 grams $NaHCO_3$, there was a marked decrease of diastase in urine which lasted two days; when 10 grams $NaHCO_3$ were injected, this marked decrease lasted for three days. This decreased activity of diastase may be due to the alkaline urine since the blood diastase remained high during this period.

Two rabbits were injected intraperitoneally with 10 grams and 5 grams $NaHCO_3$ dissolved in 50 cc. H_2O, respectively. Blood was drawn four times from these rabbits, an hour after the injection and at hourly intervals thereafter. The blood sugar and diastase increased

TABLE 4

The blood sugar and the diastatic activity of the blood in normal rabbits

RABBIT NO.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY OF THE BLOOD				
	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
37	0.14	0.14	0.14	0.13	0.14	35	35	34	35	35
39	0.15	0.14	0.16	0.15	0.15	23	24	23	21	23
40	0.16	0.15	0.16	0.16	0.16	33	32	31	31	30
41	0.17	0.16	0.14	0.15	0.17	23	23	22	22	21
Average	0.15	0.15	0.15	0.15	0.10	28	28	27	27	27

after each successive bleeding. Both rabbits died on the day of the experiment.

The normal variation of the sugar and the diastase of the blood during successive hours. The above group of rabbits was used as an aid in comparing the various types of investigations. As Table 4 shows the individual variation in blood sugar and diastase within four to five hours is very slight, although there is a wide variation between the blood sugar and diastase in different rabbits. Blood was drawn before and after the administration of the drug and the results compared.

The effect of the intraperitoneal administration of Na_2CO_3 *solution on the blood diastase.* Underhill (22) has shown that in normal rabbits the intravenous injection of Na_2CO_3 solution causes a hypoglycemia lasting for one or one and one-half hour. Underhill and McDanell (23) repeated the same experiments, but failed to obtain the above results. However, they (24) confirm the statement of Underhill that the hyperglycemia and glycosuria provoked by epinephrin are both

significantly decreased, if Na_2CO_3 is administered at a suitable period of time previous to the epinephrin introduction. We injected Na_2CO_3 into the abdominal cavity and found no appreciable change in the sugar and diastase content of the blood.

TABLE 5

The sugar and the diastatic activity of the blood before and after administration of Na_2CO_3 solution

RABBIT NO.	AMOUNT OF Na_2CO_3 SOLUTION ADMINISTERED INTO PERITONEAL CAVITY AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN THE BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
15	50 cc. of 1 per cent solution	0.14	0.14	0.16		0.16	30	31	32		30
16	100 cc. of 0.5 per cent solution	0.13	0.15	0.17		0.17	35	32	36		37
35	100 cc. of 0.4 per cent solution	0.15	0.15	0.16	0.17	0.19	16	16	16	17	16
36	50 cc. of 0.4 per cent solution	0.14	0.17	0.17	0.17	0.16	19	23	22	22	21
38	50 cc. of 0.4 per cent solution	0.14	0.14	0.16	0.18	0.20	21	23	23	21	22

The effect of HCl *solution by mouth on the blood diastase.* Acid introduction or acid production in the body exerts a distinct influence on carbohydrate metabolism.

Elias (25) states that introduction of acids into dogs and rabbits leads to hyperglycemia and glycosuria. In this investigation we only followed the changes in the blood for four hours after the ingestion of acid, and this may not be long enough to show the influence of this drug. In some cases a rise in the blood sugar was noted one hour after

TABLE 6

The sugar and the diastatic activity of the blood before and after administration of HCl solution by mouth

RABBIT NO.	HCl SOLUTION BY MOUTH AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
42	100 cc. of 0.5 per cent solution	0.14	0.16	0.14	0.16	0.17	23	22	23	26	23
43	100 cc. of 0.8 per cent solution	0.16	0.21	0.18	0.18	0.17	22	25	24	26	27
48	100 cc. of 1 per cent solution	0.16	0.21	0.17	0.17	0.16	28	28	25	27	28
49	100 cc. of 1 per cent solution	0.14	0.20	0.18	0.19	0.19	23	24	24	25	25
44	150 cc. of 1.36 per cent solution	0.16	0.17	0.16	0.17	0.18	26	26	25	25	26

ingestion of HCl, but the results were not uniform. We do not consider this rise as being due to the influence of the acid. The HCl has no noteworthy effect on the diastase content of the blood, with the exception of rabbit 43 which shows a slight rise in diastase.

The effect of the injection of epinephrin upon blood diastase. Blum (26) in 1901 discovered that adrenal extract injected subcutaneously gives rise to glycosuria. Early in 1902 the discovery was made by Herter and Richards (27) that the injection of a solution of adrenalin chloride into the peritoneal cavity of dogs was followed by an intense though transient experimental glycosuria. Herter and Wakeman (28) also reported experimental glycosuria by epinephrin injection. Metzger (29) proved definitely that the glycosuria resulted from hyperglycemia.

TABLE 7

The sugar and the diastatic activity of the blood before and after injection of adrenalin

RABBIT NO.	ADRENALIN SUBCUTANEOUS INJECTION AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
20	0.5 cc. per kilo	0.16	0.55	0.71		0.56	37	41	35		37
50	0.5 cc. per kilo	0.15	0.43	0.52	0.47	0.38	27	31	30	29	30
21	0.5 cc. per kilo	0.17	0.51	0.54	0.58	0.62	29	31	32	28	28
34	0.4 cc. to 1350 grams rabbit	0.17	0.24	0.22	0.20	0.18	20	21	18	20	20

Vosburgh and Richards (30) have made a somewhat detailed study of the sugar in the blood after the intravenous injection of adrenalin as well as after application of that substance to the pancreas. They noted that the glycosuria provoked by epinephrin was due to an increase of sugar in the blood. Later many investigators proved that the increased sugar came from the liver glycogen.

Starkenstein (31) found no appreciable difference in the diastase content after using adrenalin piqûre and various other procedures. Allen (32) stated that adrenalin, phlorizin, phloretin and asphyxia have no effect on blood diastase.

In our investigation marked hyperglycemia and glycosuria appeared, but the diastase content of the blood was not affected.

Besides the above experiments, two rabbits were injected repeatedly with 0.5 cc. to 1.0 cc. epinephrin during a period of from ten to eighteen hours. There was no effect on the diastase content but there appeared

a decrease in blood sugar after successive injections. The diastase content and blood sugar increased markedly before death in a rabbit injected with 1 cc. epinephrin.

The effect of ether anesthesia on the concentration of blood diastase. Hawk (33) and Seeling (34) noted that glycosuria appeared after ether anesthesia; other anesthetics also possess the power to induce hyperglycemia, and at the same time glycosuria. Carlson and Luckhardt (8) found a decreased diastatic activity during ether anesthesia. Blood was drawn during anesthesia which varied from twenty minutes to two hours. Bloods taken before and after anesthesia were compared and the serum drawn during anesthesia had a lesser diastatic activity than the normal serum, but this variation is not much greater than is exhibited in normal animals. They attribute this phenomenon to

TABLE 8

The sugar and the diastatic activity of the blood before and after ether anesthesia

RABBIT NO.	PERCENTAGE OF BLOOD SUGAR			DIASTATIC ACTIVITY IN BLOOD		
	8.55 a.m.	10 a.m.	11 a.m.	8.55 a.m.	10 a.m.	11 a.m.
30	0.14	0.22	0.18	29	32	28
31	0.16	0.25	0.20	25	24	22
32	0.14	0.34	0.18	26	29	25
33	0.16	0.26	0.16	28	32	28
Average	0.15	0.27	0.18	27	29	26

the inhibitory action of the anesthetic on the enzyme since there is an increased diastase production during anesthesia. In similar experiments Ross and McGuigan (35) obtained negative results. Carlson and Ryan (36) reported that during anesthesia cat's saliva contains more diastase than usual, this being due to increased concentration of the blood diastase. Carlson and Luckhardt determined the amount of diastase (1) by the rate of clearing, and (2) by the rate of the complete disappearance of erythrodextrin in starch solution.

Four rabbits were anesthetized for exactly one hour in our experiments, and the activity of the blood diastase was compared just before and after and also one hour following the anesthesia.

All rabbits showed hyperglycemia just after anesthesia. The diastase was increased slightly but not markedly on the average, and this increase cannot be considered due to the influence of ether. The blood drawn one hour after anesthesia showed a slight decrease in diastase content.

The effect of the administration of pituitrin on blood diastase. Pituitrin has no important influence on carbohydrate metabolism. In this experiment tablets were administrated by mouth in two cases and pituitrin (Parke-Davis) was injected subcutaneously in two other cases. There was no change in the blood diastase, but the blood sugar increased, especially in experiment 24. This phenomenon may be explained by

TABLE 9

The sugar and the diastatic activity of the blood before and after administration of pituitrin

RABBIT NO.	PITUITRIN ADMINISTRATIONAT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
22	Gr. III tablet by mouth.....	0.17	0.22	0.19	0.18	0.19	33	31	32	32	31
23	Gr. III tablet by mouth.....	0.14	0.14	0.14	0.16	0.16	35	33	35	36	37
24	0.25 cc. injection into subcutan...	0.15	0.22	0.18	0.24	0.25	23	23	24	22	22
25	0.4. cc. injection into subcutan...	0.17	0.21	0.17	0.18	0.18	26	26	26	25	24

TABLE 10

The sugar and the diastatic activity of the blood before and after feeding thyroid tablet

RABBIT NO.	THYROID TABLET, EACH TABLET = GR. I OF EXTRACT OR = THYROIDS GR. V FRESH SUBSTANCE AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
26	One tablet	0.16	0.16	0.17	0.18	0.18	26	26	26	27	26
27	One tablet	0.13	0.14	0.13	0.13	0.14	30	28	26	26	26
28	One tablet	0.17	0.17	0.16	0.20	0.19	29	31	28	27	29
29	One tablet	0.13	0.14	0.15	0.15	0.15	26	24	25	29	27
Average.....	One tablet	0.15	0.15	0.15	0.17	0.17	28	27	26	27	27

the fact that there is a concentration of the blood, the rabbits excreting large volumes of urine after the administration of pituitrin.

The effect of ingestion of thyroid tablets upon the blood diastase. Flesch (37) found that thyroid feeding and transplantation of the thyroid gland caused alimentary hyperglycemia. Cramer and Krause (38) reported that the glycogen content of the liver was decreased to a minimum after thyroid feeding in rabbits and rats. In dogs the toler-

ance for glucose was distinctly lowered, and they concluded that thyroid feeding inhibited the formation and storage of glycogen in the liver. Carmer and McCall (39) noted that the thyroid hormone stimulated the oxidation of glycogen and this explains the increased oxidation of carbohydrates. Recently, Kuriyama (40) stated that experimental hyperthyroidism induced by thyroid feeding produced neither a change in the blood sugar nor spontaneous glycosuria in rabbits or rats.

In this investigation tablets were given by mouth and results were followed during the first four hours after administration. This length of times does not seem sufficient to affect the body metabolism. However, if there were any changes in the blood diastase, it would at least occur in the last specimens drawn, but no changes appeared in blood sugar or diastase.

SUMMARY AND CONCLUSIONS

1. The intravenous injection of human saliva in rabbits causes an increase in the amylolytic activity of the blood.

2. The parenteral administration of soluble starch increases slightly the diastase of the blood and causes an elimination of amylase in urine a few days after the injection. Injection of large doses does not produce a sudden change in the blood but causes a successive increase over a period of several days.

3. The intravenous injection of $NaHCO_3$ (5 or 10 grams) shows a slight increase in blood diastase and sugar. When the above dose was injected intraperitoneally, a marked hyperglycemia and an increase of diastase occurred. The injection of the above dose into the peritoneal cavity caused the death of the rabbits.

4. The injection of smaller doses of Na_2CO_3 intraperitoneally had no effect on the blood diastase or sugar.

5. No marked changes in the blood diastase and sugar appeared within four hours after the ingestion of HCl.

6. The subcutaneous injection of epinephrin causes hyperglycemia and glycosuria but produces no appreciable change in the blood diastase. Intravenous injection causes hyperglycemia and at the same time an increased amylase in a fatal case.

7. Ether anesthesia is followed by hyperglycemia but the diastase remains practically constant except for a slight tendency to increase immediately after the anesthesia.

8. Pituitrin injection or ingestion has no effect on the blood diastase or sugar. The increased sugar content observed was probably due to a

concentration of blood, caused by an excessive excretion of urine after the administration of the pituitrin.

9. Thyroid feeding has no appreciable effect on the sugar and diastase content of the blood within four hours after feeding.

The author acknowledges his indebtedness to Prof. V. C. Myers for his suggestions concerning this work.

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THE DIFFERENTIATION OF THE MINIMAL CONTRACTION IN SKELETAL MUSCLE

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Received for publication October 16, 1917

With all the work published on the activity of skeletal muscle and the very frequent use of the terms *threshold* and *minimal*, there has been little if any investigation to determine directly the character of the true minimal contraction. This is doubtless owing to several reasons. In the first place, muscle has been worked upon as a continuous system, responsive in imperceptible degrees of difference to continuously varying strength of stimulus. The absolute minimal has been an ideal, infinitesimal in value and hence indeterminable by observation. In the second place, an arbitrary standard—the least perceptible contraction—has been set up, and found adequate in practice for determinations of threshold. In the light of the all-or-none principle, however, the way is open for more definite analysis which, in its turn, will further test the validity of that principle. Moreover, since the motor nerve fiber is known to actuate a multiple contractile system, the true minimal is to be sought not by indirect but by direct excitation of the unit components of the system. In order, therefore, to generalize concerning fundamental muscle activity it is not sufficient to accept conclusions drawn from the behavior of the muscle as a whole. A clear dynamic conception must be brought one step nearer by the study of the single element. Such a study has been made possible by the development in this laboratory of the capillary pore electrode, through the use of which the results here presented have been secured.

It seems probable that the smallest muscular unit capable of individual activity is the fiber. That this activity is, under varying strength of stimulus, fixed, is further to be regarded as probable (1), (2). In the directly excited muscle, therefore, it should be possible by sufficiently local stimulation to reveal a response of definite, irreducible value. Such a response should prove to be that of a single fiber.

In the course of many experiments in this laboratory, utilizing the above-mentioned apparatus, a small group of muscle fibers (often apparently a single fiber) was observed to glide independently among its neighbors, drawing upon the latter as passive structures. Again, quite frequently, a straight capillary running across the fibers would be drawn to a marked angle by the action of the fiber or fibers beneath. Such observations have led to the definite attempt, reported in this paper, to identify the minimal contraction with the functionally isolated fiber. It has recently been shown by Pratt (2) that the gradients of varying activity characteristic of skeletal muscle, including those of fatigue and staircase, may exhibit in common an all-or-none or "quantal" constitution. The work here set forth is designed to bring further light to bear upon the basic unit concerned in such effects, revealing it to direct observation.

METHODS

The apparatus for the direct stimulation of the muscle fiber is, with slight change in form but not in principle, that described by Pratt (3). The modification has been essentially for the purpose of permitting observation of the contracting element up to within two or three millimeters of the point of stimulation, and is shown in figure 1, with the omission of details of support, etc. It will be noted that the physiological solution covering the preparation is made to serve as the indifferent pole. The muscle used is the sartorius of a surviving preparation —a pithed leopard frog (R. pipiens) of medium size, uncurarized, with circulation intact. The muscle is exposed by cutting and laying back the skin on the ventral surface of the thigh. Both sides are prepared at the same time for convenience in exposing the entire length of both sartorius muscles. With the frog placed on its back and the solution just covering the bared tissues, a low-power objective can easily be brought to focus upon any portion of the muscle. In such a preparation the capillary circulation is readily observed throughout an experiment, frequently as long as six to eight hours. It is often at first very rapid, while later it may diminish considerably; the individual corpuscles may then be easily distinguished even with the direct illumination necessarily used. The illuminating system consists of two parts; one of moderate intensity for direct visual observation, and the other a very intense white spot-light produced by focussing the rays from a twelve candle-power tungsten bulb through a two-thirds microscopic objective—a method similar to that devised by Patten (4) for localized

stimulation by light. This, or a similar white light directly applied, has been used for most of the photographic records. These are secured by drawing a photographic plate over a suitable shutter arrangement placed over the ocular of the microscope, which retains its usual system of lenses. The moving mechanism consists of a plate-holder gliding very smoothly over glass slides and drawn by the spindle of a kymograph. This arrangement permits of any rate of movement or frequency of exposure needed. Records are traced, as in figure 3, by the light reflected from minute globules of mercury sprayed under high pressure from a fine orifice at the end of a glass tube. A globule,

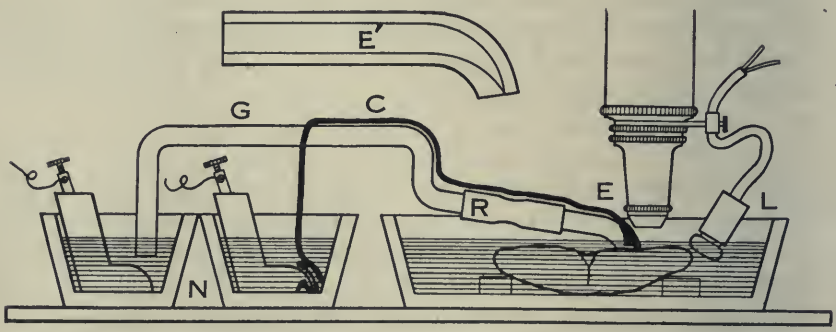

Fig. 1. Modification of the capillary pore electrode and accessory parts; preparation immersed in a physiological solution. *N*, baths of same solution, with non-polarizable boot-electrodes (Porter); *G*, glass tube supporting pore-bearing tube, *E*, and filled with the solution; *R*, rubber connection, permitting delicate and yielding pressure of electrode upon tissue; *C*, narrow strip of chamois, saturated with the solution and forming, with the liquid in contact with the preparation, the indifferent terminal; *E'*, section (actual size) of the pore-bearing tube—the active terminal.

resting immediately over the active region of the muscle and separated from it only by a very delicate fascia, moves in a majority of cases exactly with the contracting fiber or fibers. In a few instances where the globule movement is not so great as that of the fiber, owing probably to heavier fascia, the movement is strictly relative as observed with the ocular micrometer.

Tetanizing stimuli are produced by the magneto-inductor previously used in this laboratory (2) giving 50 to 60 alternations of current per second, with the intensity under very delicate control by the operator at any instant. Twitches of muscle fibers are made with induced break shocks only. The primary circuit of the inductorium, with a

single Daniell cell, includes a large straight rheocord serving two purposes: first, to reduce greatly the sparking at the platinum-mercury key; and second, to control delicately the intensity of the stimuli.

The procedure in most experiments is to apply the electrode to the surface of the sartorius at about the junction of the middle and distal thirds, shifting the electrode until a suitable contracting element can be observed. It is not difficult to select a fiber out of a very small group in any location by simply sliding the electrode carefully across the fibers by means of the horizontal adjustment. In the sartorius, however, which is generally supposed to contain a majority of long, straight fibers, it is found that many fibers which lie superficially at the point of stimulation are very shortly buried beneath the surface, many times not to reappear. The region of stimulation, distal, was first selected because of its presumable freedom from nerve filaments. However, there has never been any difficulty in avoiding indirect stimulation, even in other locations. A number of experiments have been made with the electrode applied to the pelvic end of the muscle. This is a more difficult procedure, owing to the tilt of the surface in this location, but the results have been equally good in every respect. In some experiments, where the extent of contraction has been rather limited, a greater movement has been obtained by cutting the surface fibers at one or the other end of the muscle, together with the overlying fascia. The resulting difference is that excursion is increased, with active and idle fibers more readily distinguished. The muscle may be further relaxed by flexing the leg on the thigh.

Observations are made by watching with the micrometer ocular the movement of the mercury globule, or that of a minute speck of carbon blown on the surface of the preparation; or, finally, by observing directly the movement of small capillaries that chance to cross the particular element under observation, and themselves frequently serve as clear indices for scale reading. For the purpose of this paper the muscle fibers themselves have to a large extent been observed directly with the microscope. The average width of a fiber as exposed on the surface is about 40 μ in the medium sized specimens used.

The stimulating current is always of very low intensity. The full capacity of the several generating apparatus, just perceptible to the tip of the tongue, is never used; and even further decrement must result from the high resistance at the electrode pore. An attempt has been made several times to use a pore of 3 μ diameter without success; apparently the full current was insufficient to pass so fine an orifice

and still have sufficient intensity to stimulate a fiber. The electrode now in use has a very clean-cut circular pore of 7 μ diameter; it seldom becomes plugged in use, and is of the form shown in figure 1, E and E'.

To enable localized activity to reveal itself the more distinctly by photographic recording, the following method is used. The frog is prepared as usual and the sartorius tibial tendon carefully cut without other injury to the muscle. This leaves the circulation intact and permits the surface fibers to relax. The source of illumination is placed on a level with the fibers to be observed, thus showing them in relief. The stimulating electrode is placed at about the middle of the sartorius, while the record is taken near the tibial end of the muscle where the relaxation of the fibers is greatest.

For the recognition of a minimal effect, the appearance of the activity of a single fiber is not alone sufficient; further tests must be applied. The apparent fiber must not be capable of variable response or give any evidence of a possible smaller contraction. This is determined in two ways. First, the strength of the stimulus is repeatedly increased and decreased very gradually above and below the apparent threshold. Often a very marked alteration of coil position may be made without changing the response from its uniform character. If no smaller step is in this way differentiated, a second test is used if any doubt exists. A uniform stimulation series for the apparent minimal response is maintained until the rising threshold in fatigue induces relaxation. If this occur in a single step, the test is considered satisfactory. If it take place in two or more steps, it shows the probability of being caused by the elimination of more than one fiber (2). The last step is here found by test to be all-or-none, and the single active fiber can usually be satisfactorily observed unless it has ceased to be superficial at the point of observation. Under such conditions the activity, even though it be of a single fiber, will impart a general passive movement to the fibers in that location. This movement will also be found to be all-or-none in character.

OBSERVATIONS AND EXPERIMENTAL RESULTS

The four drawings in figure 2 illustrate the test above mentioned, and are made from sketches secured during observations in an early experiment. In this experiment the pore of the electrode used was about 35 μ in diameter, much larger than that subsequently employed. Views A and B are of the same portion of a field at rest and active.

The illumination used was medium in intensity and produced, at the depression of sartorius surface along the edge of the rectus abdominis, a linear reflection of light expressed in the drawings as a broad line. The distortion of this line, as shown in drawing *B*, from tension directed nearly at a right angle, would suggest a very limited activity, if not that of one fiber. On further test, however, as shown in views *C* and *D*, more than one unit could be differentiated. In these two latter views (the same field as of the former) the illumination was greatly increased and was directed from a different point, giving the multiple reflections as shown. The same current strength was applied for both *B* and *D*;

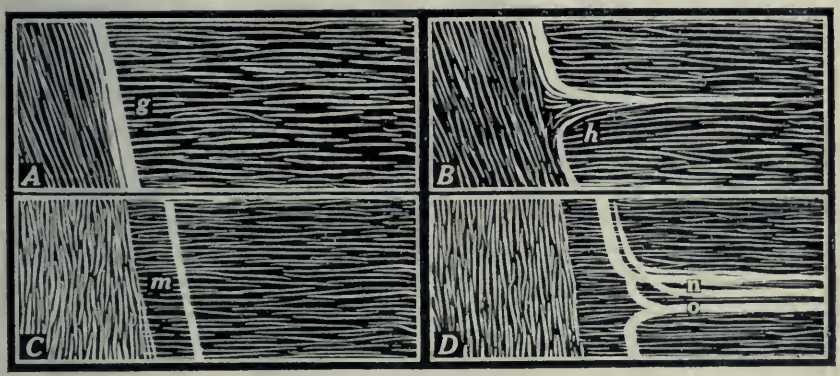

Fig. 2. Experiment of September 6, 1916. *A*, the preparation at rest; *g*, linear reflection at margin of rectus abdominis. *B*, the preparation stimulated; *h*, distorted reflection from local tension in sartorius. *C*, at rest under altered illumination; *m*, marginal reflection. *D*, again stimulated; *n*, tension lines eliminated in fatigue; *o*, residual line of minimal tension. The letters, *A*. *B*, *C*, *D*, rest upon the rectus abdominis. Further description in the text.

the difference appeared only on change of illumination. Next, the stimulus was applied continuously until the contracting field relaxed. The activity did not, however, end in one abrupt relaxation, but first *n* suddenly yielded, followed several seconds later by *o*, showing definitely that what had at first appeared as the possible activity of a single fiber was, in reality, probably that of two.

Figure 3 shows photographically the record of reflection from two globules of mercury on the surface of the sartorius. Five muscle fibers intervened between them. This interval was approximately 200 μ, or $\frac{1}{5}$ mm. The electrode stimulated the fiber beneath the upper globule, which traced twitches of two heights or steps; the higher contraction

approximately $\frac{1}{50}$ mm; and the lower, $\frac{1}{150}$ mm. The muscle fibers crossed at right angles to the abscissa. It will be noted that the lower tracing shows no interruption corresponding to the contractions, thereby indicating the very limited area of activity with stimulation by this method. By direct observation with the high-power ocular the same result was apparent; the second globule did not move even with the higher response produced by more than one fiber. This

Fig. 3. Experiment A, August 15, 1917. Photomicrographic tracing (negative) from two mercury globules on the surface of the sartorius, reading from left to right. The record was made on a uniformly moving plate. Description in the text.

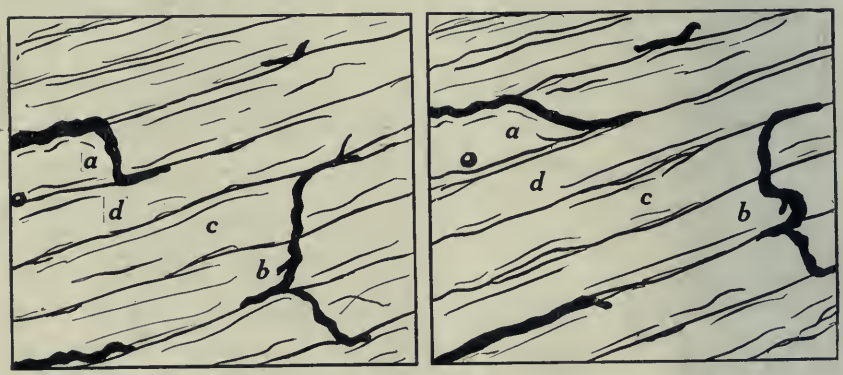

Fig. 4. Experiment A, August 2, 1917. Described in the text.

figure also illustrates the method of test for determining the minimal step by the fatigue process. The twitches were produced by the apparent minimal stimulus, using a Daniell cell with rheocord resistance. The strength of the stimulus remained constant, and was applied every two seconds. The first step, apparently minimal when the experiment began, had soon differentiated from it a lower step which on further observation proved to be all-or-none—the true minimal.

The drawings in figure 4 show the possible activity of two adjoining

fibers, both stimulated by the electrode at the point of application but separated by an intervening fiber in the field of observation. At the right, capillary *a* is pulled to a sharper angle by the contraction of fiber *d*. Capillary *b* shows a distortion to the right of the letter, from the pull of the fiber beneath. A passive fiber, *c*, lies between. At the left the muscle is at rest. An instance such as this is often encountered

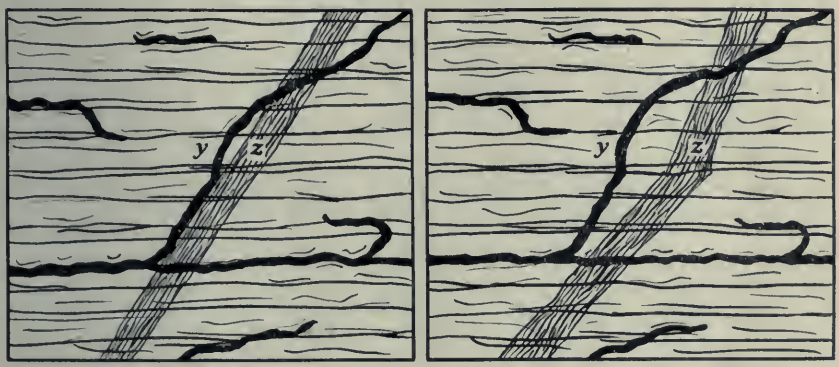

Fig. 5. Experiment of June 14, 1917. Described in the text.

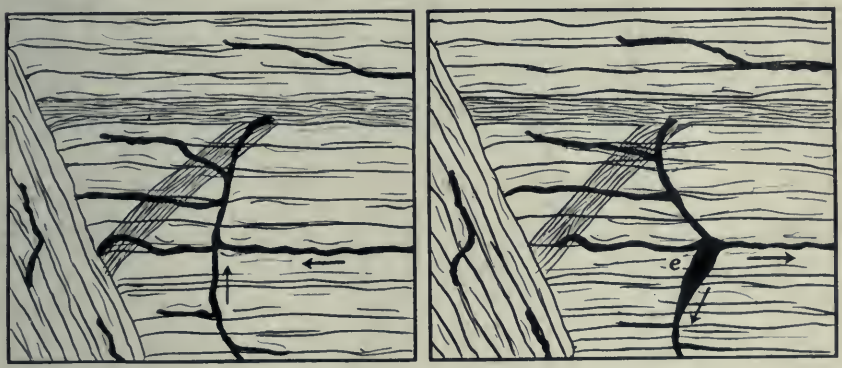

Fig. 6. Experiment of August 28, 1916. Described in the text.

when applying the electrode near the tibial end of the muscle where the fibers rapidly converge to blend with the tendon. Some of the fibers disappear beneath their neighbors to attach to the tendon deeply. Thus, a fiber appearing on the surface at the central end may not be in the field of stimulation at the tendon end. The reverse of this may also be found.

In figure 5 is seen the effect of stimulating a fiber which dips below the surface; z being a deep vessel, y a surface capillary which remains stationary. The vessel, z, is drawn to a marked angle by the hidden contracting element.

Figure 6 presents the reverse phenomenon. A surface fiber is stimulated; the deep vessel remains fixed. It is evident that the sharp angle to which the originally vertical capillary is drawn, the expansion of the capillary at e from stasis, and also the changed direction of the blood-stream as indicated by the arrows, are the result of sharply localized fiber activity.

In figure 7, at the left, is shown the muscle surface at rest with all the fibers relaxed. A deep vessel lies at v. On the right is the same

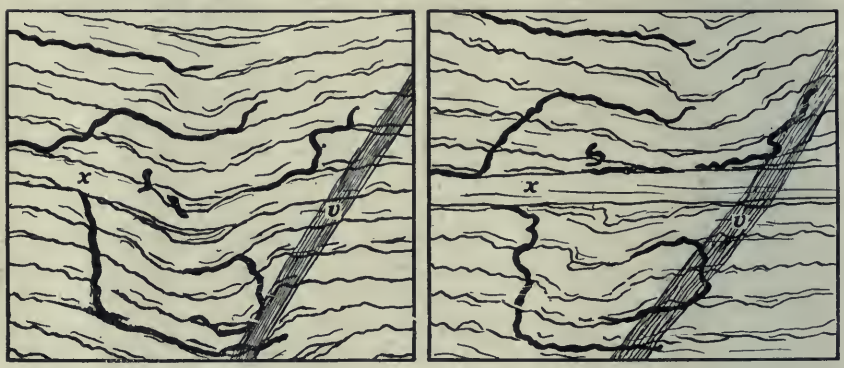

Fig. 7. Experiment of September 17, 1917. Described in the text.

field with the fiber, x, active. Note the tense appearance of x; the slight pull on the deep vessel, v; the changed relative positions of the surface capillaries. This is from a careful sketch from actual observation at the microscope. The threshold in this preparation altered rapidly and it was impossible to secure a good photographic record. The strength of stimulus was changed several times during the sketching to maintain the minimal response.

Figure 8, a photograph, shows at the left the muscle surface at rest. The fiber to contract is marginally indicated as x. The position and angle of the two capillaries crossing this fiber should be noted, as well as the heavy capillary loop, a. The latter remains stationary. At the right is the same field with the fiber, x, in a state of contraction. The abscissa is preserved in a bright spot beneath each globule; the

excursion of the lower globule is, in fact, delicately traced by this point of highest light-intensity. Note the distinctly altered angles of the capillaries crossing *x*, with the passive change as they cross the fibers to the left. The capillary, *a*, remains stationary, although the movement of the mercury globule shows that the fascia upon which it rests is decidedly deformed by the contraction of *x*. By direct observation the fiber, *x*, could be distinctly seen to slide past all its neighbors with a greater freedom of movement.

Figure 9 shows, at the left, the muscle surface at rest. The fibers are nearly straight and not particularly distinct. Capillary *a* is fairly straight. The arch of capillary *c* is quite distinct, crossing two fibers.

Fig. 8. Experiment B, August 2, 1917. Described in the text.

At the right is the same field showing the activity of fiber *x*, which now appears more tense and even straighter than at first, with a slight general movement of the field downward. The fibers on either side of the tense, active fiber have a crumpled appearance very characteristic of idling elements in a relaxed muscle. Capillary *a* is now wavy in form. Capillary *c*, drawn slightly out of focus, still retains practically the same arched contour, showing that the two fibers which it crosses, so decidedly crumpled, still retain their relative position to each other and are not themselves active.

Figure 10 shows at the left the muscle surface at rest, with all the fibers in a relaxed, crumpled position. At the right the fiber, *x*, is in a state of contraction; it appears quite tense and straight. The other fibers are still relaxed. This observation is near the cut tendon, and,

as a result of the contraction, most of the field is drawn more or less out of focus.

Figures 11 and 12 both show the same characteristics: the crumpled appearance of the fibers at rest; in contrast the tense appearance of the contracting fiber x; and the changed relative positions of prominent capillaries.

In all of these photographic records the tests mentioned earlier in this paper were used to differentiate the minimal contraction. However clear these minimal effects may appear as figured, it is entirely impossible for them to impart the conviction, afforded by even one direct visual observation, that the observer is witnessing the absolute minimal

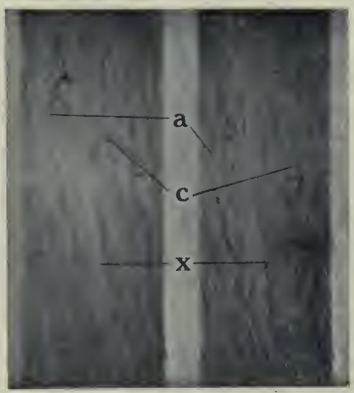

Fig. 9. Experiment C, August 16, 1917. Described in the text.

Fig. 10. Experiment C, September 17, 1917. Described in the text.

response of a muscle—a single fiber, at the will of the operator, gliding smoothly among its neighbors in a living preparation. The use of the capillaries with their changed relative positions is only a convenient aid to show the definitely limited character of the response to stimulation; filled with the circulating blood, they produce sufficient color-contrast with the fibers to record readily on the photographic plate. The capillaries, indeed, often outline the individual fibers. But by direct observation the fiber substance itself may be distinctly seen to move with a swift caterpillar-like effect of shortening, while adjoining fibers lie passive.

On consideration of the details of the method—in particular, the circulation-preparation, the width of the exposed muscle fiber as against the small diameter of the stimulating pore, and the very minute strength

of the exciting current with its probable extremely local effect (3)—it would seem that conditions have been realized highly favorable to the isolated response of a single fiber. It has been shown that in many cases the response, under direct observation, is plainly that of a single

Fig. 11. Experiment of September 18, 1917. Described in the text.

Fig. 12. Experiment of September 20, 1917. Described in the text.

fiber. In cases of doubt, even where change of stimulation strength appears to leave the extent of contraction unaffected, the minimal effect may be differentiated by elimination through the fatigue process. Thus it comes about that even when stimulation, through spread of current, is relatively diffuse, the falling out of successive contractile

units as fatigue manifests itself will tend finally to leave one element "standing." Such a remainder, as I have endeavored to show, is the fiber. Its contraction is of unit value, holding its own through rising threshold or falling stimulus until it, likewise, is abruptly degraded to zero. The minimal contraction is not an arbitrary term; nor does it reflect the abstract ideal attaching to a continuous system. It is a concrete event, readily elicitable by appropriate excitation—visible and capable of measurement.

SUMMARY

1. This paper reports a method for revealing a true minimal entity in the contraction of directly stimulated skeletal muscle. The existence of such an entity is implied in the all-or-none principle of Keith Lucas. Its determination as an observed phenomenon must serve as a further support of that principle and render possible various observations foreign to the study of the muscle as a whole.

2. The sartorius muscle *in situ* of the pithed frog, uncurarized, with intact circulation is used in all experiments. The stimulating apparatus consists in the capillary pore electrode and its accessory devices, with appropriate modifications. Microscopic and photographic apparatus for the purpose of observing and in various ways recording localized fiber activity are described.

3. The procedures involve: observation of local contraction; application of tests for minimal effect; verification, by observation and photographic record, of the uni-fibral origin of an irreducible contraction.

4. A number of drawings and photomicrographs are presented, showing the activity of single fibers, and the minimal function of these is supported by experimental evidence.

My thanks are due to Dr. Frederick H. Pratt for kindly direction during the course of this investigation.

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THE EFFECT OF ALCOHOLIC INTOXICATION ON CATALASE

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Received for publication October 19, 1917

The literature on the subject of alcohol is so large and in many respects so conflicting that no attempt will be made to review it.

We have shown that the catalase of the blood is increased during the stimulating stage of narcosis and decreased during deep narcosis. It was also shown that the increase in catalase was due to an increased output from the liver and that the decrease during deep narcosis was due to a destruction of the catalase by the narcotic. If it is true that catalase is the enzyme in the animal organism principally responsible for oxidation, possibly in the manner suggested by Bach and Chodat, then it may be that the decrease in catalase with resulting decrease in oxidation is the cause of deep narcosis, while the increase in catalase with the resulting increase in oxidation is the cause of the excitement stage. It seems to be a disputed question as to whether alcohol produces a stimulating effect or a depressing effect. If it can be shown that the initial effect of alcohol is to increase the catalase of the blood and hence of the tissues and that during alcoholic coma catalase is decreased, this would seem to argue that the initial effect of alcohol is to stimulate while the remaining effect is to depress. The object of this investigation was to determine the effect of alcoholic intoxication on the catalase content of the blood. Dogs were used in the experiments. Previous to the introduction of alcohol the animals were slightly etherized and during this period of slight anesthesia at least two determinations were made to see that the catalase of the blood had become constant. When this was found to be the case these determinations were taken for comparison as the normal catalase of the blood. Alcohol was then introduced and after this had taken effect, which required as a rule fifteen or twenty minutes, the administration of ether was discontinued. At intervals of fifteen minutes samples of blood were taken and the catalase content determined. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen

peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. After the volume of gas thus collected had been

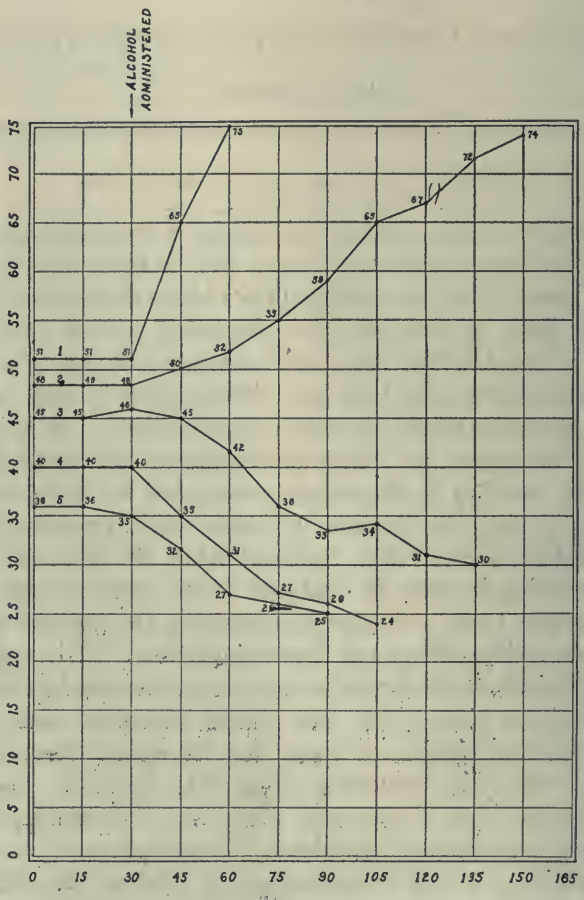

Fig. 1. Curves showing effect of alcohol on the catalase content of the blood. The figures along the abscissa (0 to 165) indicate time in minutes, while the figures along the ordinate (0 to 75) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood.

Curve 1 was constructed from data obtained from a dog after the introduction of 150 cc. of 60 per cent ethyl alcohol into the stomach of

the animal. It will be seen that 0.5 cc. of the samples of blood taken during the thirty-minute interval at the beginning of the experiment and before any alcohol had been introduced into the stomach of the animal, liberated 51 cc. and 51 cc. of oxygen respectively from hydrogen peroxide in ten minutes; that fifteen minutes after the alcohol had been introduced or at the end of the forty-five minute period, 0.5 cc. of the blood liberated 65 cc. of oxygen, while thirty minutes after the introduction of the alcohol, it liberated 75 cc. of oxygen. By comparing the amount of oxygen liberated by the blood previous to the introduction of alcohol with that after the introduction of alcohol, it will be seen that the amount of oxygen had increased from 51 to 75 cc. Curve 2 was constructed from data obtained from a dog after the introduction of 20 cc. of 30 per cent ethyl alcohol at intervals of thirty minutes. It will be seen that 0.5 cc. of blood of the two samples taken previous to the introduction of alcohol liberated 48 and 48 cc. of oxygen respectively from hydrogen peroxide; that after the introduction of alcohol the amount of oxygen liberated increased from 48 cc., the normal, to 74 cc., the amount liberated by the sample of blood after the dog had been under the influence of alcohol for one hundred and thirty-five minutes.

The question that naturally arises in this connection is, how does the introduction of alcohol into the stomach bring about an increase in the catalase of the blood? Since the alcohol is absorbed directly from the alimentary tract into the blood, the answer that suggests itself is that the increased catalase may be due to the direct action of the alcohol on the blood, or to the stimulating effect of the alcohol on the liver thus increasing the output of catalase from this organ.

The following experiment was carried out to determine if the increase in catalase was due to the direct action of the alcohol on the blood. To 18 cc. of defibrinated dog's blood 2 cc. of 95 per cent ethyl alcohol were added. The data from which curve 4 was constructed were obtained from determinations of the catalase of this blood. Two determinations of the catalase of 0.5 cc. of the blood were made previous to the addition of the alcohol, while the remaining determinations were made at intervals of fifteen minutes after the addition of the alcohol. It will be seen that 0.5 cc. of the blood before the addition of the alcohol, liberated 40 cc. and 40 cc. of oxygen respectively; that after the addition of alcohol, the catalase of the blood decreased as is indicated by a decrease from 40 cc. of oxygen, the amount liberated previous to the addition of alcohol, to 24 cc. of oxygen, the amount liberated by 0.5

cc. of the blood ninety minutes after the addition of the alcohol. This observation is interpreted to mean that alcohol of this concentration destroys the catalase of the blood. It might be said in this connection that several other experiments were carried out using much weaker as well as much greater concentrations of alcohol, and it was found that the effect of alcohol was to destroy the catalase, the rate of destruction being faster the greater the concentration.

The data for curve 3 were obtained from a dog into whose jugular vein 40 per cent ethyl alcohol was introduced at the rate of approximately 1 cc. per minute. Previous to the introduction of the alcohol, the catalase content of two samples of the blood was determined. It will be seen that 0.5 cc. of these samples liberated 45 cc. and 46 cc. oxygen respectively from hydrogen peroxide in ten minutes. Fifteen minutes after the introduction of alcohol into the blood, a determination of the catalase content of 0.5 cc. was made. Similarly determinations were made after thirty, forty-five, sixty, seventy-five, ninety, one hundred and five, and one hundred and twenty minutes respectively. It will be seen that the introduction of alcohol into the blood through the jugular vein decreased the catalase of the blood after two hours by 33 per cent as is indicated by a decrease in the amount of oxygen liberated from 45 to 30 cc. This observation is interpreted to mean that the introduction of alcohol into the blood vessels of the living animal destroys the catalase of the blood.

The question why the introduction of alcohol into the blood from the alimentary tract should increase the catalase, while the introduction of alcohol directly into the blood vessels should decrease the catalase, naturally arises. One answer that suggests itself is that when the alcohol is absorbed from the alimentary tract and is carried directly to the liver, it may stimulate this organ to an increased output of catalase. If this explanation is the correct one, then the introduction of alcohol into the portal blood should increase the output of catalase from the liver. Accordingly, experiments were carried out to see if this were true. By means of a hypodermic needle attached to a burette by a piece of rubber tube, 40 per cent ethyl alcohol was introduced into the portal blood at the rate of approximately 1 cc. per minute while the catalase of the blood taken from the external jugular was determined at intervals of fifteen minutes. For comparison the catalase of the blood was determined after fifteen and thirty minute intervals at the beginning of the experiment before the introduction of the alcohol. It will be seen in curve 5 that 0.5 cc. of these samples of blood liberated

36 and 35 cc. of oxygen in ten minutes. After the thirty-minute interval, the alcohol was injected at the rate stated. It will be seen that the effect of the injection into the portal vein was not to increase the catalase of the blood as was expected, but to decrease it as is indicated by the decrease in the amount of oxygen liberated by 0.5 cc. of the different samples of blood. Why the absorption of alcohol from the alimentary tract should produce an increase in the catalase of the blood, while the introduction of alcohol into the portal vein produced a decrease, I am not as yet prepared to state.

As a result of the experiments reported in this paper, it is assumed that in so far as the absorption of alcohol from the alimentary tract produces an increase in the catalase of the blood resulting presumably in an increase in oxidation, just so far alcohol exerts a stimulating effect, while in so far as the accumulation of alcohol in the blood in prolonged intoxication or the introduction of alcohol directly into the blood destroys catalase, resulting presumably in a decrease in oxidation, just so far alcohol exerts a depressing effect.

SUMMARY

The introduction of alcohol into the stomach greatly increases the catalase of the blood, while the introduction of alcohol directly into the vascular system decreases the catalase of the blood. The decrease in catalase produced by the introduction of alcohol directly into the blood is due to the destruction of the catalase by the alcohol. Further work is necessary before an explanation can be given for the increase in the catalase of the blood when alcohol is absorbed from the alimentary tract into the blood.

HAEMODYNAMICAL STUDIES

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Received for publication October 19, 1917

I. THE BLOODFLOW DURING IMMERSION IN COLD WATER

These experiments purposing to show the influence of immersion in cold water upon the flow of the blood, were performed upon small dogs during light ether narcosis. In each case the animal was placed in a small bath tub with the shoulders and head elevated in such a way that the body could be covered later on with water without interfering with respiration. A stromuhr[1] was then inserted in the left common carotid artery. The central cannula of this instrument was connected with a mercury manometer for the registration of the general arterial pressure. The respiratory movements were recorded by means of a simple stethographic arrangement.

The procedure followed in these experiments consisted in the determination of the bloodflow under normal conditions and during the immersion of the animal in water of 25 to 32°C. Lower temperatures than these were not employed, because the muscular reactions then usually resulting, tended to increase the general blood pressure very abruptly, and also interfered with the registration of the bloodflow in a mechanical way. I have sought to duplicate upon these animals merely those tonic reactions which one ordinarily experiences in baths of 32 to 34°C. In illustration of the results I insert in this place a table giving the values of the carotid bloodflow and of the arterial pressure as determined by experiment 2 of this series. The immersion was continued in this case during a period of about ten minutes. The water was cooled to 28°C. A comparison of the values before and after the immersion shows very clearly that the cool water exerts a favorable influence upon the bloodflow and that the increased flow is associated with a well marked rise in the systemic pressure and a slight acceleration of the respiratory movements.

[1] The recording stromuhr described by Burton-Opitz was employed (Pflüger's Arch., 1908, cxxi, 150).

TABLE 1

Carotid bloodflow during bath in cool water (Dog 9.5 kilos)

NO. OF PHASE	QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD-FLOW	PRESSURE	PROCEDURE
	cc.	*seconds*	*cc./sec.*	*mm. Hg.*	
1	18.8	12.0	1.57	106.7	Normal
2	18.5	11.0	1.69		
3	19.0	13.3	1.43		
4	19.0	12.4	1.52		
5	18.4	10.8	1.71		
6	18.8	11.4	1.64		
7	19.2	12.8	1.51		
8	19.2	11.3	1.70		
9	19.6	13.1	1.49		
10	19.0	11.3	1.67		
11	19.4	11.5	1.69		
12	18.8	12.2	1.54		
Average.....			1.59	106.7	
13	19.0	11.5	1.65	110.5	Immersion in water of 28°C.,
14	19.0	10.2	1.86		10 minutes.
15	18.2	9.7	1.87	114.3	
16	18.2	9.0	2.02	118.3	
17	18.8	9.8	1.91		
18	18.8	10.4	1.71		
19	19.2	9.5	2.02		
20	19.4	10.2	1.90		
21	19.4	9.4	2.06		
22	18.8	10.6	1.77		
23	19.4	10.8	1.80	119.5	
24	19.0	10.0	1.90		
25	19.2	10.4	1.84		
26	18.8	10.8	1.74		
Average.....			1.86	119.5	

II. THE DISTRIBUTION OF THE BLOOD DURING STIMULATION OF THE SPLANCHNIC NERVE

The quantitative measurements of the bloodflow in the portal vein and its tributaries (1) have proven conclusively that vasoconstrictor reactions in the viscera innervated by the splanchnic nerve caused a marked diminution in the influx of arterial blood into these organs. This stagnation is responsible for the increase in the arterial pressure.

Edwards (2) has shown, however, that the stagnation is not absolute and that a certain compensation is possible through the circuit of the carotid and femoral arteries, which tends to keep the venous system supplied with almost normal quantities of blood.

TABLE 2

Flow in inferior vena cava during stimulation of greater splanchnic nerve

NO. OF PHASE	QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD-FLOW	PRESSURE (MM. Hg.)		PROCEDURE
				Vena cava	Femoral artery	
	cc.	*seconds*	*cc./sec.*			
21	20.0	4.0	5.0	1.5	91.4	Normal
22	20.0	3.4	5.9			
23	19.5	3.6	5.4			
24	19.5	3.5	5.5		91.0	
Average.....			5.4	1.5	91.0	
25	19.8	4.1	4.8	1.5		Stimulation left great splanchnic nerve, 40 seconds, 15 cm.
26	19.8	3.6	5.5		111.5	
27	19.9	3.8	5.2			
28	20.0	4.0	5.0		127.8	
29	20.0	2.8	7.1			
30	20.0	3.1	6.4	0.8	132.4	
31	20.0	2.1	9.0			
32	20.0	2.4	8.3		132.0	
33	20.0	3.0	6.6			
34	20.0	2.0	10.0		132.5	
35	19.8	2.9	6.8			
36	19.8	2.0	9.9	2.0	132.0	
37	19.6	2.5	7.8			
38	19.4	2.0	9.7		128.9	
39	19.4	2.1	9.2			
40	19.3	2.0	9.6		125.8	
41	20.0	2.8	7.1			
42	20.0	2.2	9.0		118.4	
43	20.0	2.6	7.6			
44	20.0	1.8	11.0		114.4	
45	19.4	2.4	8.0			
46	19.4	2.0	9.7	4.0	108.7	
47	20.0	2.4	8.3			

This compensation in the distribution of the blood in consequence of splanchnic constriction is especially well betrayed in the inferior cava distally to the entrance of the hepatic veins. I have succeeded in showing this by calibrating the bloodstream in this vein before and

Fig. 1. Flow in inferior vena cava during stimulation of greater splanchnic nerve (Red. to ⅔ original).

during the excitaton of the left greater splanchnic nerve. The stromuhr was inserted distally to the left renal vein. The distal cannula was connected with a membrane manometer which registered the venous pressure. The general arterial pressure was recorded by a mercury manometer connected with the left femoral artery. In illustration of the results I insert at this time the calculations for a part of experiment 2 of this series, as well as a reproduction of the phases recorded by the stromuhr during this time. As is clearly shown in figure 1, the stimulation of the aforesaid nerve *A–B*, is followed by a decided increase in the venous return, the normal flow of 5.4 cc. in a second, *St*, being gradually displaced by a flow equalling 9.0 to 11.0 cc. in a second. This change occurs during the period of high arterial pressure, *C–D*, i.e., at a time when the splanchnic organs are constricted. Under ordinary conditions the results of this constriction betray themselves in the central venous channels by a drop in pressure in consequence of the lessened influx of portal and renal blood, *F*. The present experiments, however, show that this central deficiency in blood is soon compensated for by a greater influx through circuits not dominated by the splanchnic nerve. In addition this change in the distribution of the blood acts as a check upon the pressures, preventing the occurrence of an undue arterial stagnation and hence of an injurious increase in the arterial pressure.

III. THE RELATION BETWEEN THE INTRAPERICARDIAL PRESSURE AND THE PORTAL BLOODFLOW

These experiments are intended to illustrate the clinical picture observed in pericarditis as it betrays itself in changes in the dynamical conditions of the portal system. They were performed upon dogs during ether narcosis. The chest having been opened and artificial respiration instituted, a glass cannula was inserted in the pericardial sac. The stromuhr was then connected with the portal vein. The venous pressure was registered by a membrane manometer connected with the distal cannula of this instrument, and the arterial pressure by a mercury manometer connected with the carotid artery. The procedure consisted in all cases in obtaining these different records under normal conditions as well as during periods of increased intrapericardial pressure. The latter end was attained by permitting air from a pressure-bottle to flow into the pericardial sac until a very moderate degree of inflation had been established. The height of this pressure was recorded by a water-manometer.

As the results are perfectly uniform, a brief discussion of figure 2 will no doubt suffice to show their character. In the present animal, a dog weighing 14 kilos, the flow in the portal vein, *S*, amounted to 3.69 cc. in a second. The inflation of the pericardial sac was begun at *A*. It was continued during a period of about twenty seconds, i.e., to point *B*, reaching a maximal value of 4 mm. Hg. It is evident in the record that the flow decreases very markedly at *A* and continues small for some time after the cessation of the inflation, i.e., to about point *C*. The average flow during this period is only 1.81 cc. in a second, a reduction of 50 per cent.

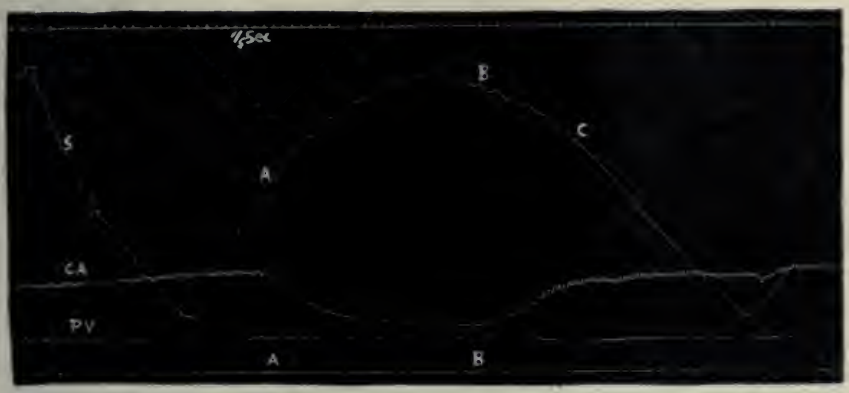

Fig. 2. Portal bloodflow on increasing intrapericardial pressure. *A* to *B* (Red. to ⅓ original).

If we now observe the records of the pressures, it is apparent that this period of decreased flow coincides with a fall in the arterial pressure, *CA*, and a rise in the venous pressure, *PV*. These phenomena of low arterial driving force and venous stagnation disappear soon after the cessation of the inflation. It need scarcely be mentioned that greater increases in pressure do not alter the character of these changes but merely tend to render them more conspicuous.

IV. THE INFLUENCE OF TRICUSPID REGURGITATION UPON THE PORTAL BLOODFLOW

The method employed to reproduce the clinical picture of tricuspid regurgitation as it betrays itself in changes in the portal system was

very similar to the one just described. The stromuhr was inserted in the portal vein centrally to the splenic vein. The arterial and venous pressures were recorded by a mercury and membrane manometer connected with the femoral artery and portal vein respectively. The procedure consisted in rendering the tricuspid valve incompetent at a certain moment after the beginning of the calibration of the normal blood flow. The latter end was attained by severing some of the chordae tendineae with a long hook-shaped knife inserted through the right external jugular vein. The chest remained closed.

This procedure was followed by an almost immediate reduction in the portal bloodflow and a fall in the general arterial pressure. The conspicuousness of these changes varied with the severity of the lesion, a gradual cessation of the flow following an absolute incompetency of this valve. In these cases the portal pressure also showed a decrease in accordance with the fall in arterial pressure. Milder types of regurgitation, however, were associated with a slight rise in venous pressure. The reversion appeared when the blood flow had suffered a reduction to about four-fifths of normal.

V. THE BLOOD SUPPLY AND INNERVATION OF THE GALL BLADDER

The attempts which I have made to determine the blood supply of the gall bladder of dogs have not been wholly successful, because the artery supplying this organ is small and rather inaccessible. To begin with, I sought to overcome this difficulty by restricting the vascular field to be experimented upon by ligating the branches of the hepatic artery, so that the main trunk of this blood vessel could be made accessible to the recording instrument. In studying the distribution of this artery with the help of paraffine injections it was found, however, that its branches are distributed not only to the gall bladder but also to the neighboring mass of the liver. Moreover, as it is practically impossible to isolate the latter under the conditions made necessary by experiments of this kind, the results of these calibrations leave much to be desired and shall for this reason not be discussed further.

There is one observation, however, to which I should like to call attention and that is the musculo-motor effect which the stimulation of the coeliac and hepatic plexuses produces. Obviously, as the contraction of the musculature of this organ tends to vary the flow through the distal blood vessels, the flow in the main channel must suffer a similar disturbance. For this reason quantitative fluctuations frequently re-

sult which can scarcely be differentiated from those having a true vasomotor origin.

The contractions of the gall bladder were registered in these experiments by a very sensitive tambour connected with the cystic duct directly below its point of origin. The hepatic plexus was then divided at a point about 2 cm. distally to the coeliac ganglion. Its distal end was placed in shielded electrodes. In another animal, the electrodes were applied to the fibers which eventually escape from the hepatic plexus to form a network around the second branch of the hepatic artery in the immediate vicinity of the common duct.

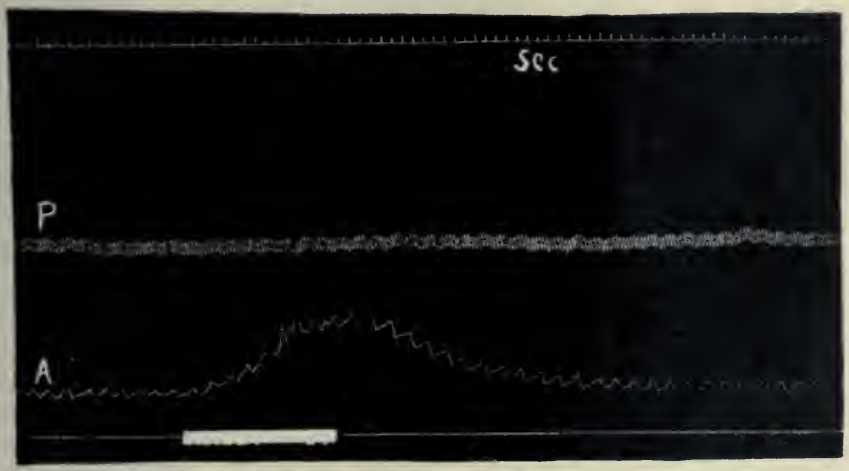

Fig. 3, Contraction of gall bladder on stimulation of coeliac plexus (Red. to ⅔ original).

In either case the stimulations resulted in well-marked contractions of the gall bladder, proving thereby that the musculo-motor fibers of this organ are derived from the coeliac ganglion and its distal plexus. The accompanying figure (3) shows the record of the tambour *A*, in relation with the blood pressure in the femoral artery, *P*. The latter presents no variations because the hepatic artery had been obstructed in this case some time before the stimulation. At other times, however, when this artery had not been compressed, the stimulations led to a decided increase in the arterial pressure, obviously because the hepatic plexus embraces constrictor fibers for the blood vessels of the liver, the excitation of which lessens the normal arterial flow into this organ (3). The

smaller oscillations upon line *A* are caused by the movements of the diaphragm. The pressures developed by the gall bladder during these stimulations did not exceed 3.0 mm. Hg.

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THE REACTION OF THE KIDNEY COLLOIDS AND ITS BEARING ON RENAL FUNCTION

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Received for publication October 19, 1917

The factors underlying the accumulation of dissolved substances in the body fluids, or their secretion, can best be studied by considering three phases in the history of the dissolved substance, namely, the origin or source of supply, the mechanism of distribution and the destination, whether storage, excretion or conversion into other substances. In all of these stages we deal with relative solubilities. Under one set of conditions substances appear to be more soluble in the blood and under other conditions in the relatively more soluble tissue constituents. At present there are two descriptions of the phenomenon of relative solubility which take into account its variations in direction and amount of action. Some emphasize the conditions which point to osmosis as an important mechanism, whereas others see in the colloidal nature of the tissue structure the clue to their behavior. Can the behavior of the tissues and organs be described both quantitatively and qualitatively on a basis of colloid behavior? If so, have we any mechanism which is adequate to accomplish the observed results, in affecting the colloids?

It is well known that many substances in the colloid state can be made to absorb or give up water under varying acid-alkali (1) or hydrogen-hydroxyl (2) concentration. That these observations held good for certain body proteins was emphasized by Martin Fischer in his application of this principle in the study of oedema (1). The question of whether this relationship of ability to hold or release, under varying degrees of "acidity,' held true for dissolved substances was the next point to be solved. Fischer found that fibrin would hold or release sodium chloride, according to whether the acid content was increased or decreased. Later Reemelin (3) showed the same to be true for fibrin and phenolsulphonephthalein. In testing out other substances and

other colloids, Reemelin and Isaacs (2) found that this same relationship held true for the kidney colloids and urea, and that the kidney colloids could be made to secrete more water and more urea, the lower the hydrogen ion concentration, and less when the hydrogen ion concentration was higher. As this extremely delicate relationship was demonstrated to be true for solutions whose strengh and hydrogen ion concentration were within physiological limits, the oft repeated criticism that Fischer's tenets held only for amounts of acid never met with in the body, was answered. This led to the formulation of a theory of *selective adsorption*, which held that a dissolved substance could be held or passed on, according to its behavior in the presence of a higher or lower hydrogen ion concentration. It thus became desirable to test out other substances and note their behavior to the kidney colloids. In this way one of the factors involved in the accumulation or decrease of dissolved substances in the body fluids could be studied. The present paper deals with the relationship between sodium chloride and the kidney colloids, under varying degrees of hydrogen ion concentration and varying strength of salt solutions. The material also allowed the taking of some observations on the excretion of albumin and its relation to the secretion of water and salts.

MATERIAL AND METHODS

In the following experiments kidneys of rabbits, under ether or chloroform anaesthesia, were isolated and an artificial circulation was set up through cannulas inserted in the arteries and veins. The "urine" was collected from cannulas tied in the ureters. The kidneys in each case were removed from the body and kept moist. The perfusion was carried out by the method described by Sollmann (4) and used by Reemelin and Isaacs (2) in the study of urea excretion. The perfusing solution consisted of sodium chloride in distilled water, and the hydrogen ion concentrations were varied by means of traces of phosphoric acid, disodium hydrogen phosphate or sodium hydroxide. From 100 to 200 cc. more or less, of each perfusing solution was used before it was replaced by another. In order to facilitate the change of solutions several reservoirs were used, each connected with the same glass tube and controlled by pinch clamps, so that a solution could be turned on or off and be replaced by a new one without danger of disturbing the kidney or admitting air bubbles. The blood pressure was kept constant throughout the experiments. The perfusing solutions were of 0.95 per

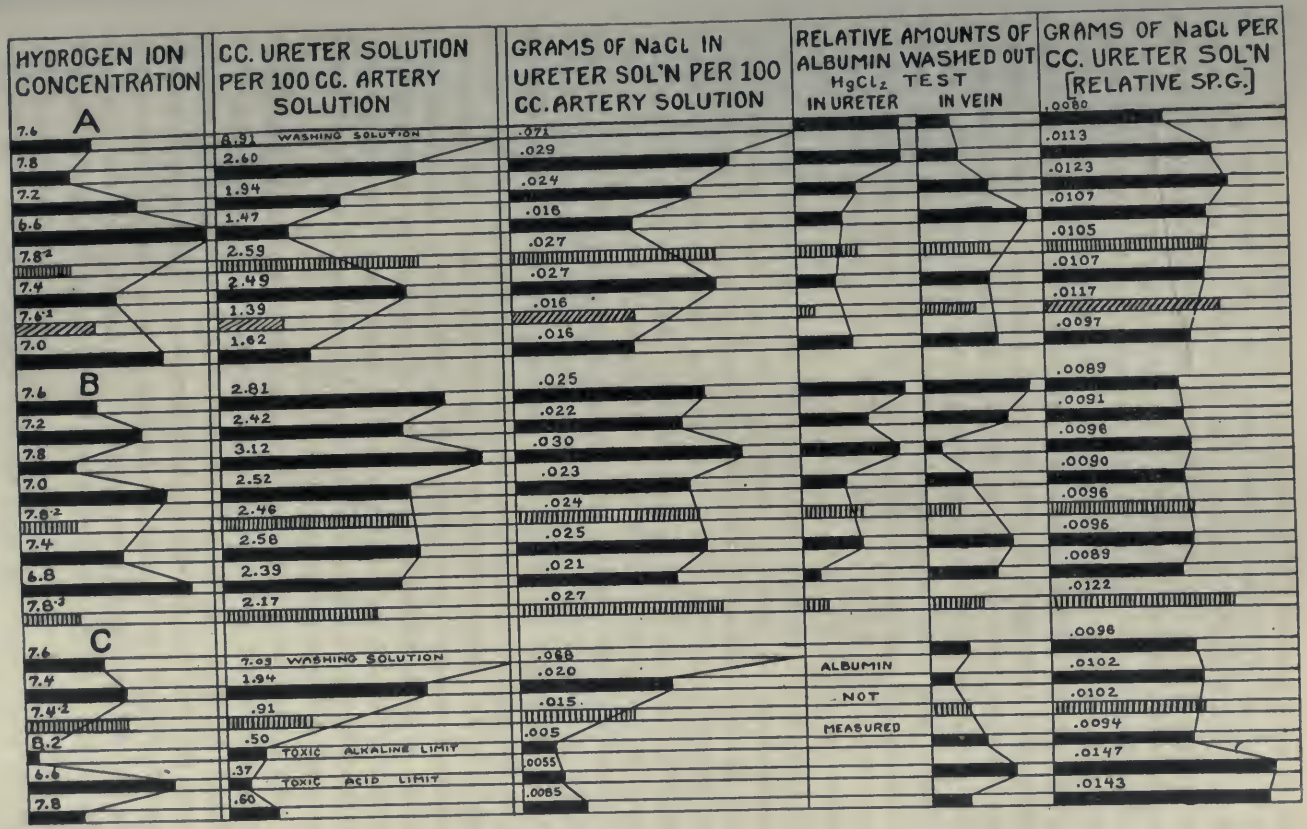

Fig. 1. Solutions are listed in the order of their perfusion. Each experiment represents a separate kidney. To read chart easily, hold page so that blackened lines are vertical.

cent, 0.88 per cent and 0.87 per cent of sodium chloride. By means of a stream of air bubbling through the solution in the reservoir, the oxygen tension was kept constant. The hydrogen ion concentration varied from pH–6.6, the most acid used, to pH–8.2, the most alkaline. The blood was washed out of the vessels with a salt solution of pH–7.6.

The hydrogen ion concentration of the artery was always retested from samples taken from a valve near the kidney, so that any change in the reaction, brought about by contact with the rubber and glass tubes leading from the reservoirs, could be detected. The hydrogen ion concentrations were determined by comparisons of measured amounts with solutions of known strength, using phenolsulphonephthalein as the indicator. The solutions were collected from the vein and ureter separately, the amounts measured and the amount of the chlorides determined by Vollhard's method. The details are given in the accompanying table.

The table and figures show the order in which the solutions were perfused, the hydrogen ion concentrations and the amount of salt and water excreted. It is to be noted that during any one experiment the chloride content of the artery solution was constant, and proteins and substances in the colloid state were absent. The solutions differed from one another in the presence of the smallest possible traces of phosphoric acid, disodium hydrogen phosphate or sodium hydroxide that would give the proper hydrogen ion concentration. This amounted to a fraction of a milligram in many cases, amounts too small to account for the results purely on a basis of osmosis. The results show that *the secretion of both sodium chloride and of water drops as the solution becomes more acid or less alkaline, within physiological limits, and rises when the order is reversed.* That this is true regardless of the order of perfusion is shown in the experiments which were arranged so that sometimes the variations were from acid to less acid or alkaline, and sometimes in the opposite direction.

A suggestive fact to be noted from these experiments is that when a solution at a given hydrogen ion concentration is repeated, that is, after about 100 cc. have been perfused, if a second perfusion of like amount is tried, the results are below normal. This is confirmed by other experiments and probably correlated with the fact that certain albumins, whose presence were shown by reagents, are more soluble in the sodium chloride solution at one hydrogen ion concentration than at another, and leave the kidney by way of the vein or the ureter. With the removal of these albumins the ability of the kidney colloids

to secrete sodium chloride and water is lessened inasmuch as no repair can take place in the kidney under the conditions of the experiment, blood being absent. In experiment B, the solution pH–7.8 was perfused three separate times, the amount of albumin per cubic centimeter as well as the total amount of albumin in the vein increased, but that in the ureter decreased. The approximate measurements of the relative total amount of albumin present in each ureter solution showed that the amount of albumin increased with the decrease in hydrogen ion concentration, and decreased as the hydrogen ion concentration increased. In this respect it followed the secretion of salt and water. This result does not at first appear to bear out some clinical correlations because of a common error sometimes used in reporting the amount of albumin in the urine, as well as the amount of sodium chloride or urea in a specimen. It is of course evident that any quantitative reading which expresses the concentration of a substance gives no clue as to the entire amount of that substance present. One commonly sees tables in the journals giving grams of urea, sodium chloride or other substance per 100 cc. of a fluid and comparisons made on this basis. Clinically it is quite common to see the direct reading of the Esbach albuminometer on one occasion compared with the corresponding reading on another occasion, and the conclusion of improvement or turn for the worse based on this. It is evident that the only factors that can be compared are the total or relative amounts of substance present in a given period of time or of equivalent function. These experiments show that, dependent upon the total amount of water excreted, the total amount of albumin lost by the kidney (and therefore, as the quantitative data show, corresponding decrease in function), may be more or less than when a different amount is excreted, although the concentration in grams per unit volume may be the same. When these facts are taken into account it is seen that when a small quantity of liquid washes out as much or more albumin than a larger quantity in a given time, it is to be expected that the disturbing cause is more marked or widespread in the first than in the second. These results during life would be modified by two factors, the repair of the kidney and the blood as a source of albumin.

The relationship of albumin to water excretion, however, is not as simple as merely a matter of mechanical solution and washing action. There is some evidence to indicate that solutions of different hydrogen ion concentrations washed out different kinds of protein in different amounts. The amounts of protein were measured in several ways.

Turbidity of columns having a proportionate relative length were compared, amount and volume of precipitate was noted as well as total amount of reagent necessary to produce maximum turbidity. Various reagents, as nitric acid, mercuric chloride, picric acid, potassium ferrocyanide, acetic acid, as well as heat, and combinations of these, gave different amounts of protein for the same solution, so that the order of concentration varied when arranged on the basis of behavior toward any given reagent. The difference was more than could be accounted for on a basis of acid albumin and alkaline albumin. Furthermore the precipitate of albumin settled to the bottom of the comparison tubes at different rates, which were not coördinate with the order of concentrations as shown by the precipitating reagent. This, together with the microscopic evidence, details of which are given below, suggests that we are dealing with solutions whose composition differed in kinds of protein present as well as in amounts.

The precipitated albumin when examined under the microscope showed characteristic precipitation patterns, which differed in complexity and structure. The microscopic picture resembled very much in form and staining qualities the fibrillar networks often seen inside the kidney cells and between them, in histological sections of "fixed" kidney tissue. This method of studying the meaning and composition of some of the structures seen in fixed tissue is now being followed and the significance of these appearances will be discussed in a later paper (see also Isaacs, (5)).

It will be noted that alkaline solutions as well as acid, washed out albumin. As we often note that clinically an alkaline urine may contain albumin just as well as an acid specimen, it is possible that the points noted on the kidney colloids in these experiments may have some relation to the condition in some living kidneys. Thus far, substances responding to the tests for serum albumin, nucleoprotein, mucin, histone and some globulins have been identified. The observations taken so far are not extensive enough to permit the publication at this time of a series showing the quantitative relation between the kind of albumin and the hydrogen ion concentration. These differences suggest that clinically the kind of albumin which predominates in the urine may be a clue to the nature of the toxic agent causing its excretion. It will be noted from the chart that as a solution at a given hydrogen ion concentration is repeated, there is a tendency for more albumin to be washed out in the vein and less in the ureter. If such a condition were present during life, one would expect a variation in the kind and amounts of albumin in the blood.

The decrease in the excretion of sodium chloride and of water, when a solution at a given hydrogen ion concentration is repeated, and the recovery, as far as other solutions are concerned, suggests that there may be *a definite relation between the kind of albumin washed out in the ureter and the hydrogen ion concentration of the perfusing solution.* This is compatible with the idea of a selective adsorption (2) as far as both sodium chloride and proteins are concerned on a basis of variation in affinity with variations in hydrogen ion concentration. In similar experiments (fig. 2) the secretion of the repeatedly perfused kidney was restored, quantitatively, to normal when defibrinated blood was added to the perfusing solution, the excreted protein being thus replaced.

The lessened effect of the repeated solution amounts in the end to a form of tolerance, which suggests a possible mechanism for drug tolerance noted clinically. The selective secretion of "Bence-Jones protein" described by Taylor, Miller and Sweet (6) may be a form of excretion similar to that noted in these perfusion experiments. The relationship of increased excretion (from the point of view of normal function) with decrease in hydrogen ion concentration is probably limited, as far as the kidney colloids are concerned, to hydrogen ion values which are within the narrow physiological limits. In these, as in other experiments, an "acid" concentration as high as pH–6.6 ($\frac{1}{3,981,000}$ gram of hydrogen ions per liter) proved toxic by reducing the secretion, whereas the alkaline pH–8.2 ($\frac{1}{158,500,000}$ gram of hydrogen ions per liter) had a similar effect, although the alkaline upper limit is probably higher than this. The secretion curve reaches its highest point between pH–7.2 and pH–7.8.

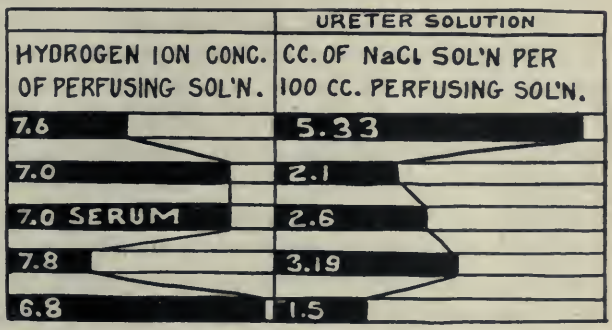

Fig. 2. Experiment D. The perfusion of a kidney with serum restores the function by replacing albumin washed out.

The biological effects of neutral salts in reducing the affinity of acidified hydrophilic colloids for water has been noted by Fischer. As it is in the swelling or reduction in size that Fischer sees the mechanism of adsorption and secretion, it is then of interest to see if neutral salts

affect the secreting mechanism in these experiments. That there is an effect, these experiments illustrate very well. In experiment B the average of the hydrogen ion concentrations was pH–7.4 which is the same as that in experiment C. However, in experiment C 100 cc. of artery solution produced only 1.367 cc. of ureter solution as compared with 2.574 cc. of ureter solution for the same amount of artery solution in experiment B. In experiment C the smaller amount of secretion was correlated with a higher concentration of sodium chloride in the artery solution, namely 0.95 per cent as compared with 0.88 per cent in experiment B. A possible explanation of the lessened secretion in experiment C may be a force analogous to that which causes neutral salts to decrease quantitatively the swelling of colloids, as taught by Fischer. In these experiments it was not possible to take quantitative data to show whether secretion changes were accompanied by swelling changes, because of mechanical difficulties. That solution of protein took place has been noted, and it is known that in many colloids the solution process runs a course somewhat parallel to the swelling process, often accompanying it. Certainly the "kidneys" after the experiments were completed showed a typical picture of "cloudy swelling," but the change in size due to the blood pressure was such that minute changes in the size of colloids or their change back and forth from a precipitated form to a soluble form could not be measured under the conditions of the experiments. Furthermore, the process of cloudy swelling, with all the changes that go with it, evidently produces a change of elasticity in the kidney so that the same blood pressure will cause the kidney volume to increase more in the later stages than in the earlier (7).

The concentrations of the ureter solutions taken in grams of sodium chloride per cubic centimeter (see fig. 1) indicate that the secretion of sodium chloride is probably quantitatively not dependent on the secretion of water as, in the presence of a constant amount in the artery solution, the concentrations of some ureter solutions increased with the decrease in hydrogen ion concentration, whereas in others it decreased. In experiment C there is a consistent variation of the concentration of sodium chloride with that of the hydrogen ions, both increasing and decreasing together. This direct variation held true for only some of the other perfusions. In two cases (pH–7.4 in experiment C and pH–7.8 in experiment B) the repetition of a solution gave ureter solutions of practically the same concentration (number of grams of sodium chloride per cubic centimeter) but the relative amounts

excreted (amount of sodium chloride per 100 cc. of artery solution) were different, as pointed out before. If these results apply to the living kidney one would not expect to correlate the concentration of the urine directly with its acidity or with the hydrogen ion concentration of the blood, as the chart clearly shows. A third factor, namely, the colloid most affected at the hydrogen ion concentration in question, evidently must be taken into consideration.

The experiments on urea and on sodium chloride have shown that in the presence of a constant concentration of these substances in the artery solution, the amount secreted varied and the variation was closely related to the hydrogen ion concentration of the artery solution. They have also shown that although a chemical analysis may show the presence of a certain amount of urea or sodium chloride in the blood, yet the amount *available* for secretion may not be the same. Thus although an artery solution contained a constant amount of an aqueous salt solution, a variation of over 36 per cent excretion was noted in one experiment, the only variable factor being a change in hydrogen ion concentration from pH–7.2 to pH–7.8. While secretion of both urea and sodium chloride increases as the hydrogen ion concentration decreases, this relation does not hold true for all the colloids in the body. Fischer (1) as stated before, finds that fibrin will hold sodium chloride, the more acid it is, while Reemelin finds that this does not hold for fibrin and urea. On this basis we can expect diminution in the chloride secretion ("salt or chloride retention") in the presence of an apparently good urea output, as, of the blood and kidney colloids, both tend to hold the chlorides, while of the two, only the kidney colloids tend to hold the urea. This condition is noted clinically in some conditions of chloride retention. Wolferth (8) notes that chloride retention in eclampsia may be marked while the retention of urea may be very slight.

The results of experiments on glucose, creatin, creatinine, uric acid and phenolsulphonephthalein will be reported later.

SUMMARY AND CONCLUSION

1. In isolated rabbit kidneys, perfused with salt solutions of constant and known composition, the secretion of sodium chloride and of water increases with the decrease in hydrogen ion concentration and decreases as the hydrogen ion concentration increases.

2. This relationship holds true for hydrogen ion concentrations within physiological limits.

3. pH–6.6 probably represents the acid limit for function while the alkaline limit is pH–8.2 or higher. Variations beyond these are highly toxic to the kidney colloids and reduce secretion. The optimum hydrogen ion concentration lies between pH–7.2 and pH–7.8.

4. The amount of sodium chloride found in the blood on chemical analysis does not indicate the amount available for secretion. The amount available varies with the hydrogen ion concentration.

5. The effect of neutral salts (sodium chloride) in preventing the kidney colloids from taking up water and salt and secreting it can be shown quantitatively by varying the strength of the perfusing solution.

6. That the kidney colloids can be the source of albumin in the urine is shown by the fact that an artificial albuminuria, responding to the clinical tests, can be produced even though the artery solution contains no protein.

7. The amount of albumin washed out varies with the hydrogen ion concentration and follows the secretion of salt and water in which, under the conditions of the experiment, it becomes soluble.

8. There is evidence that some of the kidney proteins are more soluble in solutions of one hydrogen ion concentration than in another. This may account for the variations in kind of "albumin" found clinically in urine analyses, as well as the selective secretion of proteins.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLIV. The Origin of the Epigastric Pains in Cases of Gastric and Duodenal Ulcer

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Received for publication October 20, 1917

The genesis of the pains of gastric and duodenal ulcers is not yet satisfactorily demonstrated. The view that *the pains are due to acid irritation of hyperexcitable nerve endings or exposed nerve fibers in the ulcer area* has probably the greatest number of adherents at present. The essential facts in support of this view are: 1. Analogy. Acid irritation of abraded areas of the skin, mouth or nose epithelium, etc. produces pain. 2. The frequent occurrence of so-called gastric hyperacidity in gastric and duodenal ulcers. 3. The temporary alleviation of the ulcer pain by food and alkalies.

The following facts appear to run counter to, or are at least not readily explained by this acid corrosion theory:

1. Gastric ulcer with or without clinical hyperacidity[1] may be present without pain.

2. Gastric ulcer and ulcer pain may be associated with normal acidity, and even with hypoacidity.

3. The pains of gastric ulcer may be present and be temporarily relieved by food or alkalies, even though the stomach contents are alkaline (1).

4. Introducing acids (0.5 per cent HCl) into the stomach does not, or at least not invariably, induce or augment the ulcer pains in gastric ulcer patients (7).

[1] While there is no evidence that Alexis St. Martin at any time had acute or chronic ulcer of the stomach or duodenum, Beaumont on several occasions observed raw patches on the gastric mucosa from which blood or pus exuded. In most instances this condition of the mucosa did not cause pain or discomfort, even though the abrasions of the mucosa persisted for several days. Beaumont remarks (p. 240): "It is interesting to observe to what extent the stomach may become diseased without manifesting any external symptoms of such disease."

5. The ulcer pains usually show a periodicity (being described as "gnawing" or "boring"), and the periods are too short to be explained by variations in the gastric acidity.

The other leading view *ascribes the ulcer pains to contractions of the stomach, the pylorus and possibly the upper end of the duodenum, the excessively painful character of these contractions in ulcer cases being due either to hyperexcitability of the gastric pain nerves or to abnormally strong contractions.* This theory has been fortified by clinical observation and experimental data especially by Hertz (7), and striking confirmatory findings on ulcer patients have recently been reported by Ginsburg, Tumpowsky and Hamburger (5). The following facts appear to support this theory:

1. Strong contractions or a certain type of contractions of the stomach and intestines give rise to varying degrees of pain in the absence of ulcer ("hunger pangs," "colic," tenesmus, various forms of gastralgia, etc.).

2. Pain nerves appear to be absent from the gastric mucosa (4).

3. The evident synchrony of the ulcer pains with gastric and possibly pyloric contractions, so far as this phase has been studied by the balloon and X-ray methods (4), (5), (7), (13), (14).

4. The frequent association of typical gastric ulcer pains with appendicitis, cholecystitis and even achylia gastrica, (6) not complicated with gastric ulcer.

5. The similarity of the moderate ulcer pains as regards periodic exacerbation with the strong hunger pangs of normal persons, a similarity that lead Moynihan and others to designate the pains of gastric and duodenal ulcers as "hunger pains."

But while the gradually accumulating reliable data thus point to gastrointestinal contractions as the immediate cause of the ulcer pains, we do not know: (1) what part of the digestive tract (stomach, pylorus or duodenum) is primarily concerned; (2) whether the site of the painful contraction varies with the location of the ulcer; (3) or what is the relation of gastric acidity to the initiation of the contractions. The observations here reported were undertaken in the hope of securing clearer knowledge of these factors.

Mr. G. H. M., age 25, the subject in these studies, was at the time a medical student in our laboratory. Six months before coming under our observation he suffered an acute attack with all the typical symptoms of "peptic ulcer," including hematemesis. After several weeks in a hospital on medical treatment and an ulcer diet, he recovered suf-

ficiently to resume his medical studies.[2] But despite the improvement the ulcer had evidently not healed, as he suffered periodic exacerbations of the ulcer syndrome, even though he continued the ulcer diet fairly consistently. These attacks usually lasted from three to six days and came at irregular intervals, a few days to several weeks apart. Dietary indiscretions or excessive mental work appeared to induce or aggravate these attacks.

During the three months of these observations Mr. M. experienced two of these relapses, one of them being very severe. Between these attacks his gastric motility and gastric sensations were those of a normal person.

1. When the gastric hunger pains are of moderate severity there is practically complete synchrony of the appearance, intensity and duration of the ulcer pains and the stomach contractions (fig. 1)*; and these contractions are not stronger or more prolonged than the stomach contractions felt as ordinary hunger pangs on days when the subject has no ulcer symptoms.*

That the gastric contractions give rise to the ulcer pains, and not *vice versa*, is shown by the fact that the pains are not felt until the contractions are well developed, or frequently not until the fundic part of the stomach has begun to relax or is completely relaxed. That is to say, the gastric contractions precede the ulcer sensation, just as they precede the hunger pang. The reader will recall that in our graphic method of recording the gastric contractions the balloon is lodged in the fundus and body of the stomach. And since the ulcer or hunger contraction sweeps over the entire stomach as groups of peristaltic waves beginning at the cardia (10), it is evident that when the fundus starts to relax the pyloric end of the stomach will be strongly contracted. The fact that Mr. M. in most cases did not begin to feel the ulcer pains until the contractions involved the pyloric end may indicate that it is mainly this region of the stomach, together with the pylorus, that gives rise to the ulcer pains by contraction. If this is a fact it would help to explain the frequent absence of ulcer symptoms when the ulcer is located at the fundic and cardiac regions.

2. The ulcer pains are felt both on the empty and the filled stomach.

[2] February 26 to March 18, 1917, the Nebraska Methodist Hospital, Omaha. At that time Mr. M.'s stomach content and feces showed occult blood. The x-ray examinations revealed gastric hyperperistalsis and rapid emptying. The Roentgenologist, Dr. C. R. Kennedy, made the diagnosis of duodenal ulcer. The internist in charge of the patient, Dr. E. L. Bridges, made the diagnosis of "peptic ulcer," the exact location of the ulcer being uncertain.

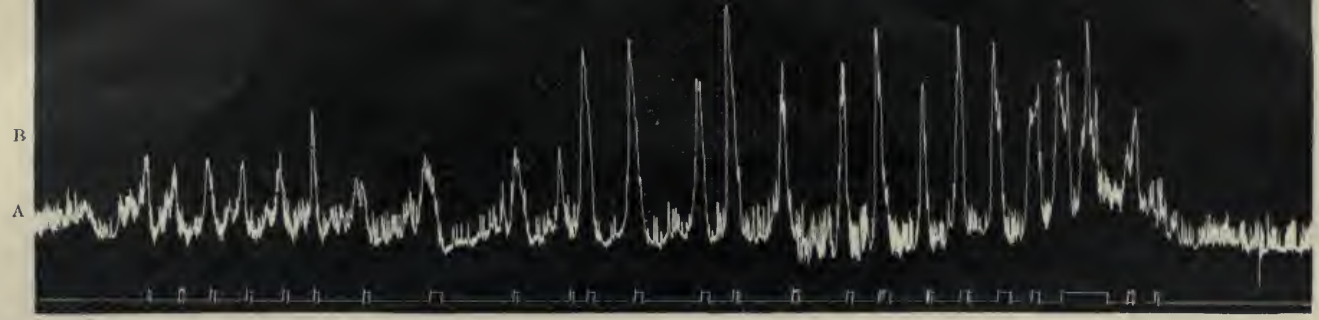

Fig. 1. G. H. M. August 10, 1917. Record of the sensation of gastric ulcer by means of a recording signal, *b*, parallel with the recording of the contractions of the stomach by the balloon method, *a*. Chloroform manometer. Contents of the stomach at the beginning of the record 65 cc., trace of bile, but no food remnants. Free acidity = 0.18 per cent, total acidity = 0.24 per cent. Stomach contents at the end of the record 25 cc. + bile. Free acidity = 0.15 per cent; total acidity = 0.21 per cent.

This record shows that in ulcer patients the contractions of the empty stomach—felt as hunger pangs by normal persons—induce the sensation of gastric ulcer pains.

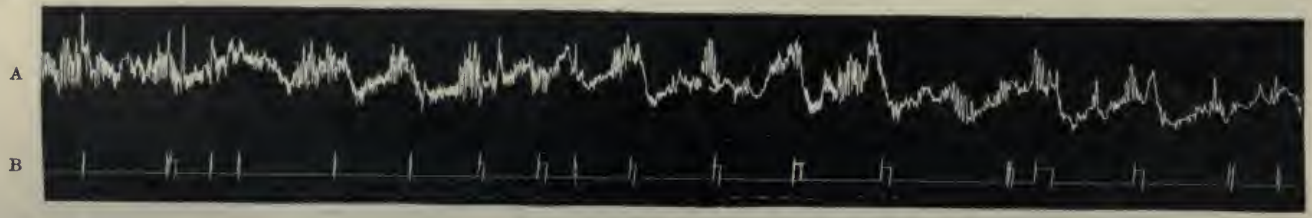

Fig. 2. G. H. M. August 3, 1917. Record of the sensation of gastric ulcer pain by means of a recording signal, *b*, parallel with the recording of gastric contractions by the balloon method, *a*. Mr. M. had a bowl of tomato soup and a glass of milk at 12.30 p.m. He began to feel the ulcer pains distinctly at 1.30 p.m. The graphic observations were made from 3 to 6 p.m. At 3 p.m. 100 cc. of chyme containing tomato débris, some milk curds and a few fat droplets, were removed from the stomach. Free acidity = 0.34 per cent; total acidity = 0.44 per cent. At the end of the observation period 35 cc. of nearly clear fluid was removed from the stomach. Free acidity = 0.34 per cent; total acidity = 0.41 per cent.

The ulcer pains of moderate severity felt on the partly filled stomach are caused by a gastric tonus rhythm apparently identical with that seen in the normal digestion peristalsis (fig. 2).

The degree of tonus rhythm of the partly filled stomach felt as ulcer pains by Mr. M. gave rise to no sensations at all during the time of well-being between the two periods of attack. This is typically normal. We have studied the gastric fundus rhythm of normal digestion peristalsis in ourselves and a number of other normal persons. These contractions produce no effects on consciousness, except toward the very end of gastric digestion when they gradually develop into hunger pangs.

The reader will note in figure 2 that the ulcer pains, as a rule, are felt toward or at the end of each tonus contraction, a fact which again points to the antrum and the pylorus as the main factors in the genesis of the ulcer pains, at least in Mr. M. But it should not be forgotten that the relatively feeble or normal digestion contractions of the fundus may be no index of the intensity of the antrum and pylorus contractions.

3. Gastric acidity bears no direct relation to the onset or the severity of the gastric ulcer pains (figs. 3 and 4). This appears to be conclusively established by the following facts:

1. On certain days of the two periods of relapse Mr. M. was disturbed by ulcer pains only at certain times of the day. On recording the gastric acidity and motility it was always found that when he was free from the ulcer pains the stomach was relatively atonic and quiescent; when the ulcer pains were present gastric contractions were also present (fig. 3). The acidity of the gastric content remained the same, or it might be either lower or higher during the pain period.

2. The same fact is brought out by comparing the gastric acidity on days free from ulcer pains with that on days of marked pain (fig. 4). Thus on the morning of July 28 (no breakfast) the stomach content was 90 cc. with an acidity of 0.20 per cent free and 0.29 per cent total. The balloon showed the stomach quiescent and Mr. M. felt no gastric distress. On the morning of July 31 (no breakfast) the gastric content was 85 cc., free acidity 0.20 per cent, total 0.27 per cent. The stomach showed vigorous contractions and Mr. M. felt the ulcer pains as very severe.

3. On August 8 Mr. M. had breakfast of oatmeal and cream at 8 a.m. The ulcer pains appeared about 9 a.m. and continued all morning. At 12 o'clock noon the stomach contents were 120 cc. of chyme with an acidity of 0.34 per cent free and 0.43 per cent total. At 1 p.m. he ate

two soft boiled eggs, one slice of toast and a glass of milk. The ulcer pains appeared at 2 o'clock and continued throughout the afternoon. At 5 p.m. 150 cc. of chyme were taken out of the stomach, having an acidity of 0.33 per cent free and 0.47 per cent total. On this day Mr. M. evidently showed clinical hyperacidity with some gastric retention.

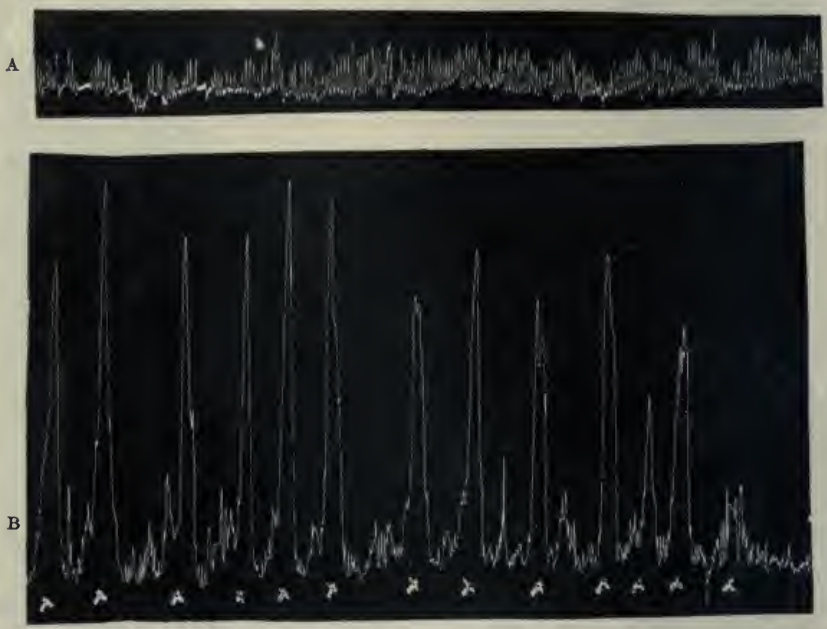

Fig. 3. G. H. M. July 24, 1917. Record of the stomach contractions by means of the balloon method. No breakfast. Stomach contents at 9 a.m., 95 cc.+ trace of bile, no food remnants; free acidity = 0.20 per cent; total acidity = 0.28 per cent. Tracing A taken 9.30 a.m. Stomach atonic and quiescent. Mr. M. felt no ulcer pains. The acidity of the stomach contents was: free = 0.20 per cent; total = 0.27 per cent. Tracing B taken at 11.30 a.m. The time when the ulcer pains are felt is indicated by *X*. The acidity of the stomach contents was: free = 0.18 per cent, total = 0.26 per cent. These tracings show that the ulcer pains are correlated with gastric motility, not with gastric acidity.

But contrast this record with the observations on August 14. On the evening of August 13 Mr. M. had dinner (two soft boiled eggs, two slices of toast and a glass of milk) at 6 p.m. The ulcer pains appeared at 7.30 p.m. They were relieved for a time by taking a glass of water. At 9 p.m. the pains were eased for thirty minutes by taking soda; at 11.30 the pains were relieved by taking two glasses of milk. At 2 a.m.

the ulcer pains woke him up from his sleep and he took 2 gm. soda without relief. At 2.30 he took two glasses of milk, the pains eased up, but he woke up at 5 a.m. with severe pains. Temporary relief obtained from two glasses of water. More water was taken at 7 a.m. No breakfast. At 8 a.m. the stomach content was 120 cc. + trace of bile with an acidity of 0.04 per cent free and 0.10 per cent total. A glass of water was taken at 10 a.m., the ulcer pains persisted throughout the morning. At 1.30 p.m. the stomach content was 65 cc. + bile and mucus with an acidity of 0.03 per cent free and 0.07 per cent total. There was no remission in the ulcer pains. Mr. M. stated that so far as he could make comparisons the ulcer pains on August 14 (stomach empty and actual hypoacidity) were just as severe as on August 8 (gastric food retention and clinical hyperacidity).

4. There is some evidence that the contractions of the pylorus and the upper part of the duodenum may in certain cases contribute to the ulcer pains.

In tracing B, figure 4, the reader will note that Mr. M. records the feeling of ulcer pains, *x*, in some cases when there is no evidence of contractions in the stomach itself. What is the cause of these pains? We believe they are due to contractions of the pylorus and the upper end of the duodenum, on the following grounds:

1. Observations by Dr. A. B. Luckhardt in collaboration with the author have brought out a new fact in the control of the pylorus, namely, a correlation of the opening of the pyloric sphincter and the tonus rhythm of the stomach. *In normal men and dogs the pyloric sphincter opens not with each peristaltic wave but at the end or at the height of each tonus contraction of the stomach.* We do not yet know to what extent this normal mechanism is modified by gastrointestinal disorders or by experimental changes of gastric acidity. It is evident, however, that the mechanism fails to work regularly in cases of pyloric spasms. But pyloric spasms are not an invariable concomitant of gastric and duodenal ulcer.

2. Again, referring to tracing B, figure 4, we call attention to the fact that in many cases at least the moderately severe ulcer pains are felt toward the end or at the very end of a gastrotonic contraction, that is, at the time of the opening of the pylorus and its immediate closure by the acid reflex from the duodenum.

3. It is a fact well known to physiologists that acids or chyme with an acidity of 0.3 per cent to 0.5 per cent acting in the upper end of the duodenum induce not only a reflex closure of the pylorus but a strong

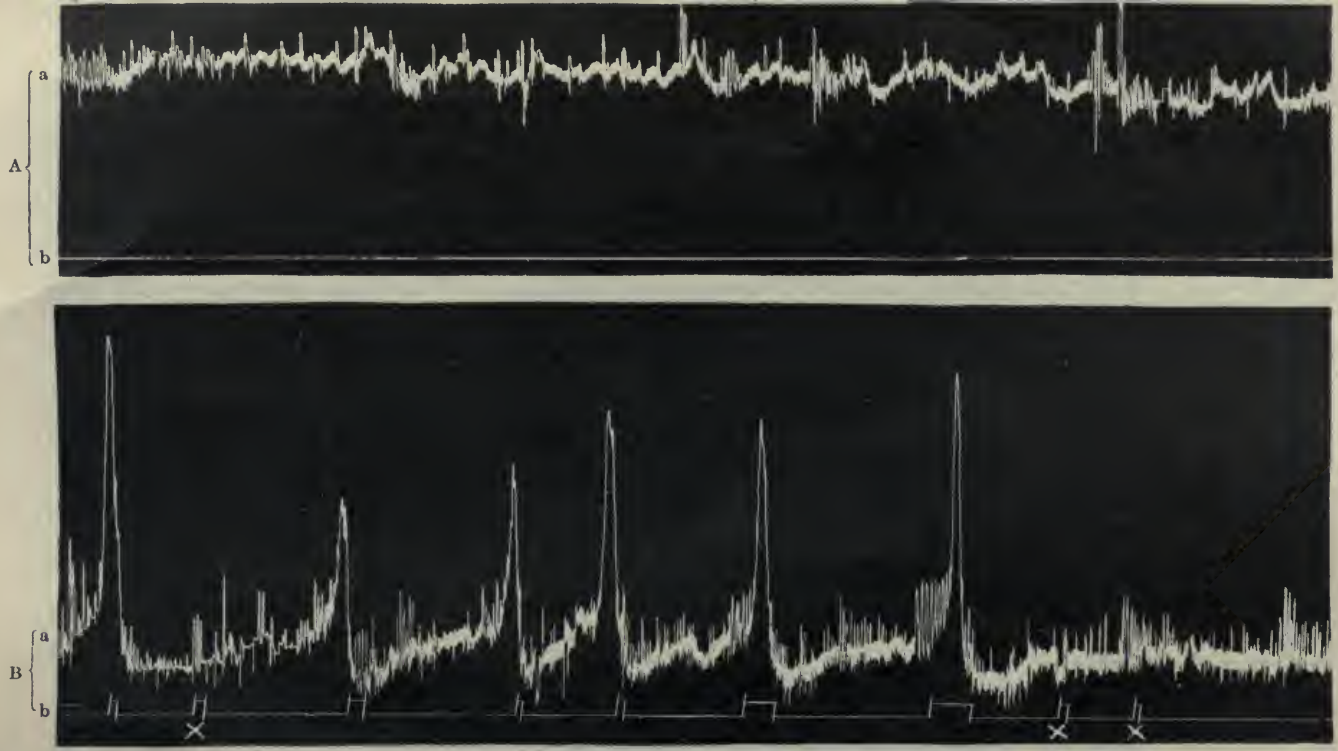

Fig. 4. G. H. M. Record of the sensation of gastric ulcer pains by means of a recording signal, *b*, parallel with the recording of the gastric contractions by the balloon method. Chloroform manometer. Tracing A, July 28, 1917, 11.30 to 12.30. No breakfast; a glass of water at 8 a.m. 11.30 a.m., contents of the stomach 90 cc. + bile. Free acidity = 0.20 per cent; total = 0.29 per cent. At 12.30 p.m. contents of stomach: 85 cc. + bile. Free acidity = 0.22 per cent; total = 0.31 per cent; stomach quiescent and no sensations of ulcer pains.

Tracing B, July 31, 10 to 12 a.m. No breakfast, a glass of water at 8 a.m. At 10 a.m. stomach contents = 65 cc. + bile. Free acidity = 0.20 per cent; total = 0.27 per cent. At 12 m. stomach content = 55 cc. + bile. Free acidity =

tonic or tetanic contraction of this part of the duodenum itself. This is at least true of dogs under light ether anesthesia. The higher the acidity the stronger and the more prolonged both the pyloric closure and the duodenal spasm (8). It is reasonable to infer that weaker acids, 0.1 to 0.2 per cent, will induce these strong contractions in cases where the duodenal and pyloric nervous mechanism is rendered hyperexcitable by ulcer or other pathological states.

4. Pyloric hypertonicity and spasms in infants may or may not be painful, but internists are generally agreed that in adults pyloric spasms are painful in proportion to their intensity and duration. And if the sensory nerves of the pylorus are hyperexcitable, the ordinary pyloric tonus and contractions may be felt as pain, just as is the case with the normal digestion peristalsis of the stomach in gastric ulcer.

DISCUSSION

All the evidence thus points to the fact that the pains of gastric and duodenal ulcers are contraction pains arising either in the stomach or in the pylorus and upper part of the duodenum. In the case of the stomach the contractions are usually not stronger than those of normal digestion peristalsis of the filled or the hunger tonus rhythm of the empty stomach. This points clearly to a condition of hyperexcitability of the gastric pain nerves in the ulcer patients experiencing the typical ulcer pains.

Since the ulcer pains are due to tension of excessive contractions or of normal contractions on hyperexcitable pain nerves, it is evident that pathological states other than ulcer, inducing such hyperexcitability or hypermotility, will cause symptoms of gastric ulcer pains practically identical with those of ulcer, as appears to be the case in many instances of appendicitis, cholecystitis and achylia.

The contraction origin of the ulcer pains also serves to explain the frequent, if not usual, lack of parallelism between ulcer pains and gastric acidity. Within certain limits the motility of the stomach is independent of the chemical reaction of the stomach contents (3). The influence of the chemical reaction of the gastric content on the pylorus is more complicated (2, 3, 10, 12), but high acidity will intensify and prolong the duodenal reflex contraction of the pylorus as well as induce strong contractions in the duodenum itself. Clinical hyperacidity may in that way indirectly aggravate the ulcer pains from the contraction of these parts of the alimentary canal, and any measure (protein

food, water or alkalies) that temporarily lowers gastric acidity will temporarily ease these pains, provided there is sufficient relaxation of pylorus to allow the weakened gastric content to reach the duodenum.

When the ulcer pains are due to the tonus and contractions of the body of the stomach, any measure which inhibits or decreases the gastric tonus (ingestion of food, water, alkalies, or acids, passing of the stomach tube, etc.) will temporarily ease the pains, irrespective of the chemical reaction of the stomach content.

While it is true that in the gastric ulcer patients, so far carefully studied by the balloon method, the stomach contractions causing the pains have not been stronger than those of healthy persons, there may be cases of ulcer pains actually due to excessive contractions, especially in the case of the pylorus. The continuous epigastric pain at times present in ulcer is probably due to persistent hypertonus of the stomach or the pylorus, and the more severe pains that cause the patient to double up are probably due to pyloric spasm.

The contraction origin of the ulcer pains also helps to account for the many cases of gastric ulcer not accompanied by pain. The variable factors are the gastric, pyloric and duodenal contractions, the proximity of the ulcer to the main branches of the vagi, the extent of inflammation and edema in the ulcer area, and the degree of inherent stability of the autonomic nervous system of the individual. It is obvious that further advance of our knowledge in this difficult field requires a combination of the balloon and the fluoroscope method of objective registration parallel with careful introspection on the part of the intelligent patient.

These newer aspects of the ulcer pains also serve to emphasize the fact that elimination of the ulcer pains is no criterion of healing of the ulcer. This criterion satisfies only the ignorant patient and the careless clinician.

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A NOTE ON SOME OBVIOUS CONSEQUENCES OF THE HIGH RATE OF BLOOD FLOW THROUGH THE ADRENALS

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Received for publication October 25, 1917

It has been shown by a number of observers that the rate of blood flow through the adrenal glands is exceptionally great. According to Neuman (1), it amounts in cats to 6 to 7 cc. of blood per minute per gram of organ, with a blood pressure of 130 mm. of mercury, a greater flow than that through any other organ.

Stewart and Rogoff (2) have published results on the rate of filling of a cava pocket from the adrenal veins in cats, from which the rate of flow through the glands with unopened blood vessels and in the absence of hemorrhage can be calculated. For example, in five successive observations while the circulation was fairly good, the blood flows were 2.8 cc., 2.6 cc., 2.2 cc., 2.3 cc., 2.5 cc. per gram of gland per minute. As the mesenteric and coeliac arteries were not tied and the arterial blood pressure was rather low in this experiment (38 to 42 mm. of mercury during the observations quoted), these rates of flow are doubtless below the average with blood pressures more nearly approaching the normal.

In other papers (3), we have published numerous observations in which the blood flow through the adrenals was estimated by collecting the blood through a cannula. In the great majority of these experiments the coeliac, mesenteric and renal arteries and the abdominal aorta were tied. Therefore the arterial pressure at the commencement of collection of the blood was generally high.

In twenty-three cats the blood flows in grams per minute per gram of gland were: 3.3, 4.2, 6.4, 11.3, 5.0, 7.0, 5.6, 4.5, 3.0, 1.4, 2.7, 3.2*, 7.9*, 6.0*, 4.5*, 4.2, 9.1*, 4.9*, 6.6*, 2.0*, 2.5*, 3.1, 6.0. Average, 5.0 grams.

The numbers marked with an asterisk are from animals in which only one adrenal remained, the other having been extirpated some weeks

previously. The average of these results shows practically the same flow as the average of the whole. In all the experiments several samples of blood were collected through the cannula. The rate of blood flow is usually calculated for the second sample, the first being rejected if much higher than the others because of the possibility of some blood being included in the cava pocket at the time it was clipped off, which would of course increase the apparent rate of outflow in the first sample. The results may accordingly be considered as certainly not too high for the given experimental conditions (ligation of alternative arterial paths, relatively high arterial pressure and almost zero pressure in the vein).

Burton-Opitz and Edwards (4), working with a stromuhr, obtained in dogs an average blood flow of 4.9 grams per gram of gland. This, as they point out, is very much more than the flow through any other organ with the possible exception of the thyroid. In connection with the figures given in the literature for the thyroid, it may be remarked that although there is no doubt that the blood flow through the normal thyroid is large in comparison with that of most organs, some of the experiments were probably performed on hyperplastic glands.

From the relatively great quantity of blood passing through the adrenals, it might be expected that the blood of the adrenal veins would differ less from arterial blood than ordinary venous blood does. It would hardly be credible, for instance, that the percentage loss of oxygen by the arterial blood in the adrenals should be as great as in the generality of tissues. It might, therefore, be looked for that adrenal vein blood should be richer in oxyhemoglobin than ordinary venous blood obtained, say, from the jugular vein.

If 5 grams of blood pass through a gram of adrenal in a minute, and the oxygen consumption of the gland is taken as 0.05 cc. (Neuman gives 0.045 cc.) per gram of organ per minute, the blood issuing from the adrenal veins would contain only 1 cc. of oxygen per 100 cc. of blood less than the arterial blood. In order that the difference should become as great as in ordinary mixed venous blood, say 8 volumes of oxygen per cent, the consumption of oxygen would have to rise to 0.4 cc. per gram of adrenal substance per minute. This would be five times as great as the oxygen consumption given by Barcroft for striated muscle during maximal activity. Yet a recent writer (5) thinks it worth while to demonstrate that the oxyhemoglobin bands in a dilution of adrenal vein blood are stronger than the oxyhemoglobin bands in jugular vein blood from the same animal, diluted to the same degree with oxygen-

free saline solutions, and to seek for an explanation of this fact in some action exerted by adrenalin in "augmenting the oxygen-absorbing capacity of hemoglobin."

The facts that adrenal vein blood often "develops an arterial appearance" in the vein when the latter is clamped at its outlet into the vena cava, and that on dilution with salt solution it becomes red sooner than ordinary venous blood are easily understood as soon as it is recognized that adrenal vein blood is nearer to arterial blood than ordinary venous blood. There is no evidence and no likelihood that adrenalin has anything to do with the matter at all.

"The observation that the addition of adrenalin to the perfusion fluid favorably affects the oxygen intake of the heart in perfusion experiments" ought not, I think, to suggest "that the base may play a similar rôle in relation to the oxygen-absorption of hemoglobin in the adrenal vein." For anything which strengthens the action of the heart may be expected to increase its oxygen consumption.

Again, it is known that the H-ion concentration of ordinary venous blood is somewhat greater than that of arterial blood. It might be expected from the great blood flow through the adrenal gland that the adrenal vein blood would be somewhat more alkaline than ordinary venous blood since it is nearer to arterial blood, and in particular cannot be supposed to have acquired as much carbon dioxide in its passage through the glands as mixed venous blood. The realization of this would probably have modified the statement in another paper (6)[1] that "the increased alkalinity (in the adrenal vein blood) is due to the dissolved adrenalin which it contains."

It is not easy to gather in what way these writers suppose that the change in the reaction of the adrenal vein blood is produced by the adrenalin in it. It is scarcely necessary to point out that it is highly improbable that the mere addition of the base adrenalin in the concentration of a $\frac{1}{500,000}$ molecular solution (which would correspond to a normal concentration of adrenalin in adrenal vein blood) to a liquid with the buffer properties of blood could produce an effect on the H-ion concentration measurable by the gas-chain method.

[1] The paper referred to, although headed "From the Cushing Laboratory for Experimental Medicine, Western Reserve University," was not seen by me until recently. The work was not done under my direction.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 45 JANUARY 1, 1918 No. 2

THE EFFECT OF ORGAN EXTRACTS UPON THE CONTRACTION OF VOLUNTARY MUSCLE

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From the Johnston Livingston Fund for Experimental Medicine in the Department of Medicine, Cornell University Medical College

Received for publication November 6, 1917

In previous communications in this journal we have attempted to show some of the physiological actions of certain endocrine glands. For this purpose we employed alkaline saline extracts (1) of the pituitary, thyroid, parathyroid, thymus, pancreas, spleen, adrenal, liver and ovary. Such an extract of an organ contains several different materials, each of which has been tested and compared to ascertain, if possible, which portion of the extract is most active. In our experiments, the fresh extract, made with normal salt solution, after straining through gauze and filter paper, has first been treated at room temperature with 10 per cent acetic acid to remove the nucleoproteins. After their separation the clear filtrate has been boiled, filtered and made slightly alkaline with sodium hydroxide; then boiled and filtered again. More or less hydrolysis must therefore occur. The final filtrate represents the non-coagulable part of the original extract and for convenience has been designated as the "residue" of that organ.

The coagulable portion of an organ extract contains, of course, simple proteins such as nucleoproteins, and derived proteins such as acid and alkali albumins, and coagulated proteins. Of these we have tested the nucleoproteins and acid and alkali albumins, and found them to be practically inert except in the case of the pancreas and adrenal (2). The nucleoproteins as well as the "residue" of the pancreas and adrenal glands, show considerable influence upon gastric activity, and in this

respect are exceptional. But the non-coagulable portion of an alkaline saline extract of every organ, on the other hand, (its "residue," as we have designated it), is uniformly active in that it produces certain immediate and characteristic effects. That is, the intravenous injection of each "residue," when the dose is standardized by its nitrogen content, is followed by a quite characteristic fall in blood pressure (3). The "residue" of the adrenal gland alone causes a rise in blood pressure. Each "residue," including those from the pituitary and adrenal glands, produces a more or less characteristic effect upon the contractions of the unstriated muscle fibre of some portion of the intestinal tract (4). When the "residue" stimulates the contractions, the addition of the commercial 1:1000 solution of adrenalin produces an immediate relaxation. Some "residues" increase the flow of pancreatic secretion, while the adrenal "residue" like adrenalin, checks it (5). Others, especially the thyroid "residue," stimulate the amount and acidity of the gastric secretion. The "residue" of the pituitary, as well as of the adrenal gland, inhibits gastric activity (2).

Having previously demonstrated the effect upon unstriated muscle fibre of the intestinal tract, of the "residue" of the endocrine glands, it was thought desirable to study their effect upon striated muscle. The question first arose as to whether the muscle of cats in which the thyroid gland had been removed was more readily fatigable then the normal. A number of cats were accordingly tested with this in view. In several cats the thyroids were removed some days prior to the experiment (care being taken to preserve the parathyroids), and they were allowed to recover from the operation before the experiment was performed. In other cats the glands were removed at the time of the experiment.

In both cases, however, the variation of the limits of the fatigue period were within the limits of variation of the normal cat. This is what one would expect since it has been shown that when the parathyroids remain intact, thyroidectomized animals may live for a long period without exhibiting any abnormal symptoms (6).

The general effect of thyroidectomy on the fatigability of the organism, the parathyroids remaining intact, was also studied in another manner. A number of cats were thyroidectomized and after their necks were healed, or in about two weeks, they were made to run in an electrically turned (wheel) treadmill until they were fatigued. A corresponding number of normal cats were run under the same conditions as controls. There was, of course, considerable variation in the period before the onset of fatigue, depending upon the age and size of the animal,

but within allowable limits of variation there was no appreciable difference between the normal cats and those in which the thyroids had been removed.

Following is a typical protocol of such an experiment, June 13, 1917.

Female cat, (operated on, thyroids removed, parathyroids intact, May 29.)
10.45 a. m. Placed in wheel; wheel started revolving.
11.25 a. m. Cat fatigued so that it slid in wheel instead of running.
11.40 a. m. Normal female cat placed in wheel; wheel started revolving.
12.28 p. m. Cat fatigued so that it slid in wheel instead of running.

Such finding corroborates the other experimental data. Apparently within such a period of time a sufficient amount of the thyroid "hormone" still remains in the circulation to be effective, or some other mechanism is finally able to bring about a compensation. Whether this compensation is permanent is, of course, another question. The time factor is probably an important element here but experimentation has not yet been carried out as to the ultimate effects of thyroidectomy on striated muscle.

After it had been ascertained that the fatigability of the muscle was apparently not influenced by more or less immediate thyroidectomy, the effect of the intravenous injections of various extracts upon the contraction of partially fatigued muscle was studied.

Normal adult cats were etherized, then tracheotomized and etherization continued through a tracheal cannula. The rectus abdominis muscle of one side was then freed from its attachment to the ribs and fastened at the upper end to a writing lever by means of a series of pulleys. As the portion of the rectus abdominis below the ribs was undisturbed, the blood and nerve supply remained fairly intact. One electrode from the induction coil was attached to the free end of the muscle and the other to the muscle near the pubic symphysis. The entire muscle was thus stimulated from a storage battery through an induction coil at the rate of one shock a second, on the break only, with a current of 0.7 amperes. An ammeter indicated that the stimulus was constant. The diminishing excursions of the writing lever in response to the same stimulus indicated the increasing fatigue of the muscle.

The dosage of each extract was measured by its nitrogen content calculated as protein so that the standard dose contained 0.1 gram protein. Blood pressure from the carotid artery was taken at the same time that the effect of intravenous injection upon the muscle was studied. When the excursions of the writing lever had decreased to a magnitude of less

than one-half of their original height, a standard injection of a thyroid preparation was given by the femoral vein. The following materials obtained from the fresh thyroid gland were tested in these experiments: The non-coagulable "residue" of the alkaline saline extract; nucleoproteins; coagulable proteins; Kendall's so-called "active principle" (7); a thyroid extract prepared according to Eddy's method for pancreas-vitamines (8); a "residue" made from commercial desiccated thyroid powder; and finally "residues" made from diseased human thyroids. These were obtained at operations and were of two kinds: *a*, consisting of simple goitre tissue removed from patients who gave no evidence of hypo- or hyperthyroidism; *b*, consisting of diseased thyroid tissue excised from patients who presented marked evidences of hyperthyroidism or expothalmic goitre.

Following is a typical protocol of the way in which the action of the glands was studied. The effects of a single organ extract were first observed, and it was then found that the action of several extracts could be studied without any complication in the effect upon the muscle or the blood pressure. The combinations in which the extracts were given were numerous. No set rule as to order was followed, in order that any deviation in results might be more readily checked up.

Protocol of July 23, 1917. Male cat, ether, tracheotomy

10.15 a. m. Femoral vein exposed for injection.

10.35 a. m. Blood pressure apparatus set up, with cannula in left carotid. Normal blood pressure taken.

10.55 a. m. Left rectus abdominis attached to pulleys, electrodes fastened and stimulation begun.

11.20 a. m. 3 cc. thyroid residue injected in femoral vein. Note fall in blood pressure, lasting two minutes. Note increase in muscle contractions.

11.40 a. m. 2.8 cc. alcoholic thyroid extract (95 per cent) injected. Note fall in blood pressure and lack of effect on muscle.

11.55 a. m. 4 cc. simple goitre residue injected. Note very slight fall in blood pressure and lack of effect on muscle.

12.15 p. m. Trachea occluded. Cat dies.

Of all these extracts, the non-coagulable "residue" of the alkaline saline extract, made from fresh thyroid glands, was found to be most effective in reënergizing fatigued voluntary muscle (fig. 1). The "residue" made from commercial desiccated thyroid powder showed only a fraction of the activity of the "residue" made from fresh glands (fig. 2). None of the other "extracts" or preparations mentioned above, including those from diseased thyroids, had any effect upon voluntary muscle, although it is interesting to note that the 95 per cent alcoholic thyroid extract is a greater stimulant of gastric secretion than the "residue" of

the alkaline saline extract which is so potent for striped muscle—a point which is at present being studied in a research to be published later.

It might have been expected that that "residue" would be inert which was made from the simple adenomata, or cyst-adenomata, of thyroids which in life gave no constitutional symptoms. Tumors of secreting structures are not generally believed to produce any active material. But that a "residue" of true hyperthyroid tissue should also be inert is somewhat surprising. The thyroid gland of exophthalmic goitre ap-

Fig. 1. The failing contraction of the fatigued muscle is shown at the left. At the point on the left indicated by the cross, 5 cc. of thyroid "residue" containing 0.1 gram of protein was injected. The succeeding increased excursions of the writing lever show the effect of the injection upon the previously fatigued muscle.

parently produces too much secretion. Is it possible that this excessive product is of extremely poor quality?

A study of the blood pressure tracings brings out another interesting variation. Organ extracts, with the exception of those from the adrenal gland are almost uniformly vaso-depressor. As may be seen from the tracings (fig. 3), blood pressure falls immediately on injection of the thyroid "residue," then rises again to the normal level within a short time

or from two to five minutes. The thyroid extract prepared according to Eddy's method for pancreas-vitamines, however, appears to have a pressor effect. Blood pressure rises and remains up for from five to seven minutes after injection, before it descends to the normal level again (fig. 4). The curve which it presents is very similar to the blood

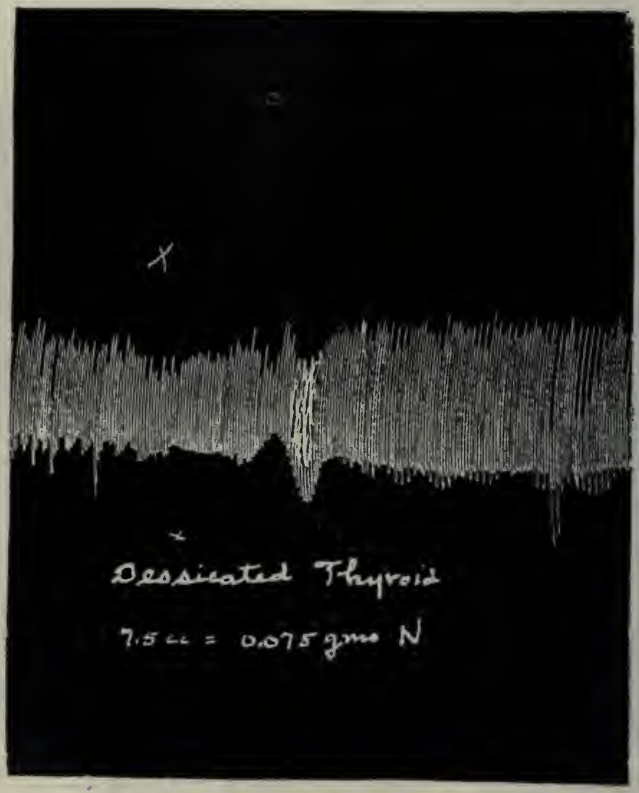

Fig. 2. On the left is shown the decreasing contractions of the muscle in the process of fatigue. On the right is shown the slight invigoration produced by the injection of a "residue" made from commercial desiccated thyroid.

pressure curve shown after intravenous injection of adrenalin or the adrenal residue (figs. 5 and 6).

Clinically, one of us (J. R.) has observed several cases of hyperthyroidism with high blood pressure in which the blood pressure fell to normal immediately or very soon after the enucleation of one or more encapsulated tumors of the thyroid. This experience, in conjunction

with the pressor effect of the thyroid "vitamine," (?) suggests that the pathological tissue excised from patients with high blood pressure may secrete a similar toxic substance.

After ascertaining that our thyroid "residue" was the only thyroid preparation which was capable of energizing fatigued muscle, we have

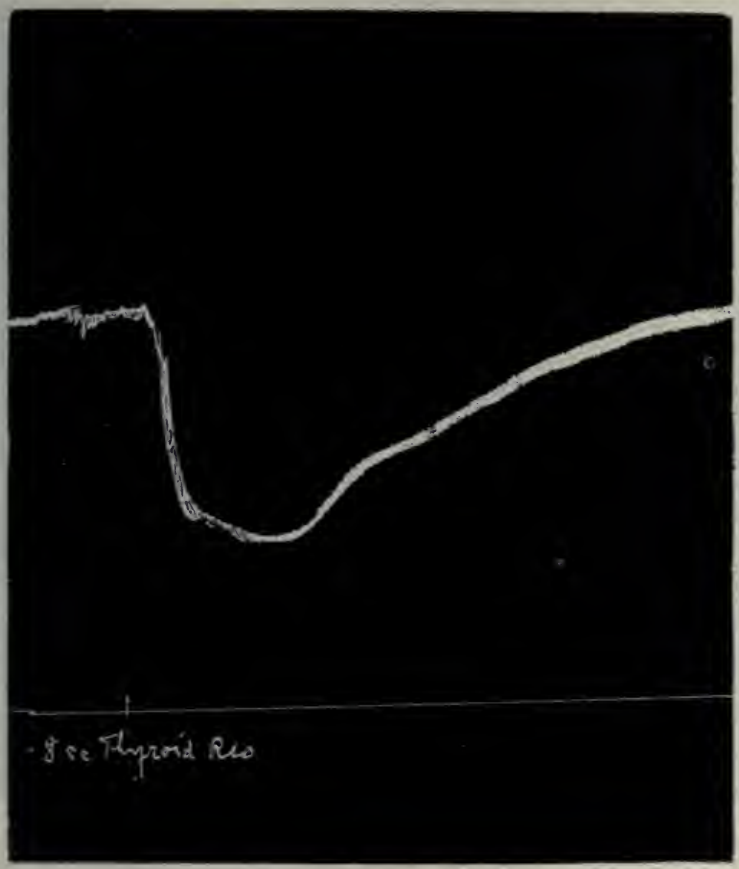

Fig. 3. This shows the characteristic fall in blood pressure which is produced by the intravenous injection of the thyroid residue.

similarly tested corresponding materials derived from the other endocrine glands including liver, spleen, ovary and pancreas. We have found that the "residues" of the parathyroid (fig. 7) and the adrenal gland (fig. 8) and also the commercial 1:1000 solution of adrenalin (fig. 9)

show a power like that of the thyroid, or reënergizing fatigued muscle. No other materials or extracts obtained from these or other organs exhibited any of this kind of activity.

The thyroid, parathyroid and adrenal glands alone seem capable of invigorating fatigued muscle. It should be noted here that our adrenal

Fig. 4. This shows the rise in blood pressure produced by the intravenous injection of the thyroid "vitamine" (?) It is preceded by a slight initial fall in pressure.

"residue" responds to the tests for adrenalin but apparently contains some other active material. Our "residue" is derived from the whole gland, while adrenalin or adrenin is obtained only from the medulla. Nevertheless, when the adrenin doses of the adrenal "residue" and of the 1:1000 adrenalin solution are the same, the effects correspond.

This, however, does not necessarily indicate that our adrenal residue is the exact physiological equivalent of adrenalin.

The next question concerns the mechanism or manner in which the thyroid, parathyroid and adrenal glands act upon the voluntary muscles. If, in the experimental animal, the spinal roots of the last six dorsal and all five lumbar nerves are sectioned on the side on which the rectus abdominis is being stimulated, fatigue of the muscle takes place somewhat more rapidly than in the normal or thyroidectomized animal.

Fig. 5. This shows the effect upon the blood pressure tracing of the injection of the commercial 1: 1000 solution of adrenalin.

This may be due either to the general exhaustion produced by the rather severe operation, or to lack of control of the peripheral by the central nervous system, or to both influences.

After section of the spinal roots of the nerves which supply the rectus abdominis muscle, however, the effects upon the fatigued muscle of the intravenous injection of the extracts is the same as in the muscle with the nerve supply intact. The point of action of the gland extracts must, therefore, be near the muscle fibre itself. Gruber (9) has demonstrated

in the case of adrenalin that the threshold stimulus of a denervated muscle was unaffected by curare and that adrenalin had no effect upon the curare threshold in this muscle.

The threshold of a curarized muscle with the nerve intact, however, is affected by fatigue which adrenalin counteracts. If curare acts upon Langley's "receptive substance" (10) between the nerve endings and the muscle, fatigue and adrenalin [as well as our "residues"] must act at another point nearer the muscle than the "receptive substance."

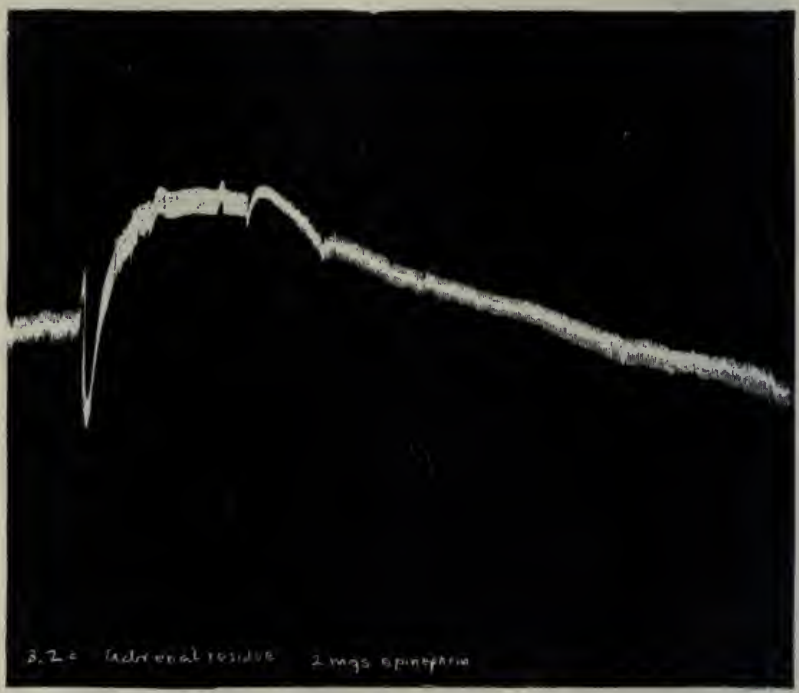

Fig. 6. This shows the effect upon the blood pressure tracing of the intravenous injection of an amount of adrenal residue which contained the same quantity of adrenalin as was given in figure 5.

In these experiments we have endeavored to demonstrate that materials derived from the thyroid, parathyroid and adrenal glands, and from those endocrine glands only, have a direct influence upon the contraction and fatigue of voluntary muscles. We have further sought to determine chemically the general group of substances in which the active principle of these endocrine glands is to be found. Such work must, however, at this stage be regarded as purely suggestive, since it calls

for a very thorough working out from physiological, chemical and clinical viewpoints.

Tests made with the various materials derived from the thyroid, parathyroid and adrenal glands show that fatigued muscle can best be invigorated by the non-coagulable or "residue" portion of an alkaline saline extract of each organ. This "residue," it should be noted, con-

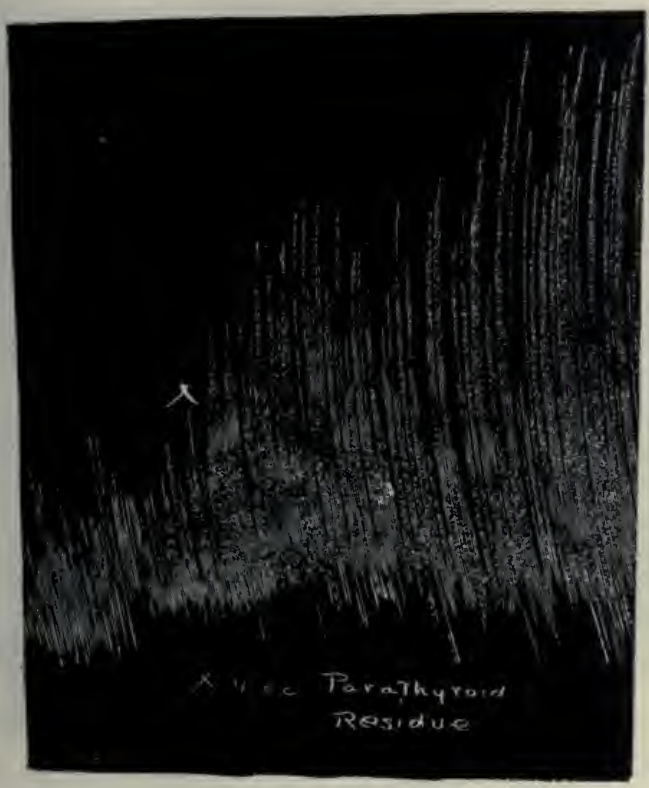

Fig. 7. This tracing shows the effect upon the failing contraction of fatigued voluntary muscle of the injection of the parathyroid "residue." 4. cc. = 0.1 gram of protein.

tains slightly hydrolized material, and in our experiments has always been made from "fresh" glands. A "residue" of desiccated commercial thyroid material is not nearly so active as extracts made from the fresh gland and, therefore, the usual thyroid medicament must be more or less unsatisfactory.

Muscular weakness is quite evident in the clinical condition commonly

described as that of hypothyroidism. Hypofunctionation of endocrine organs other than the thyroid and the adrenal is not so well recognized but, as a rule, seems also to be accompanied or manifested by asthenia; that is, muscular weakness is a constant and striking symptom not only in hypothyroidism and hypoadrenalism (Addison's disease) but also seems to occur in many other analogous conditions.

Fig. 8. This tracing shows the effect upon the failing contraction of fatigued muscle of the intravenous injection of the adrenal "residue." The 4 cc. contained approximately the same amount of adrenalin as the 3 cc. of adrenalin 1:1000 shown in figure 9.

From our experiments we infer that the thyroid gland, through its secretion, affects the neuro-muscular junction and thus in some unknown manner invigorates muscular contractions. It is, therefore, reasonable to suppose that the marked muscular weakness of hypothyroidism, at least in part, is due to the deficiency of thyroid secretion and consequent absence of the usual neuro-muscular activation or stimulus. This as-

thenia of hypothyroidism can generally be relieved by thyroid feeding, which usually means the administration by mouth of small amounts of the desiccated gland. It is scarcely conceivable, however, that digestion of this material can result in the introduction into the circulation of a substance which is so closely equivalent to the thyroid product as to act directly upon the neuro-muscular apparatus or junction as our

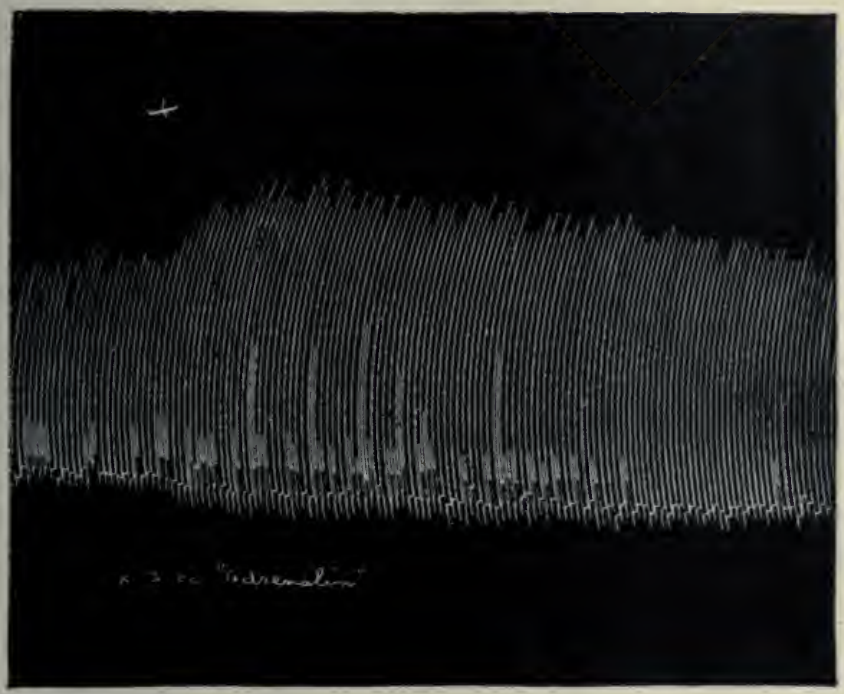

Fig. 9. This tracing shows the effect upon the failing contraction of fatigued muscle of the intravenous injection of 3 cc. of the commercial 1:1000 solution of adrenalin.

thyroid "residue" apparently does. In hyperthyroid conditions there is the same or a worse asthenia, and it is generally intensified by thyroid feeding. According to our tests, the extract of the hyperthyroid gland is entirely inert. Consequently, the usual muscular weakness which is present in these conditions appears to be virtually that of hypothyroidism. There are undoubtedly some cases of hyperthyroidism in which all the symptoms, including the muscular weakness, can be relieved and not intensified by thyroid feeding. Nevertheless, these cases are

exceptional. One can only conjecture the meaning of these apparent contradictions. In the absence of thyroid secretion, or in the presence of a very poor quality of secretion, there should, according to our experiments, be a deficiency in the muscular energy. More than this is unknown.

CONCLUSIONS

1. Intravenous injection of the non-coagulable portions of the alkaline saline extracts of the thyroid, parathyroid and adrenal glands increase the vigor of contraction of fatigued voluntary muscle.

2. The commercial 1:1000 solution of adrenalin shows a similar stimulant effect.

3. No other materials tested, derived from the thyroid, parathyroid and adrenal glands show any stimulant effect upon fatigued voluntary muscle.

4. Materials from the other endocrine glands show no effect upon voluntary muscle contraction.

5. Desiccation of the thyroid gland appears to lessen or destroy its activity.

6. Excision of the thyroid appears to have no immediate effect upon the fatigability of voluntary muscle.

7. "Residues" made from adenomatous or cyst-adenomatous thyroid material, as well as those made from the supposedly overactive gland of hyperthyroidism, are inert.

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ADRENALIN VASODILATOR MECHANISMS IN THE CAT AT DIFFERENT AGES

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Received for publication November 13, 1917

It has been established by a number of investigators (1), (2) that in the cat the normal response of the vascular system to small doses of adrenalin is such as to cause a fall in blood pressure. This effect is accomplished by a dilatation in the blood vessels in the muscles (3). Moreover the dilatation is controlled by a central nervous mechanism (4).

We accidentally discovered that adrenalin fails to produce a fall in blood pressure in young kittens with any dose, however small. The only reaction given is a rise. Inasmuch as the fall in blood pressure is controlled by a central nervous mechanism it seemed possible that the failure of this mechanism to develop at an early age might account for the reaction in young kittens.

With the hope of throwing more light on the nature of the adrenalin vasodilator mechanism we have made a study of cats at different ages.

The methods employed in this research were similar to those used in previous researches (4) but with the following modifications: Much smaller bellows than those used in adult cats were found advantageous in registering volume changes of the limb or intestine of kittens. Bellows with a base 26 mm. by 13 mm. were used in a majority of the experiments, while occasionally in the youngest kittens a smaller bellows with a base 17 mm. by 10 mm. was tried. All animals were under the influence of ether.

When the age of the kittens was unknown, it was necessary to make an estimate from the animal's weight. These estimates were based upon the weights of five kittens, whose ages were known, ranging from 0.3 kgm. to 1.3 kgm. in weight. The ages so determined are close enough for our purpose because the earliest occurrence of the adrenalin vasodilator mechanism is unquestionably variable.

BLOOD PRESSURE REACTION AT DIFFERENT AGES

A study of the blood pressure responses might be expected to give us an idea of the age at which the adrenalin vasodilator mechanism begins to appear. We therefore sought to answer our problem by means of the blood pressure reaction.

The youngest kittens employed were of known age (three weeks) and weighed 0.3 kgm. and 0.32 kgm. respectively. The threshold for blood pressure response to adrenalin was high in both cases. In the first, 0.1 cc., 1:100,000 caused a rise from 46 mm. to 49 mm., while less than that produced no effect. Even larger doses produced a smaller percentage rise than that from proportional doses in older kittens, although the duration of the effect might be as long (see *a*, fig. 1).

Eight older kittens weighing from 0.6 kgm. to 0.67 kgm. (about eight weeks of age) possessed a lower threshold for adrenalin, in some instances being as low as 0.2 cc., 1:1,000,000. In only two of these animals was there an occasional fall of blood pressure succeeding the rise. When it did occur it was small in amount. A depressor effect at this age was exceptional (see *b*, fig. 1). Kittens even older failed to give a fall in blood pressure with adrenalin. Seven individuals weighing respectively 0.72 kgm., 0.75 kgm., 0.9 kgm., 0.9 kgm., 0.95 kgm. and 1.05 kgm. (estimated ages, nine to eleven weeks), gave a rise without a fall in every injection, however small.

On the other hand animals weighing 1 kgm. or more usually gave a rise and fall in blood pressure with small doses, although repeated injections in the same animal might not always do so (*c* and *d*, fig. 1). It might be suggested that the failure of a depressor reaction in these cases was due to the easy fatigue of an incompletely developed mechanism. Kittens of the following weights gave the depressor reaction: 1.0 kgm., 1.0 kgm., 1.1 kgm., 1.3 kgm.

As the animals became older the pressor effects from small doses of adrenalin became gradually less and less while the fall in blood pressure became greater and more prolonged. Finally in the adult animal the rise became insignificant or in some cases a pure fall resulted (see fig. 1).

A study of the blood pressure reaction has shown that the depressor response to adrenalin begins to appear at the age of eleven or twelve weeks. From that age onward the depressor response encroaches more and more upon the pressor effect until finally the latter may almost disappear, provided small doses of adrenalin are injected. This graded increase of the depressor response with the growth of the animal indicates a gradual development of the adrenalin vasodilator mechanism.

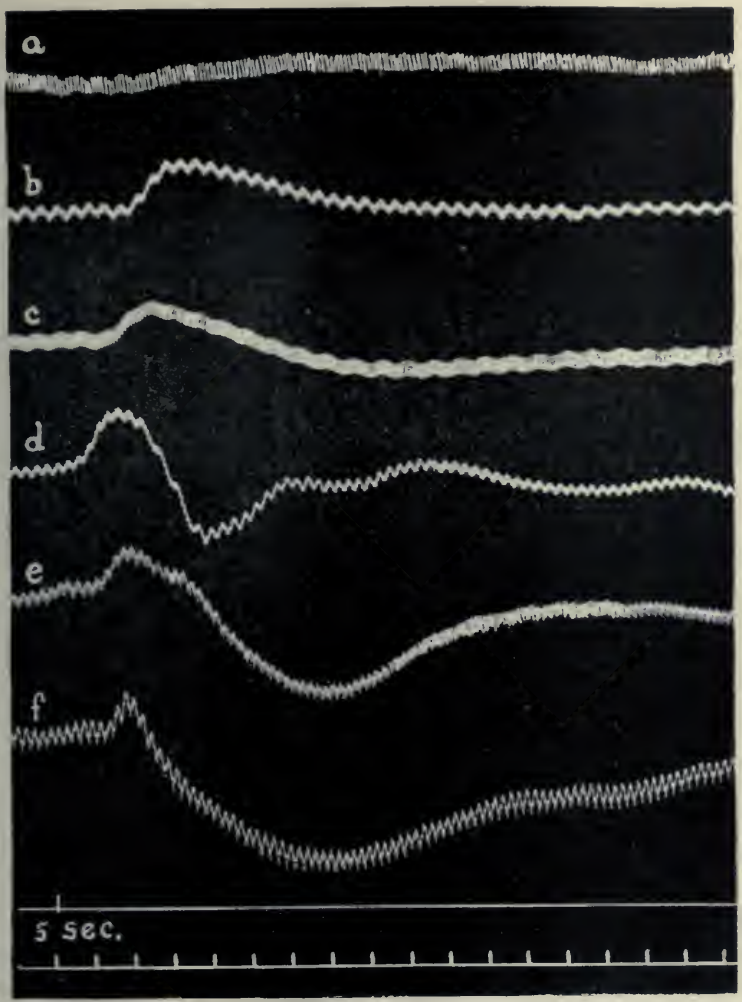

Fig. 1. Different types of blood pressure curves produced by adrenalin in cats of different ages.

	WEIGHT	AGE	DOSE OF 1:100,000 ADRENALIN	INITIAL HEIGHT OF BLOOD PRESSURE
	kgms.	*weeks*	*cc. per kgm. of body weight*	*mm. of mercury*
a	0.30	3	1.67	60
b	0.62	8	0.16	67
c	1.0	11	0.20	119
d	1.3	14	0.076	170
e	1.8	24	0.17	150
f	2.8	adult	0.071	151

It seemed at first that we had settled the question as to the age at which the adrenalin vasodilator mechanism first appears by a study of the blood pressure reaction. But in work on adult cats, which is to be published soon, we found later that an adrenalin vasodilator mechanism might be acting in an animal although the blood pressure response was a rise. Therefore without a study of the volume changes in the organ concerned, we cannot be certain of our solution.

For reasons given in another research (4, p. 366) we are led to treat separately the adrenalin vasodilator mechanism for the limb and the intestinal adrenalin vasodilator mechanism. It might be well to briefly repeat those reasons. First, there is a difference of threshold, i.e., the mechanism for the intestine has a higher threshold on the aver-

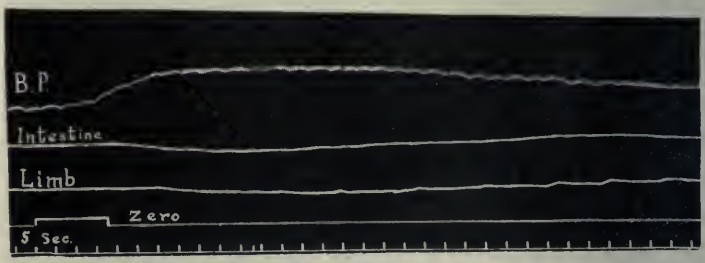

Fig. 2. Effect of 0.4 cc. adrenalin, 1:100,000 on the volume of the intestine and hind limb in a kitten three weeks old (0.32 kgm.). Smaller doses produced similar though less marked effects. (Reduced ½.)

age than that for the limb. Second, a difference in reversal, i.e., no increase in the dose of adrenalin ever changes intestinal dilatation to constriction when once the dilatation threshold is passed, while such an increase does cause a reversal from dilatation to constriction in the limb. These observations suggest different types of mechanisms. We will therefore discuss them separately.

THE ADRENALIN VASODILATOR MECHANISM FOR THE LIMB

Although we have employed the hind limb in this study, there is ground for assuming that its reaction (provided skin effects are negligible) represents the reaction for the skeletal muscle throughout the organism. We have therefore considered active adrenalin dilatation of the hind limb as proof of the presence of the adrenalin vasodilator mechanism for skeletal muscle.

We have sought for the presence of this mechanism in nine kittens at varying ages. The smallest to give undoubted evidence of its existence was nine or ten weeks old (0.85 kgm.) (see fig. 3). However the repeated injections of similar doses did not always produce active dilatation. This finding is parallel to the observation on the inconstancy of the depressor response in blood pressure in kittens first to show the reaction. Five younger kittens from three to eight weeks old gave no active dilatation of the limb. (See fig. 2.)

Two kittens about eleven weeks old (1 kgm.) reacted by limb dilatation more easily than did the nine-weeks-old kitten. As the animals

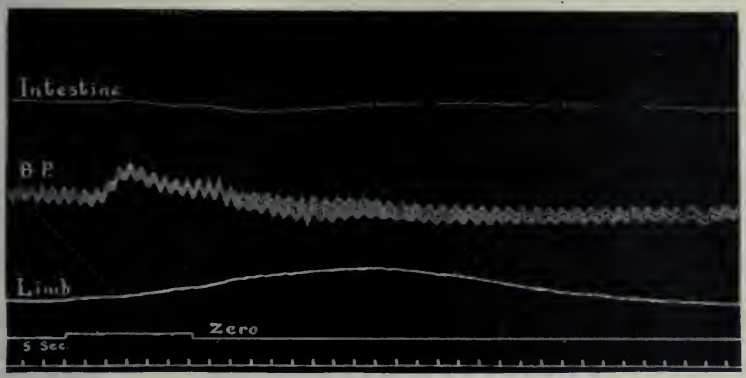

Fig. 3. Active vasodilatation in the limb of a kitten about nine weeks old (0.85 kgm.). Dose of adrenalin 1 cc., 1:100,000. (Reduced ½.)

grew older the reaction was elicited with greater constancy. At six months the response resembled more that of the adult.

In general we may say that the limb mechanism begins to function at or possibly before the eleventh week. Inasmuch as the fall in blood pressure is due to the action of an adrenalin vasodilator mechanism for skeletal muscle, we should find that the depressor response of blood pressure and the active limb dilatation begin to appear at the same age. Within the limit of experimental conditions we have found this to be true.

THE INTESTINAL VASODILATOR MECHANISM

Although the intestinal vasodilatation from adrenalin may contribute to the fall in blood pressure with doses that do not produce constriction in skeletal muscle, as soon as these doses are exceeded, intestinal vasodilatation merely subtracts from the pressor effects of the con-

stricting skeletal muscle. Therefore we cannot expect to throw much light on the blood pressure reaction by a study of the development of this mechanism. But because it seems to be of a different type we were anxious to compare its development with that of the limb mechanism.

Fig. 4. Intestinal reaction to a large dose of adrenalin, 0.4 cc., 1:20,000 in a three-weeks-old kitten (0.32 kgm.). (Reduced ½.)

The volume changes in the intestine, resulting from the injection of adrenalin into the general blood stream, were observed in eleven kittens ranging in age from three weeks to six months. The amounts of adrenali ninjected varied from that just sufficient to give a response to massive doses. The absence of the intestinal adrenalin vasodilator mechanism was considered proven if massive doses failed to cause dilatation.

All kittens up to about eleven weeks of age (eight) failed to show the presence of an adrenalin vasodilator mechanism for the intestine. Three of about this age showed nothing but constriction, while a fourth gave a marked dilatation (see fig. 6). The character of the constriction differed somewhat with the age of the animal, younger kittens showing a more prolonged effect than older kittens (see figs. 4 and 5). This might be due to the vasodilator effects beginning to appear in the older kittens because we know that in adults the constriction is cut short by dilatation. At that stage the vasodilator mechanism is fully developed.

On account of differences already mentioned we have been led to consider the adrenalin vasodilator

mechanisms as of two different types. If this is true, the question arises as to whether the limb and intestinal adrenalin vasodilator mechanisms begin to function at the same age. This could best be answered by seeking for them in the same individual. Ten kittens were tested for the presence of both the limb and intestinal adrenalin vasodilator mechanisms. The youngest to show the presence of either mechanism was about nine weeks old. There was positive evidence of the presence of the adrenalin vasodilator mechanism for the limb, but absolutely no trace of the other mechanism. Two kittens about

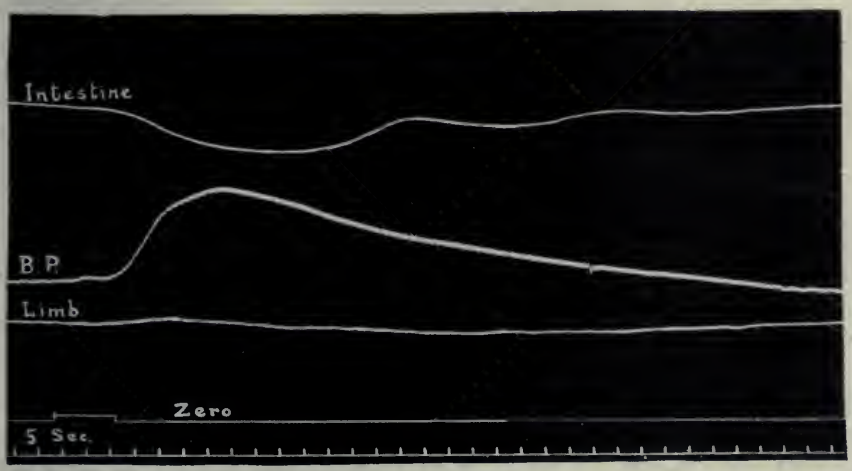

Fig. 5. Less prolonged intestinal constriction from a large dose of adrenalin (0.5 cc., 1:10,000) in an older kitten than in the previous figure. Age eight weeks, weight 0.67 kgm. (Reduced ½.)

eleven weeks of age gave active limb dilatation with adrenalin but absolutely no intestinal dilatation. We may conclude from these results that the two mechanisms may begin to function at different ages in the same individual. Moreover in every case so far noted (three) the adrenalin vasodilator mechanism for the limb functioned earliest. These observations lend support to the idea that the two mechanisms are of different types.

In conclusion we may ask: Why do the adrenalin vasodilator mechanisms develop so late in the life of the individual? Does it mean that the mechanism is one of the last to appear in the evolution of the cat? If so, it might be that they are specialized mechanisms occurring

Fig. 6. The youngest kitten to give evidence of the presence of the intestinal adrenalin vasodilator mechanism. Age, eleven to twelve weeks. Weight, 1.1 kgm. Adrenalin injected, 0.5 cc., 1:10,000. (Reduced ⅔.)

only in the carnivora. (Their presence has been proven in the dog.) A systematic survey of the vertebrates for the presence of these mechanisms is in progress in this laboratory.

SUMMARY

1. The smallest effective doses of adrenalin produce only a rise in blood pressure in young kittens.

2. The threshold for adrenalin blood pressure effects is high in young kittens, decreasing as they grow older.

3. The response to adrenalin of a fall in blood pressure begins to appear at about eleven weeks.

4. The increasing of the depressor effects from the slight fall succeeding a rise in younger animals to a marked almost pure fall in adults indicates a gradual development of the adrenalin vasodilator mechanism.

5. This fall in blood pressure seems to be due to vasodilatation in skeletal muscle, for the two begin to appear simultaneously in most instances.

6. The intestinal adrenalin vasodilator mechanism often develops later than the adrenalin vasodilator mechanism for the limb. This supports the view that the two mechanisms are of different types.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLV. Hunger, Appetite and Gastric Juice Secretion in Man During Prolonged Fasting (Fifteen Days)

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Received for publication November 15, 1917

There are two phases of the physiology of prolonged fasting that require further investigation on man, namely *a, the hunger mechanism and the hunger sense*, and *b, the gastric juice secretion.*

The reliable literature on prolonged fasting in man is practically unanimous on the point that the fasting person feels little or no hunger after the first three or four days. In the thirty-one days' fast of Levanzin, carried out in the Nutrition Laboratory of Carnegie Institution in 1912, the subject claimed he felt no hunger at any time (3). The observations of Boldireff (4) on fasting dogs appear to support these reports on man. It is now established that the sensation of hunger is induced by a certain type of tonic and peristaltic contractions of the empty or nearly empty stomach (1), (2). Boldireff concluded that the stomachs of fasting dogs become atonic and quiescent after the third or fourth day of fasting. No investigation of the motor activity of the stomach of fasting persons seems to have been made previous to the report of the writer in 1914.

The following facts appear to question the validity of the view that fasting leads in a few days to paralysis of the hunger mechanism and consequent absence of hunger sensation.

1. It has been noted by physiologists and surgeons that the stomach of man and animal after a prolonged fast or on death from starvation is in a state of strong tonic contraction. Other factors being normal, a stomach in such state of contraction will give rise to hunger sensations.

2. The observations of the writer on two normal persons showed that the gastric hunger contractions and the hunger feeling persisted with practically normal intensity at least to the end of the fifth day of starvation.

3. The studies of Patterson, Rogers and the writer on fasting dogs and rabbits failed to confirm Boldireff's conclusions. The studies from our laboratory show that the gastric hunger contractions continue normal or even stronger than normal almost to the stage of death from starvation. Similar results were obtained by Patterson on fasting turtles and frogs.

4. If hunger and appetite disappear after a few days' fasting in animals below man, it is difficult to understand what induces the fasting animal to resume eating or to search and fight for food. A fasting person may resume eating from a sense of propriety or duty, but can we assume similar motives to action in the starving wolf or the starving caterpillar? Or is it likely that failure of the hunger mechanism after a few days' fasting obtains in man alone, in view of the complete parallel of other features of inanition in man and the lower animals? Wodsedalek has recently reported that a beetle (Trogoderma tarsale) will resume feeding after four to five years of enforced starvation during which period he may lose seven-eighths of his body substance.

Most of the evidence tending to show suppression of hunger after a few days' fasting consists of the statements of professional fasters or persons fasting to improve their health. Many of these persons have a fixed faith in fasting as a cure-all, hence they tend to ignore or deny most discomforts of starvation. The professional faster may do so in the spirit of bravado, and where the elements of faith or bravado are not in evidence the statement of the fasting person is obscured by the usual confusion of the sensations of the *pangs of hunger* with the *appetite for food*. In prolonged fasting the latter is interfered with in many persons by a persistent bad taste in the mouth.

We must recognize the possibility that the hunger mechanism may be depressed or altered in an individual by emotions and auto-suggestions. Observations on fasting animals are therefore not conclusive as the subject must be able to report his sensations synchronously with the presence or absence of the gastric hunger contractions.

A most striking case of prolonged suppression of hunger by emotional states in mammals has recently been recorded by Osgood, Preble and Parker. It would seem that the Alaskan fur-seal bull neither eats nor drinks during the entire breeding season (May to August).

> Many of them (the bulls) have been on the beaches from May and during the period from their arrival to the end of July or the early part of August, they touch no food. This fast of well over two months coupled with their incessant activity (fighting, sex activity) drains them of all their stored energy. They are reduced to skin and bones (p. 393).

From the time the bulls take their places on the beaches till they leave about early August, it is impossible to drive them away and they are never seen in this period in the water so that I think it perfectly safe to say that most bulls get no food for two months (June and July). If some come in April, as is stated by the islanders, the period may be as long as four months. The weather is cool or misty or rainy so that loss of water is not favored and abstinence from water is not so remarkable as that from food.[1]

The seal and the whale obviously obtain the water requirements for the body from the food itself (fish). In the absence of activity of the sweat glands the loss of water (from the lungs) in the seal is probably not much greater on land than in the sea. Does the tissue catabolism of the fasting seal yield a sufficient amount of water for the work of the kidneys for two to four months? Assuredly, a mature fur-seal bull confined in a suitable metabolism cage for two to four months during the breeding season would furnish very interesting data.

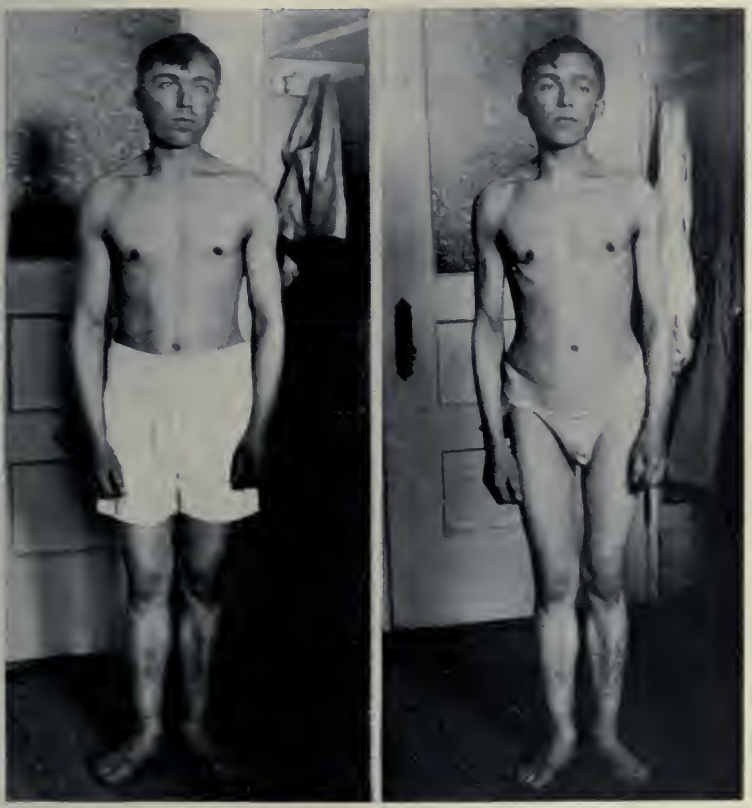
According to Boldireff, the stomach of the fasting dog exhibits occasional periods of secretion of gastric juice during the first three days of fasting, and after the third or fourth fasting day the gastric glands secrete continuously. Boldireff is inclined to ascribe the motor quiescence of the stomach of his fasting dogs to a reflex inhibition from the acid of this continuous secretion. The observations of Beaumont on Alexis St. Martin and those of Pavlov on dogs are largely responsible for the generally accepted view that in normal individuals the gastric glands are quiescent when there is no food or secretagogues in the stomach, and the appetite mechanism eliminated. We have shown that this view is erroneous for adult man and dogs. Hess and Taylor have shown that there is a continuous secretion of gastric juice in the newborn infant before the first feeding, and in young infants in the absence of food in the stomach. A continuous secretion of gastric juice by the normal empty stomach is therefore established. Is this continuous secretion augmented in prolonged fasting, as indicated by Boldireff's work on dogs? So far as we know this phase of inanition has never been investigated in man. This question is of importance not only in relation to the nature of inanition and to gastric physiology but as bearing on the possible rôle of the alimentary tract enzymes in starvation metabolism.

Mr. Frederic Hoelzel, the subject of this fasting study, is a man twenty-eight years old, graduate of a first class technical high school, and well

[1] Prof. G. H. Parker, personal communication. Prof. W. H. Osgood informs me that the bull may occasionally enter the water to reclaim an escaping cow, but he does not go far from land and does not feed.

versed both in the scientific and the popular literature on fasting, dietetics and nutrition.

During his last year in the high school (age 18), he suffered a breakdown in health, with insomnia, digestive disorders, great mental depression and a loss of 30 pounds in weight in the course of a year. The



A B

Fig. 1. Photograph of Mr. F. Hoetzel. *A*, before; *B*, at the end of the fifteen days' fast.

specific cause of this breakdown was not determined. The digestive disorders directed his attention along dietetic lines. He states that he has never completely recovered the mental and physical wellbeing enjoyed before this breakdown. He believes that starchy foods particularly disagree with him (causing flatulence). He has tried from time to

time to improve his health by starvation, fasting many times for periods of three to four days, at one time for a period of eight days, and once, three years ago, for a period of twenty-six days.

With the view of obtaining cure for these real or fancied digestive disorders as well as to render fasting more easily carried out, he has tried such food substitutes as "loam, sand, glass beads, silk, agar-agar, whole grain and seeds, bran, raffia, artificial cellulose and cotton fiber or kapoc."[2] He found the most satisfactory food substitute to be cotton fiber, cut into short lengths and flavored with salt, vinegar, citric acid, fruit juices or other food extractives. For the last year his diet has consisted mainly of fruits, vegetables and nuts, with some cream and eggs, and cotton fiber to make up bulk or "staying" quality.

Before starting this fast Mr. H. was given a thorough physical examination, including x-ray of the stomach by Dr. A. B. Luckhardt and Dr. F. C. Becht. The only defects discovered were some enlargement of the lymph glands of the neck, a tooth abscess and an unusual thickening and roughness of the skin, particularly of the lower extremities. He appeared somewhat nervous and diffident in the presence of strangers but looked the part of a well-nourished man, despite his own statement that he felt below par physically and mentally. He also felt convinced that his health would greatly improve after a prolonged fast. He thus undertook the fasting in the interest of his health and not primarily for the physiological observations made during the fast, although he appreciated the importance of the latter and coöperated in them with splendid intelligence and ability and with scrupulous honesty.

During the entire observation period—June 11 to September 1—Mr. H. lived in the laboratory.

Before starting the fast daily observations were made of the gastric hunger contractions and the gastric secretion for one week while Mr. H. continued on his regular diet. He states that for the past year he has practically taken only one meal a day, that is, from 6 to 11 p. m. The food taken June 11 to 17 was consumed in the same manner from 6 to 11 in the evening each day. There was always some food in the stomach at 8 o'clock the following morning. The stomach was usually empty of food (including the cotton) at 11 to 1 o'clock.

[2] Kapoc is the trade name for a very soft or silky cotton from the orient, used mainly for mattress filling.

Diet during control period, June 11 to 17

DATE AND MATERIAL	QUANTITY	CALORIES
June 11.		
Cream	1 pint	822
Eggs	132 grams	220
Nuts	77 grams	650
Flavor (maple sugar and cocoa with water)	276 grams	552
Oranges	311 grams	155
Tomatoes	841 grams	200
Strawberries	1020 grams	387
Cotton fiber (Kapoc)	21 grams (dry)	0
		2986
June 12.		
Eggs	141 grams	231
Cream	1 pint	822
Nuts	26 grams	181
Oranges	147 grams	73
Tomatoes	877 grams	212
Strawberries	942 grams	359
Flavor (maple sugar and cocoa)	231 grams	463
Cotton fiber (Kapoc)	21 grams (dry)	0
		2341
June 13.		
Eggs	177 grams	289
Cream	1 pint	822
Nuts	40 grams	288
Oranges	200 grams	100
Pineapple	440 grams	200
Tomatoes	845 grams	200
Flavor	212 grams	624
Cotton fiber (Kapoc)	27 grams (dry)	0
		2523
June 14.		
Eggs	256 grams	245
Cream	1 pint	822
Nuts	26 grams	181
Oranges	140 grams	70
Peaches	165 grams	69
Cherries	136 grams	96
Strawberries	432 grams	165
Tomatoes	905 grams	217
Flavor		560
Cotton fiber (Kapoc)	24 grams (dry)	0
		2425

Diet during control period, June 11 to 17—Continued

DATE AND MATERIAL	QUANTITY	CALORIES
June 15.		
Eggs	100 grams	165
Cream	1 pint	822
Strawberries	732 grams	279
Blackberries	484 grams	280
Tomatoes	715 grams	172
Oranges	166 grams	75
Nuts	28 grams	185
Flavor		584
Cotton fiber (Kapoc)	18 grams (dry)	0
		2562
June 16.		
Cream	1 pint	822
Eggs	95.5 grams	160
Raspberries	481 grams	255
Strawberries	800 grams	304
Blackberries	132 grams	79
Tomatoes	706 grams	168
Nuts	70 grams	505
Flavor		423
Cotton fiber (Kapoc)	10 grams (dry)	0
		2716
June 17.		
Pineapples	704 grams	316
Oranges	335 grams	267
Cherries	278 grams	227
Strawberries	457 grams	172
Bananas	224 grams	224
Cantaloupe	716 grams	340
Tomatoes	706 grams	176
Peaches	176 grams	72
Raspberries	226 grams	119
Apricots	127 grams	106
Flavor		182
Maple sugar	38 grams	155
		2356

This was the last meal before the first fasting period. The quality and the quantity of the food (and cotton) consumed each day were determined by his appetite and hunger. The heat value of the foods was

not figured until a later date. But it will be seen that even on such an unusual diet and only one meal per day the average heat value of the food consumed each day for this preliminary period was 2,544 calories.

The motility of the empty stomach was recorded by means of a small balloon in the stomach connected with a chloroform manometer. The contents of the empty stomach and the continuous secretion of the stomach were collected by a modified Rehfus tube. Mr. H. had no difficulty in swallowing these instruments and retaining them in the stomach without discomfort for several hours (four to ten) at a time.

The neuro-muscular strength and endurance tests were made on a Story type of ergograph, the abductor muscle working against a weight of 700 grams.

Mr. H.'s daily routine during the two fasting periods was, in general as follows: Sleep or rest in bed until 9 to 10 a.m. Taking of stomach content and continuous secretion. Recording of the hunger contractions (several hours). Ergograph record. A detailed diary especially of his sensations and emotions written by Mr. H. himself. A short walk in the nearby park or a ride in an automobile. Reading in the evening from 8 to 10. He sometimes attended a nearby motion picture theatre evenings. Towards the end of his fifteen days' fast he became less inclined to leave his room, spending most of the time resting on the couch when not engaged in the physiological tests.

I. THE GASTRIC HUNGER CONTRACTIONS DURING THE PRELIMINARY CONTROL PERIOD. BULIMIA

The record showing the end of a typical period of gastric hunger contractions of Mr. H. during the preliminary or control week is reproduced in figure 2. There is nothing abnormal in these records for a man of his age and physical condition, except that the periods were, on the whole, unusually long (one to two hours). The periods frequently ended in incomplete gastric tetanus lasting from ten to fifteen minutes. Shorter periods of incomplete tetanus or strong tonus waves also frequently appeared before the end of the period.

It was quite evident, however, that Mr. H. felt these contractions as more painful and uncomfortable than the average normal person. Before coming to our laboratory he had ascribed these pains to the pressure of gases chiefly in the large intestine. He did not consider them hunger pangs although he knew that he could abolish them by taking food or filling the stomach with indigestible material.

Fig. 2. Record showing the end of a period of gastric hunger contractions of Mr. F. H., June 13, 1917, twenty hours after a large meal (see p. 127). These apparently normal contractions induced severe abdominal discomfort or pain and a mental condition probably identical with bulimia. Chloroform manometer, time = 50 minutes.

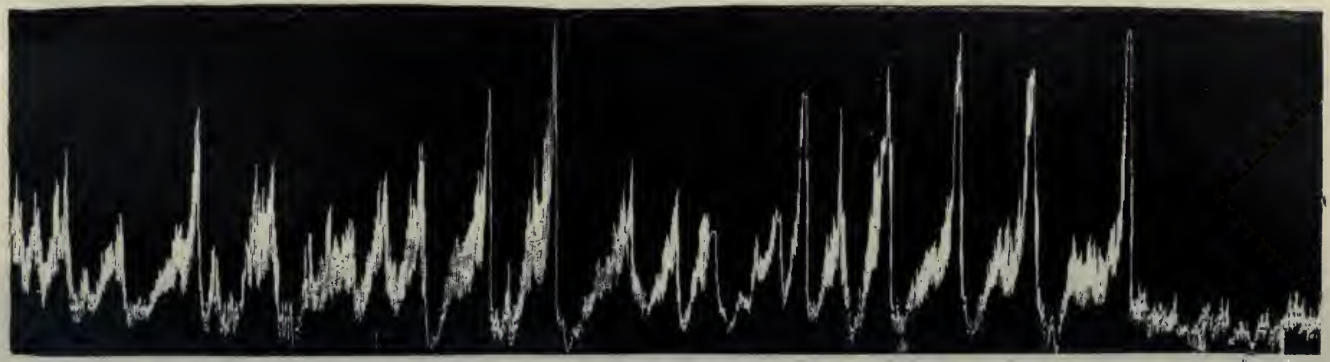


Fig. 3. Mr. F. H., June 20. End of a period of gastric hunger contractions on third day of fasting.

On June 13, when the record in figure 2 was taken, Mr. H. wrote in his diary: "These pains have frequently compelled me to end a fast, or deterred me from starting a fast. I don't see how any one could consider such pains as normal. When these contraction pains are most intense they appear to involve the entire abdomen and cannot be localized in the stomach." Mr. H. occasionally felt the pain, but less severely, even after beginning the ingestion of food.

It is quite clear that essentially normal gastric hunger contractions induced hunger pangs of abnormal painfulness in Mr. H. The reason for this condition is at present a matter of conjecture. There was no evidence of gastric or duodenal ulcers or of tabes. Hence the excessively painful character of the hunger contractions must have been due either to a more or less chronic hyperexcitability of the sensory nerves of the alimentary canal, or to a type of neurosis that led Mr. H. to give undue prominence to the visceral sensations in his conscious processes.

Mr. H. seems also to be deficient in the sensation of satiety.[3] The quantity of food in the form of bulky fruits and vegetables, in addition to bulky cotton, ingested by Mr. H. in order to feel "satisfied" would in the normal individual of the same size produce discomfort from overdistention of the stomach. Hence, unless Mr. H. takes indigestible material, like cotton fiber, together with his food, he necessarily overeats if he is to fill the stomach to the stage of satiety.

These facts lead me to think that Mr. H. presents a case of true bulimia. I have never seen a case of true bulimia, so diagnosed by a competent clinician, nor do I know of a case of true bulimia whose gastric motility has been studied by the balloon method. But these results on Mr. H. seem to show that bulimia does not necessarily involve an increase in the strength and duration of the gastric hunger contractions.

II. THE GASTRIC HUNGER CONTRACTIONS DURING FASTING

Our results may be given by the following analysis of the daily observations, together with the typical tracings reproduced in figures 3 to 9.

[3] Mr. H. states that food like bread or potatoes fails to satisfy him even when taken in very large quantities. The end of a meal is determined by the discomforts of an overdistended stomach, without any feeling of real satisfaction or satiety. The latter feeling usually does not come till the following morning.

First control period

June 11, 2–4 p.m. Two strong hunger periods (26 contractions) both ending in tetanus.

June 12, 12–4 p.m. Two strong hunger periods (37 contractions) both ending in tetanus.

June 13, 12–6 p.m. Three strong hunger periods (45 contractions) all ending in prolonged tetanus.

June 14, 12–6 p.m. Strong tonus and almost continuous hunger contractions (80) with short periods of tetanus.

June 15, 2–4 p.m. Two hunger periods (45 contractions).

June 16, 2–4 p.m. One prolonged hunger period (18 contractions) ending in tetanus lasting 10 minutes.

First fasting period

(Last meal 10 p.m., June 17)

June 18, 12–4 p.m. Three hunger periods (50 contractions), one ending in tetanus.

June 19, 5–8 p.m. Two mild hunger periods (37 contractions).

June 20, 5 hours. Four hunger periods (88 strong contractions).

June 21, 6 hours. Three hunger periods (53 strong contractions), one period ending in tetanus.

June 22, 6½ hours. Four hunger periods (40 fairly strong contractions).

June 23, 1½ hours. One strong hunger period (12 strong contractions).

June 23, 9 p.m. to June 24, 5 a.m. Five fairly strong hunger periods (50 contractions). No tetanus. Mr. H. slept during part of this observation period, becoming restless in his sleep and tossing about at the height of the strongest hunger contractions.

June 24, 3½ hours. Two moderately strong hunger periods (20 contractions).

June 25, 5 hours. Three strong hunger periods (38 contractions).

June 26, 5 hours. Three very strong and prolonged hunger periods (40 contractions).

June 27, 5 hours. Four moderately strong hunger periods (47 contractions), one period ending in moderate tetanus.

June 28, 8 hours. Three fairly strong hunger periods (35 contractions).

June 29, 4 hours. Three fairly strong hunger periods (25 contractions).

June 30, 4 hours. Three fairly strong hunger periods (35 contractions).

July 1, 5 hours. Three fairly strong hunger periods (30 contractions), one period ending in tetanus.

July 2, 5½ hours. Four fairly strong hunger periods (37 contractions).

July 3, 4 hours. One fairly strong and two feeble hunger periods (30 contractions), no tetanus, but marked and prolonged tonus variations, some of the tonus contraction periods lasting 10 minutes.

Second control period (July 4 to August 10)

During this period records of the motility of the empty stomach were taken for two to four hours each day. The tracings are practically identical with

Fig. 4. Mr. F. H., June 23. End of a period of gastric hunger contractions on sixth day of fasting.

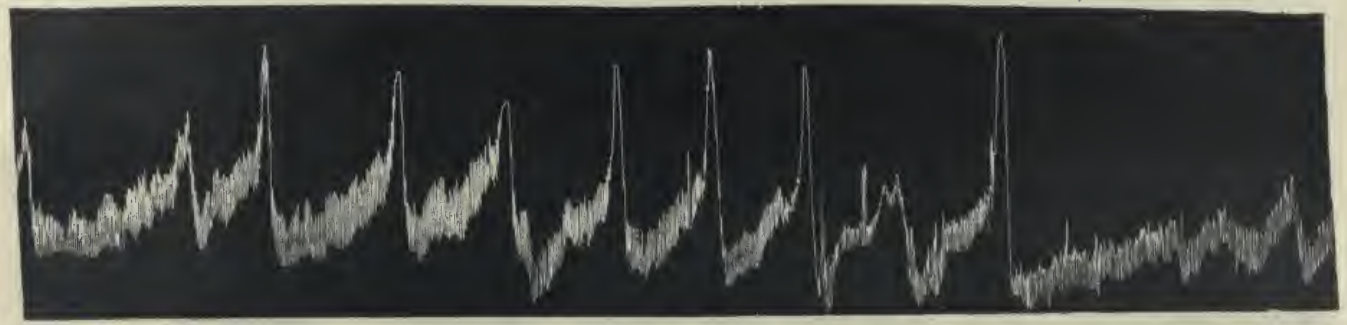


Fig. 5. Mr. F. H., June 26. End of a period of gastric hunger contractions on ninth day of fasting.

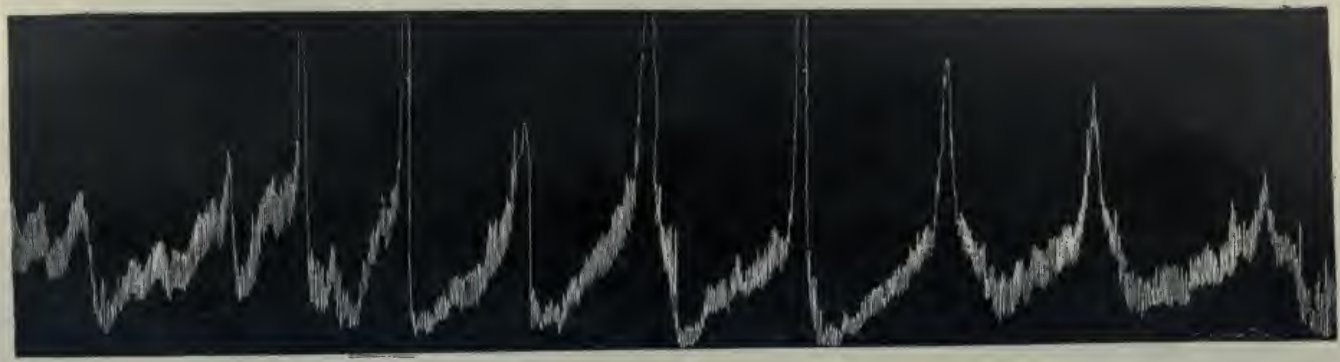


Fig. 6. Mr. F. H., June 29. End of a period of gastric hunger contractions on twelfth day of fasting.

Fig. 7. Mr. F. H., July 2. End of a period of gastric hunger contractions on fifteenth day of fasting.

Fig. 8. Mr. F. H., July 3. End of a period of gastric hunger contractions on sixteenth day of fasting.

Fig. 9. Mr. F. H., August 18. End of a period of gastric hunger contractions on the eighth day of the second fasting period.

those of the first control period, so it is not necessary to give the data in detail. Periods of strong tonus and incomplete tetanus were again of frequent occurrence.

Second fasting period with daily ingestion of cotton moistened with gastric juice (August 11 to 18)

August 11, 10–12 a.m. One moderate hunger period (11 contractions).
August 12, 12–5 p.m. Four strong hunger periods (36 contractions).
August 13, 12–7 p.m. Six strong hunger periods (58 contractions).
August 14, 10.30 a.m.–2 p.m. Three strong hunger periods (33 contractions).
August 15, 11–12 a.m. One strong hunger period (15 contractions).
August 16, 11.30 a.m.–5 p.m. Four strong hunger periods (60 contractions).
August 17, 12.30–3.30 p.m. Practically continuous hunger period (40 contractions and 3 incomplete tetany periods, one lasting 20 minutes).
August 18, 7–11 a.m. Practically continuous hunger periods (45 contractions and 2 incomplete tetany periods).

Third control period (August 19 to September 1)

The daily record of the gastric hunger contractions during this period are practically identical with those of the previous control periods. Detailed analysis is therefore superfluous.

At the rate that Mr. H. was losing weight during the first fast (nearly 500 gr. per day) his second fast, reducing his body weight to 47.5 kgm., is equivalent to adding six to eight fasting days to his first fasting period, as his body weight at the end of that period was 50.57 kgm. Our results thus permit the conclusion that *the gastric hunger contractions persist with no appreciable decrease or increase in vigor, at least during the first twenty days of complete fasting in man.* This conclusion is further strengthened by the previous studies on fasting in man and dogs reported from our laboratory.

III. THE SUBJECTIVE FEELINGS OF HUNGER AND APPETITE DURING THE FAST

Mr. H. was conscious of the gastric hunger contractions throughout the fasting, and the periods of strong continued gastric tonus and incomplete tetanus were felt as a continuous uncomfortable tension. The more severe tetany periods made him very restless and uncomfortable and occasionally induced sweating. But on the whole *the gastric hunger contractions, although of normal duration and intensity, were less uncomfortable or painful than during the control periods when he consumed sufficient food to maintain or even increase the body weight.*

Mr. H. is also convinced that he felt the gastric hunger contractions throughout his twenty-six day's fast, carried out three years earlier. He is inclined to the belief that fasting men who report feeling no hunger

after the first three days' abstinence from food do not associate the sensation produced by the gastric hunger contractions with hunger, but regard it as pain or discomfort from gastro-intestinal disorders.

The state of the appetite for food during fasting appears to depend on the condition of the mouth. The mouth is frequently foul and the tongue coated, despite repeated washing of the mouth and brushing the teeth. The following notes from Mr. H.'s daily record are typical of his appetite condition:

Third fasting day. No salivation on seeing fruit. Rather indifferent to eating.

Fourth fasting day. No salivation on seeing food or seeing people eat.

Fifth fasting day. Seeing food induces some salivation. No strong desire for food.

Sixth fasting day. No strong desire for food.

Seventh fasting day. Some salivation on thinking of food during a period of hunger contractions.

Eighth fasting day. Fruit displays look very good to me, but the bad taste in the mouth seems to prevent appetite.

Eleventh fasting day. Would be glad to resume eating.

Thirteenth fasting day. Odor and sight of food very agreeable, but mouth conditions seem to repress appetite.

Fifteenth fasting day. Seems to crave bulk and flavor, but not necessarily nutrient foods.

First day of breaking the fast. Surprised that I do not crave food very much,—want flavor and bulk.

On the first day of breaking the fast he wrote: "Food (fruit) does not taste as good as I anticipated." Nevertheless he stated at the end of both fasting periods that "*the dominant element in consciousness throughout the fast is ideas or thoughts of food and eating.*"

IV. THE CONTINUOUS SECRETION OF GASTRIC JUICE DURING PROLONGED FASTING

Our data on this phase are summarized in tables 1 and 2. These data permit the following conclusions:

1. There is no increase in the quantity of the contents of the empty stomach in fasting but the gastric content is more frequently mixed with bile and shows, on the whole, a higher acidity.

2. There is no definite increase in the rate of the continuous gastric secretion in fasting but the secretion shows, on the whole, a higher acidity. This is evident during the fifteen days' fast, but there is no increase in the acidity during the second fast when Mr. H. ingested cotton fiber daily.

These results on man are corroborated by studies on fasting dogs recently completed in our laboratory by Dr. G. F. Sutherland. It must be admitted, of course, that individuals may show gastric hypersecretion during fasting owing to special conditions induced by the fast. But Boldireff is evidently in error in concluding that fasting invariably leads to continuous hypersecretion after the third or fourth day.

TABLE 1

Summary of observations on the contents of the empty stomach of Mr. F. H.

	Number of observations	Contamination bile	Quantity cubic centimeters			Acidity					
						Free			Total		
			Low	High	Average	Low	High	Average	Low	High	Average
		per cent	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Period before fasting	6	16	10	50	26	0	0.09	0.05	0.03	0.18	0.12
First fasting period (15 days)	23	92	10	65	28	0.09	0.34	0.13	0.11	0.34	0.22
Period after fasting (27 days)	25	28	10	70	27	0	0.13	0.07	0.08	0.21	0.13
Second fasting period (8 days)	10	80	12	55	30	0	0.16	0.08	0.10	0.25	0.14
Period after second fasting (10 days)	10	30	10	45	31	0	0.16	0.08	0.06	0.22	0.14

TABLE 2

Summary of observations on the continuous secretion of gastric juice in prolonged fasting, Mr. F. H.

	Number of observations	Contamination bile	Secretion rate per hour			Acidity					
						Free			Total		
			Low	High	Average	Low	High	Average	Low	High	Average
		per cent	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Before fasting	4	50	25	75	50	trace	0.15	0.10	0.10	0.25	0.15
First fasting period (15 days)	21	85	28	126	64	0.08	0.32	0.19	0.12	0.39	0.27
After fasting (27 days)	25	12	40	224	140	0.04	0.15	0.08	0.08	0.20	0.14
Second fasting period (8 days)	10	50	40	120	72	trace	0.16	0.06	0.06	0.21	0.12
After second fasting period.	10	20	40	168	92	trace	0.16	0.07	0.06	0.19	0.14

V. ADDITIONAL NOTES ON THE PHYSIOLOGY OF FASTING

The blood pressure, the strength and endurance tests and the total nitrogen elimination during the two fasts were recorded, not with the view of revealing anything new, as we have a number of well-controlled studies on fasting men in the literature, but for the purpose of *a*, checking up on any special peculiarity in Mr. H., and *b*, as a control of his actual fasting. It will be remembered that Mr. H. was not locked up when not under my observation.

A. The blood pressure

DATE	DIASTOLIC	SYSTOLIC	PERIOD
June 18	95	110	First fasting period
June 25	86	100	
June 26	88	92	
June 27	86	90	
June 28	86	94	
June 29	86	92	
June 30	72	88	
July 1	74	90	
July 3	76	94	
July 6	72	84	Period of very low protein diet (essentially fruits and vegetables)
July 9	80	94	
July 10	80	92	
July 13	86	100	
July 14	86	94	
July 15	80	98	
July 17	88	104	
July 20	76	88	
July 23	88	104	
July 30	88	102	
August 19	78	89	End of second fasting period
October 16	84	110	

The data show the same tendency to a lowering of the blood pressure during the fast as has been noted by previous investigators.

B. The ergograph records. (Figs. 10 to 13.) The thirty minutes daily ergograph records show some apparent increase in strength and a marked increase in endurance gradually developed during the fifteen days' fast. Cramplike pains in the abductor muscles while taking the

Figs. 10 to 13. Ergograph records (abductor indices muscle working against 700 grams) of Mr. F. H. Duration of test = 30 minutes.

Fig. 10. June 17, day before starting first fast.

Fig. 11. June 22, fifth day of fasting.

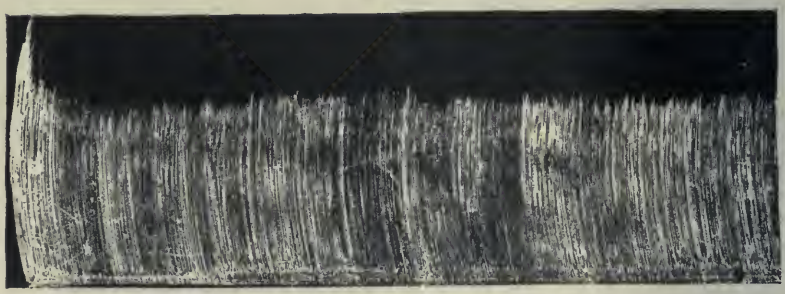


Fig. 12. June 28, eleventh day of fasting.

Fig. 13. July 3, fifteenth day of fasting.

records became less. This evident improvement in the ergograph records during the first fast were probably due in part to training of the particular neuro-muscular group involved and the mental factor or conviction on the part of Mr. H. that starvation improves the physical condition of a person. Taking Mr. H.'s physical condition as a whole there is no question that the fast produced gradual weakness and disinclination to physical or mental effort, such as reading, walking or working.

C. The water intake (tables 3 and 4). The daily ingestion of water was governed not by the thirst sensation primarily, but by the desire to increase elimination and the hope to improve the condition of the digestive tract.

D. The nitrogen elimination (tables 3 and 4). During the interval between the two fasting periods (July 4 to August 10) Mr. H.'s diet consisted essentially of fruit juices and vegetables (mainly tomatoes), with an occasional pint of cream and cotton fiber. On this diet the daily excretion of nitrogen in the urine varied from 2.5 to 4.5 grams., representing a protein metabolism of 15 to 30 grams per day. On this diet he gained nearly 2 kilos in the thirty seven days.

Both fasting periods showed a marked drop in urine nitrogen on the second and third days. A similar drop in the urine nitrogen appeared in Levanzin's fast under Benedict. During the fifteen days' fast the average daily output of urine nitrogen was 7.18 grams or 0.132 grams per kilo body weight, representing a daily average protein metabolism of about 45 grams. During the second fast of eight days the nitrogen output was considerably lower, or 5.70 grams per day (0.116 grams per kilo body weight), representing a daily metabolism of 35.62 grams of protein.

The striking feature in the present case is the low protein metabolism in the fasts. Benedict's subject, Mr. Levanzin, with a body weight practically identical with that of Mr. Hoelzel, excreted more than 8 grams of nitrogen in the urine up till the end of the thirty days' fast, and during the first fifteen days of the fast the nitrogen output averaged about 10 grams per day.

E. Movement of the bowels. During the fasting period June 18 to July 3 movements of the bowels occurred on the following days:

June 18. Ordinary feces.
June 19. Ordinary feces.
June 23. Mucus and food debris (strawberry seeds, etc.). About 3 cc.
June 26. Mucus and food debris (vegetable fibers, seeds, etc.). About 8 cc.
June 27. Mucus and a few traces of food debris. About 10 cc.

June 28. Mucus and a few traces of food debris. About 15 cc.
June 29. Mucus and a few traces of food debris. About 10 cc.
June 30. Mucus and a few strawberry seeds. About 2 cc.
July 1. Mucus and a few strawberry seeds. About 15 cc.
July 2. Mucus and a few strawberry seeds. About 25 cc.
July 3. Mucus, no trace of food debris. About 60 cc.

Mr. H. made special effort to force a bowel movement each day. No enemas were used. After June 19 the material passed was mostly soft bile-stained (brown) mucus with a few vegetable fibers and fruit seeds from his food taken before fasting. The material was semi-liquid, but there was no diarrhoea at any time. Gas was passed per rectum every day, and the solids passed had fecal odor (on some days very offensive) even on the fifteenth day of starvation.

The striking fact is the retention of traces of food débris (strawberry seeds) in the alimentary tract for at least fifteen days.

After June 19 Mr. H. had at no time the normal desire to defecate. All the bowel movements were forced, several attempts usually succeeding in one passage each day except as noted above.[5] The quantity of fecal material passed each day was very small, and without the special effort Mr. H. might have gone through the entire fast without a passage, as has been recorded in the case of other fasting experiments.

During his second fasting period, August 11 to 18, when he ingested a certain quantity of cotton moistened with gastric juice, there were bowel movements as follows:

August 11, 6.20 a.m., 1.00 p.m., 11.10 p.m.
August 12, 6.45 a.m., 12 noon.
August 13, 7.30 p.m. (Food debris and cotton.)
August 14, 11.15 p.m. (Cotton with a few grape seeds.)
August 15, 11.15 p.m. (Cotton; no food debris.)
August 16. Attempts at bowel movement without results.
August 17, 11.30 a.m. (Cotton; no food debris; slight fecal odor.)
August 18, 11.30 a.m. (Cotton; no food debris; slight fecal odor.)

The ingestion of cotton evidently sweeps out completely the food débris in the alimentary tract in three to five days. It may also reduce the quantity of intestinal bacteria by mechanical action. The form in which cotton taken by mouth is passed per rectum may be gathered by the fact that the smallest bolus passed measured 0.5 cm., the largest 3.5 cm. in diameter.

[5] Mr. H. states that during his twenty-six days fast three years ago he usually succeeded in forcing one bowel movement per day.

TABLE 3

The water intake and output of urine and urinary nitrogen of Mr. F. H. during the first fasting period, June 18 to July 3

DATE	ROOM TEMPERATURE	BODY WEIGHT	URINE			WATER INTAKE
			Quantity	Specific gravity	Total nitrogen	
1917			*cc.*		*grams*	*cc.*
June 18		57.72	1200	1016	11.03	700
June 19		56.37	630	1020	3.97	900
June 20	64–70	55.56	800	1023	8.02	1350
June 21	70–72	54.86	1110	1015	10.07	1400
June 22	64–66	54.56	1060	1020	10.53	1100
June 23	69–73	53.89	580	1030	8.85	1300
June 24	64–66	53.59	1020	1020	9.31	1000
June 25	68–74	53.10	760	1022	8.23	1700
June 26	76–78	52.62	910	1017	7.97	1400
June 27	74–76	52.14	1300	1014	7.27	1800
June 28	74–76	51.82	1250	1012	6.81	1500
June 29	68–70	51.52	1225	1015	6.99	1300
June 30	68–74	51.11	1100	1016	6.50	1300
July 1	68–70	51.08	1210	1012	6.22	1800
July 2	64–68	50.89	1000	1012	6.34	1100
July 3	68–70	50.57	1090	1013	6.85	700
July 4	68–20		550	1020	4.73	

TABLE 4

The water intake and the output of urine and urinary nitrogen during the second fasting period, August 11 to 18

DATE	ROOM TEMPERATURE	BODY WEIGHT	INGESTION OF COTTON	INGESTION OF WATER	URINE		
					Quantity	Specific gravity	Total nitrogen
1917			*grams*	*cc.*	*cc.*		*grams*
August 10					2600	1009	3.70
August 11	66–70	52.12	15	200	2200*	1011	2.52
August 12	70–72	51.09	19	400	560	1018	1.78
August 13	73–74	50.38	35	700	225	1030	3.18
August 14	72–74	49.80	55	500	400	1022	5.87
August 15	70–72	49.07	35	500	380	1025	6.70
August 16	74–78	48.38	48	700	310	1028	5.81
August 17	72–74	48.16	42	700	370	1027	6.39
August 18	74–80	47.83	50	900	410	1026	6.70
August 19	78–80	47.50			650	1018	6.69
August 20					500	1016	2.29

* On August 10, the day before starting the second fast, he consumed the following quantity of food: ½ pint cream; 5 ounces maple sugar; 10 bananas; 1 apple; 9 oranges; 1 pound cherries; 1¾ pound grapes; 2 cantaloupes; 3 lemons; 1 tomato; 1 quart red raspberries; 2 ice cream "sundaes;" 25 cc. water.

F. The mouth condition. In the first fasting period Mr. H. noted a disagreeable or foul taste in the mouth, coated tongue and tenderness of the gums (spontaneous bleeding or bleeding on rubbing the gums with the tongue). These conditions persisted throughout the fasting period and undoubtedly modified or suppressed his appetite for food as appetite involves the memory of pleasant gustatory and olfactory sensations.

Similar mouth conditions developed during the second fast, August 11 to 18. They are not due to lack of salivation or to neglect of common mouth hygiene.

G. General mental condition. The prevailing feeling during both fasting periods was one of gradually increasing weakness. The degree of depression varied from day to day, but the expressions, "feel all in" or "all fagged out" recur again and again in his diary. This depression was evident both in his mental and physical behavior. A certain degree of mental disturbance by the fasts is also indicated by the fact that Mr. H. became readily provoked and discouraged. But despite the feeling of weakness the mind was frequently unusually clear.

His sleep was frequently disturbed by the hunger pangs, by headache and by vasomotor irregularities ("hot flashes"). He was not able to sleep at all when the hunger contractions were severe.

Mr. H. was convinced that his sense of smell became more acute during the fasts. The *odor* of foods was the most potent stimulus to appetite. Sex interest and sex emotions were depressed.

SUMMARY AND CONCLUSIONS

1. During the fifteen days' complete fast and the subsequent eight days of abstinence from food with daily ingestion of cotton fiber, the gastric hunger contractions of Mr. Hoelzel continued with practically normal rhythm and intensity, but the subjective sensations induced by the gastric contractions appeared to be somewhat weakened and tinged with an element of general epigastric distress or sick stomach. The view that the hunger mechanism fails early in prolonged fasting is therefore not tenable as a general law.

2. The appetite sense or desire for food was modified or obscured by a tendency to a persistent bad taste in the mouth that developed during the fast. But Mr. Hoelzel states that the dominant element in consciousness during the fast was nevertheless thought of food and eating.

3. The contents of the empty stomach and the continuous gastric

juice secretion during the fasts show a tendency to a slight increase in acidity and greater frequency of regurgitation of duodenal contents into the stomach, but there is no significant increase in secretion rate over that of the control periods. In other words, the normal process of continuous gastric secretion of the empty stomach in not noticeably augmented in prolonged starvation.

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ADDENDUM

Chicago, September 1, 1917.*

Dr. A. J. Carlson,
Chicago, Ill.

Dear Sir:

As I may not find it convenient to resume some of the past experimentation at a later date I am taking this occasion to give a summary of the feelings or ideas which have prompted me to take the somewhat irregular course regarding feeding and fasting in the past few months.

The first week (the control) fairly represents my feeding habits during the previous three months—with the exception that on Sundays and holidays I always began eating within an hour or two after rising. In order to hold to the regime represented I would ordinarily need to be occupied with work involving

* At the end of six weeks of hard physical labor (harvesting). On this date Mr. H's body weight was 61.84 kgm.

partly physical activity so as to distract my attention from the practically constant mild headaches and abdominal pains (gastric and other).

My first fast (fifteen days) was taken and carried to the end of the fifteenth day mainly because I felt that I had pledged myself to go without food that long. If no one but myself had knowledge of this resolve I believe that the general weakening and depression would have led me to stop before the sixth day. I never carried a pure fast longer than four days when no one else knew my intentions.

Although I entertained the secret hope of fasting continuously for thirty days or more I began eating on the sixteenth day to get rid of the depression and weakness. I also discontinued this fast because I expected to find fasting more agreeable later while using cotton fiber. As the fast progressed there developed a growing conviction or feeling that food would be agreeable, but this indication of hunger seemed to be overshadowed by the general depression, weakness and foul mouth condition.

The eight-day-fast (using cotton fiber) with and without my own gastric juice) is the longest fast I have taken where I felt that I was free to stop as soon as it became disagreeable. If I had not been underweight with the disagreeable experiences of the first fast fresh in mind, I might have continued this fast much longer.

My subsequent attempts to use only lemon juice, etc., with fiber could not be carried out because it seemed that the small amount of nutriment used coaxed the appetite or hunger and made it harder to abstain from more substantial food.

As already indicated in earlier discussions with you I believe that the so-called hunger pangs or intense gastric contractions are not an indication of hunger but reflect a more or less abnormal gastro-intestinal condition. It seems to me that hunger has mainly a psychic or mental indication originating from the chemical blood and tissue state. However this mental hunger may be intensified by gastric contractions. The contractions impress me as a form of local pain when hunger (mental) is vague or absent.

A muscular or tissue sense of hunger may be indicated by the feeling of emptiness which is particularly prominent the first few days of fasting. But as this feeling disappears as the fast progresses (probably by readjustment of muscle and tissue tone over the abdomen) I believe this is abnormal (habit hunger?). However hunger appears to be a complex phenomenon which can probably be separated into hunger for proteins, for carbohydrates, etc., as it does not seem as though satiety can be produced until all the elements of hunger indicated at any time are satisfied by the amount and kind of food craved.

The foregoing opinion of what hunger is does not prevent me from making attempts to allay the gastric pangs. The most simple and direct method is to stop these intense contractions by eating food, whether hungry or not. But as eating without hunger makes gastro-intestinal conditions worse it has seemed wiser to allay the pangs by taking some food substitute, as cotton fiber, etc. And as I believe that the gastric contractions would go on without affecting consciousness under ideal physiological gastro-intestinal conditions, I attempt to regain this state by fasting, adjustment of diet, etc.

Respectfully yours,

FREDERICK HOELZEL.

AEQUIRADIO-ACTIVITY

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Received for publication October 3, 1917

It is remarkable that the animal tissues are made up of a comparatively small number of elements. All in all there are twelve (of the first *Mendejeleff* group *H*, *Na* and *K*; of the second *Mg* and *Ca*; of the fourth *C*; of the fifth *N* and *P*; of the sixth *O* and *S*; of the seventh *Cl*, and of the eighth *Fe*). Two of them possess a very characteristic atomic property, which no doubt enables them to exert an influence of their own. They are iron, which is ferro-magnetic, and potassium, which is radio-active.

The ferro-magnetism of iron is often concealed in the compounds (also in those in which iron occurs in the human body, namely, hemoglobin) behind the diamagnetism of the other atoms, so that the whole complex becomes diamagnetic again. The radio-activity of potassium, however, remains in the compounds so that it may be expected to reveal itself in the organism anywhere and at any time.

True, the radio-activity in potassium is but little pronounced. According to the discoverer, N. R. Campbell, (1) it renders a photographic plate appreciably black only after fifty-six days. It emits exclusively β-rays of a high penetrating power, but their number is so small that the ionising power exerted by a layer of potassium-salt upon the air of the ionising chamber is a thousand times lower than that of a similar layer of uranium-oxid, insofar as the β-radiation is concerned. α-rays are not emitted by potassium at all, so that potassium-salt, unlike uranium-salt, in such an experiment need not be screened from the ionising chamber by tinfoil.

Upon the basis of these facts it is possible to establish by calculation the intensity of the radio-active radiation of this element to be found in all animal tissues, which determination is of great moment for physiology. The ionising-power of potassium is one thousand times weaker than that of uranium which, in its turn, is again about a million times weaker than radium. The more correct ratio is 4: 10^9 (thousand millions) (2). But the radium under

discussion contained the elements to which it is progressively converted. Its radio-activity, irrespective of the accessory products, is estimated at 1.38×10^6 erg per second and per gram. The β-rays come in for 3.2 per cent or 4.4×10^4 erg per second (3). This was the β-energy with which we had to do in the determination of the ionising-power through aluminium foil. To establish the ionising-power of potassium we must divide the energy-quantum of radium by 250,000,000, the result being 17.6×10^{-5} erg per second. This, however, applies only to the case when the β-rays of potassium are of the same penetrating power as those of radium. It is, however, eight times stronger and the energy of each ray about four times greater. Consequently the energy-value per gram of metal rises for potassium to 7×10^{-4} erg per second. Meanwhile we have still to correct an error, for in our calculation we presumed to have to do with pure radium (4) whereas we actually worked with radium and accessory products when the parallel was drawn. The energy of this radium is supposed to be fifty times that of pure radium (4). The energy of one gram of potassium lies, according to my calculation, in the neighborhood of 3.5×10^{-2} erg per second. Should it be supposed that the considerable penetrating power of the potassium-radiation is not sufficiently established, or that it ought not to be taken up in the calculation, the last value is reduced to 0.8×10^{-2} erg. In our case we took the round value of 3.10^{-2} erg.

Closely allied to the radio-activity of potassium is that of rubidium. A salt of this metal renders the photographic plate black in one hundred days (5). The ionising-power is one five-hundredths that of uranium-oxid for β-rays (6). The two light metals have in common that each of them gives off exclusively negative particles. According to Rutherford, this absolute lack of α-radiation precludes the hypothesis that heavy radio-active elements are at the bottom of the phenomenon. Nor does the exposure to the action of light modify in any way the radio-activity peculiar to potassium and rubidium.

The ionising-power of rubidium being one five-hundredth of that of uranium-oxid through tinfoil, the energy of 1 gram of rubidium would be as great as that of about 1 gram of potassium, if not the penetrating power were found to be ten times smaller (Campbell). In this connection I think the energy of 1 gram of rubidium is to be fixed upon $2 \times \frac{1}{5} \times 3.5 \times 10^{-2}$ erg per second or about 1.7×10^{-2} erg per second. So the number of the β-rays sent out by radium is larger indeed but their effect is slighter because the velocity of the mobile negatively-charged particle is less.

The heavy radio-active elements are not found in the animal body unless they have been purposely introduced. Also rubidium fails. As a radio-active element, therefore, potassium stands entirely by itself in the organism, so that the question arises whether any specific effect can be detected.

With the intention to collect the necessary data to settle this question, I enjoined my assistant, Mr. T. P. Feenstra, to ascertain whether in the artificial circulating fluids of S. Ringer and his followers potassium could be replaced by the other radio-active elements. A similar investigation, but not guided by radio-activity, had been previously made by Ringer (7) himself for the first group of *Mendejeleff* and he compared his results with those obtained with potassium in aequimolecular concentrations. Rubidium proved to be the only element that answered the purpose. Our new point of view now urged us to work not with aequimolecular but with aequiradio-active dosages. We therefore calculated as well as we could, in the way indicated above, the radio-activity of the potassium and the rubidium that occurs in Ringer's solutions and subsequently measured the doses of the other radio-active elements to be examined, so as to make their total radio-activity agree with that of the potassium doses. The α-, the β- and the γ-rays were taken together, as it seemed to me hardly judicious to make an *a priori* selection of one of them. Most helpful was Rutherford's excellent textbook containing direct indications about the total radio-activity of uranium, thorium and radium, so that there was no necessity to calculate them in our own clumsy way. This, indeed, evolved a seeming inconsistency in the calculated K- and Rb-values on the one hand and the physically determined U- and Th-values on the other. Moreover, experimentation had to decide. Starting from the values we had calculated, Mr. Feenstra patiently and tactfully tried to find the practicable doses. After some shilly-shallying, my coworkers hit upon the following metal-doses per litre of circulating fluid, that is applied to the Kroneckered frog's heart (winter frog) (8):

Metal doses in milligrams per liter

K	Rb	U	Th	Ra
53	105	12	24	3.10^{-6}

In our estimation the following energy-quanta correspond to these metal doses (9):

Energy-quanta come in per litre and per second (in 10^{-4} erg.)

K	Rb	U	Th	Ra
16	73	96	72	41

It will be seen that the metal doses are not perfectly aequiradioactive. They are of the same order, though. It should not be forgotten, however, how they originated, viz., first theoretical conjecture, then experimental searching. The said metal doses were contained in the following amounts of salt:

Potassium in 100 mgm. KCl per litre	53 mgm.
Rubidium in 150 mgm. KCl per litre	105 mgm.
Uranium in 25 mgm. $UO_2 (NO_3)_2$ per litre[1]	12 mgm.
Thorium in 50 mgm. Th $(NO_3)_4$ per litre	24 mgm.
Radium in 5 micromgm. radium salt per litre	3 micromgm.

Besides the radio-active ingredients they were in S. Ringer's artificial circulation fluids.

Calcium chlorid[2]	200 mgm.
Sodium bicarbonate	200 mgm.
Sodium chlorid	7 grams
Water	1000 grams

These five artificial circulatory fluids can keep the isolated Kroneckered frog's heart (winter frog) beating for hours. It is decidedly necessary, however, to take one precaution: in passing from one fluid to another, *to pass through a solution quite free from potassium.*

We proceeded as follows: First the Kronecker inflow-cannula, "à double courant," was slipped into the sinus venosus. The ligation was then performed midway between sinus and atrioventricular boundary. It now seemed as if the heart had been tied up by Stannius ligatures and so we had to make sure of the heart beating forcibly and regularly for a quarter of an hour. It was accordingly suspended after Gaskell-Engelmann and registered graphically, if necessary. Then followed a circulation with common Ringer's mixture deprived of potassium-salt. The result was a more or less abrupt or a gradual standstill in diastole, on the average after a half hour. Great attention was given to the utmost purity of the various salts and above all due care was taken to use a real potassium-free Ringer's solution when procuring the standstill of the heart. This was far from easy as commercial sodium chlorid, even when obtained in a supposed pure condition of well-known factories, mostly contains potassium. In this procedure the colorimetrical method recommended by Howell and

[1] Inclusive of water of crystallization.

[2] Sometimes quite free from water, then again with 25 per cent water.

Duke (10) stood us in good stead. We deemed it sufficient if the potassium-free mixture contained less than 1 mgm. of potassium per litre.[3]

The precaution indicated above is indispensable for, if omitted, the forcibly beating heart will cease its pulsations directly when a K- or Rb-fluid is succeeded by one with the heavy metals or the reverse. The light and the heavy metals may, however, be *inter*changed without a potassium-free interval. This does not interfere with a continuance of the regular and forcible pulsation. The solutions may even be mixed in equal amounts. Contrariwise, if one administers a mixture in equal amounts of a Ringer's solution with light metal and a Ringer's solution with heavy metal, the heart will come to a complete standstill at once (11) if one should experiment with the fresh organ or after a short potassium-free circulation. This I have named "the first paradox" of the three that we have encountered in this study (12).

A heart accidentally brought to a standstill in one of these procedures resumes its action the moment when the interfering fluid has been replaced by one that has been deprived of potassium and uranium both. During the standstill the susceptibility to a mechanical stimulus is most often retained. A peculiarity regarding electrical stimuli has been described by us in detail in a previous paper (13).

As indicated at the beginning, we tried to find out whether in the artificial circulating fluid the potassium is to be substituted by the other radio-active elements. This we found to be the case. The remarkable aequi-activity manifesting itself in the appropriate doses gave rise to the presumption that the substitution is feasible only on the ground of the radio-active, atomic property of these elements and is not to be ascribed to any other factor. This also led us to try the addition of emanation to the potassium-free Ringer's mixture. A quantum of 100 Mache-units proved to yield a positive result. Moreover here also the first paradox was obtained, so that we could place emanation on the same level with the heavy radio-active metals that in previous experiments we had added to the artificial circulating fluids.

It seems to me that an important conclusion may be deduced from the experiments with radium-containing fluids and those with emanation. Apparently the influence that comes into play here is of a different nature from that dominating the famous experiments on

[3] In some experiments potassium was present to the amount of 2.5 mgm. per litre.

balancing ions of J. Loeb (14) and his coworkers, as is evident from the fact that the mass may, so to speak, be eliminated both in the case of radium-salt, which was added only to an amount of 5 micromgm. (5×10^{-9} gram) per litre, and in the case of emanation. If the balance is caused by the concourse of mass effects between the various ions, in regard to the proteins, when there are no masses the influence of chemical affinities and valences cannot clinch the matter. In this case there are no masses, consequently we have to seek the influence at play elsewhere. Nevertheless in others when the quanta are easily measurable, with uranium as well as with thorium, the balancing power remains. When the calcium in the circulating fluid was augmented, the uranium and the thorium doses had to be increased. Nay, even the mixture-ratios between potassium and uranium which, being mutually antagonistic caused a standstill instead of automaticity of the heart, were slightly modified under the influence of the calcium (15). Also balancing appeared when calcium was replaced by strontium. W. H. Jolles (16) established this by testing the electrocardiogram as well as the movements. With strontium in place of calcium, circulating fluids may be made from the radio-active elements, in which the entire frog's heart excised from the body, continues to beat yielding a normal electrocardiogram. We started similar experiments with summer frogs but did not complete them. Evidently the composition of the circulating fluid has to be modified. The calcium content has to be increased a little (to 250 mgm.) per litre exclusive of the water of crystallization) whereas the potassium content is to be largely diminished (from 100 mgm. of potassium chlorid to 30 or 50 mgm. per litre). In the same way in summer frogs the uranium or the thorium content is to be diminished (from 25 mgm. of uranium salt to 5 mgm. and from 50 mgm. thorium salt to 10 mgm. per litre).[4]

The foregoing renders it more and more probable that the beneficial influence of potassiun in Ringer's circulating fluid is due to the radio-activity of the element. Positive certainty may be obtained through mesothorium- and radium-radiation.

Together with C. E. Benjamins and T. P. Feenstra (17), I made initially thirty-four experiments, afterwards a larger number, in which the Kroneckered frog's heart was exposed to a short-distance radiation. The heart had beforehand been deprived of its diffusible potassium by allowing a potassium-free fluid to run through it until a standstill

[4] S. de Boer will publish these experiments more fully.

ensued. This precaution is essential for if the diffusible potassium is not removed at all or only in part by employing a fluid whose salts are contaminated with potassium or a fluid, originally free of potassium, that has been contained in phials of common glass, which gives off potassium, the radiation is ineffectual. As the conditions are arranged well, however, the otherwise irrecoverably motionless heart is seen to recover its contractility abruptly, forcibly and regularly after half an hour's radiation when, as was done by us, 3 mgm., 5 mgm. or 6 mgm. of radium, respectively, mesothoruim is applied. The radiation-times for the various cases depend entirely on the past history of the organ (natural frequency of the pulsations; velocity of circulation; the tonus, in consequence of which the lacunae are more or less open; the time required by the heart to stop its beats through deprivation of diffusible potassium, etc.). The duration of the renewed, perfectly normal rhythm is also varying. A prolonged radiation generally evolves a secondary standstill.

It will be understood that during the experiment the circulation with potassium-free Ringer's solution goes on unintermittently. Jannink (18) found that in those cases about 1 mgm. of potassium per litre from the heart is added to the circulating fluid. In some degree a continual mobilization, therefore, takes place of the potassium from the solid form in which it occurs in the muscle[5] to the diffusible form. There is no appreciable difference in this respect between winter and summer frogs. In either case the outflowing liquid hardly contains any potassium when at the inflow it was potassium-free.

When recovery has been effected through radiation, it is not difficult to render visible "the second paradox" that we encountered in the course of our experiments. It consists in the arrest of the cardiac action directly when, instead of potassium-free mixture, the original Ringer's solution has been administered. During the initial pulsations this paradoxical phenomenon is still absent, but after about twenty or thirty pulsations it is sure to make its appearance. After the fluid, free from potassium, is allowed to run through, the heart recovers itself. This induced us to believe that the normal amount of potassium, suddenly entering the heart-cells, evolves in it a quantum of radioactivity that, together with the electricity already accumulated through radiation, increases to an extent that is incompatible with a normal automaticity. For the same reason the standstill in diastole occurs

[5] The *permanent* radiation of the non-diffusible potassium, present in the muscle, does not seem to exert a noticeable influence.

which is familiar to us from potassium-intoxication. The reaction is reversible. Circulation with potassium-free Ringer's solution restores the beats rather soon.

In this connection it is a question of vital importance to know the quanta of radio-activity concerned in such cases. A certain quantum most likely causes contractility. Maybe double that quantity proves to be too much. The adequate quantum can no doubt be computed from the circulation experiments with Ringer's mixture to which is added 5 micromgm. of radium-salt so that per litre and per second 41×10^{-4} erg is sent through. However, at the most only 0.0001 litre passes per second. Then the quantum of energy that causes recovery is at any rate less than 4×10^{-7} erg. With this we approach the energy quanta capable of stimulating our senses close to the threshold. Thus the result of this calculation does not seem to be improbable (19).

The results of the experiments with potassium-uranium mixtures, reported above, have induced me to study the behavior of uranium hearts toward radiation. They cannot stand radiation. After a short interval the rhythm ceases to return again after the removal of radium (or mesothorium). Such experiments succeed best when starting from a potassium-uranium equilibrium, i.e., a condition in which the heart does not beat because it is fed with a Ringer-solution that contains antagonistic amounts of potassium and uranium. We took per litre 40 mgm. of potassium chlorid and 10 mgm. of uranylnitrate (inclusive of the water of crystallization) in the hearts of winter frogs; and 30 to 50 mgm. of potassium chlorid and 5 mgm. of uranylnitrate in the hearts of summer frogs. A heart in a state of complete quiescence and relaxation, fed with such a solution for some time, say five minutes, will resume its beats in a comparatively short time when radium or mesothorium is brought close to it. Upon increasing the uranium-dosis in the fluid by slow degrees, the cardiac action is soon arrested again and will recommence once more on further uranium increment. So there is a radiation-uranium antagonism in the same sense as there is a potassium-uranium antagonism. Quantitative relations (20) may even be traced between the distances of the radium or the mesothorium and the amounts of uranium used to counteract them. No account is taken here of time relations, the gist of the matter being the distances and the uranium-quanta. Those distances are surprisingly small, all falling within 1 cm. Furthermore, the uranium-quantum, taken as a counterpoise, is considerably diminished by placing thin aluminium screens between mesothorium and heart. I inferred from these ex-

periments that the biological activity is due to the extremely weak β-rays shot out by the radium and the mesothorium.

In the radiation experiments just made mention of, in which radiation was contrasted with the uranium administered internally, we have encountered a third paradox. A prolonged experiment, in which an overbalance of radiation or of uranium has alternately been acting on the heart, evokes a paradox different from the two paradoxes we have hitherto met. The heart will ultimately stand still as well with a normal Ringer's solution as with that containing uranium, and nevertheless pulsate with a potassium-free circulating fluid. In a number of such cases this paradoxical behavior has been overcome by an excessively large uranium-dosis.

SUMMARY

In summarizing the above we arrive at the conclusion both simple and startling that, provided that only radio-active quanta be taken, all radio-active elements administered to the isolated heart internally are capable of sustaining the automaticity. So is also radiation, with the understanding that it is applied in a certain dosage. In some of these cases α-rays come into play, in others β-rays. Both are active, but when acting in conjunction they counterbalance each other. Obviously it is the electric charge, imparted by the α- and β-particles, to which the results are to be ascribed. Whether the γ-rays also come into operation I dare not decide.

Recently also a screen-effect of the diffusible potassium has shown itself. Excess of radiation is detrimental to the heart that is free from potassium, but harmless for a heart that has been fed with normal Ringer's solution. The non-diffusible potassium of the heart muscle is wanting in a screen-effect. It seems to have in our sense no perceptible working at all.

The hypothesis that the significance of radio-activity for the automaticity of the heart rests on the application of an electric charge has occasioned a number of new experiments. They have brought to light that a galvanic current arrests a beating uranium heart suddenly and temporarily, leaving it in a state of utter relaxation, and that in such a uranium heart extrasystoles can be generated only by mechanical stimuli (not by induced electrical). We meet, however, with many theoretical difficulties. So aequiradio-activity is equivalency of energy, but electrical charge is a factor of quantity in energy. The dimensions are not the same.

It has also been proved that the effect of radio-activity, in the case described above, is decisive not only for the automaticity of the heart but also for a number of other functions. In my laboratory this has been established for the vascular endothelium (21), for the irritability of the muscle, directly and indirectly (22), recently for the intestinal movements (with a reservation though), for the regulating functions of the extracardiac nerves (23), and in the laboratory of Hamburger (24) for the glomerulus-epithelium.

The results described induced me to project a provisory working-hypothesis, which was communicated by me on the Congress of Dutch Physiologists in Amsterdam, December, 1916. It served me and my coworkers for guidance and is given a brief exposition in Ned. Tijdschr. voor Geneeskunde, 1917, i, 1178.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 45 FEBRUARY 1, 1918 No. 3

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 80, AND FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, NO. 305

THE PHYSIOLOGY OF ASCIDIA ATRA LEUSEUR

III. The Blood System

SELIG HECHT

Received for publication November 6, 1917

CONTENTS

I. INTRODUCTION

Ascidia atra is a large tunicate possessing an opaque, blue-black test. It is common in the Bermuda Islands and may be collected in sufficient numbers for experimentation in the immediate vicinity of Agar's Island.

In the previous papers of this series (Hecht, '18 a and '18 b), and in an earlier paper (Hecht, '16), the activities of this species have been described. The reader is referred to these accounts of the general and sensory physiology for the more important aspects of the life of Ascidia.

The vascular system of Ascidia atra consists essentially of an arrangement of continuous cavities within which the blood is kept in constant movement by the contractions of a muscular heart. By means of this shifting of the blood, the respiratory, nutritive and secretory products, which are produced by the activities of different portions of the organism, are made available for their general and local utilization. As a result, any adequate consideration of the physiologic relations of the vascular supply would mean a treatment of almost the complete physiology of the animal. To avoid this I propose, therefore, to treat the blood system merely as an organic mechanism by itself, and to describe as far as possible the physiology of the tissues and organs which compose it.

II. BLOOD

1. Path of blood stream. The cavities through which the blood flows represent those remains of the primitive blastocoel of the embryo which have not become filled with connective tissue strands and jelly. In the large monascidians, to which A. atra belongs, the connective-tissue cells near the blood spaces arrange themselves so as to form tubes with fairly definite walls (Seeliger '93–11, p. 531). It is through these that the vascular fluid moves. The vessels of the test are formed by the evagination of the ectoderm into the body of the test.

In spite of this apparently indefinite mode of origin, the blood channels of the adult present quite a uniform development, and in Ascidia their pattern and direction show individual variations only in the smaller vessels. There are present three large and well-defined vessels which determine the major distribution of the blood to the body. Two of these may best be described in their relation to the heart; the third in relation to the branchial sac. Examination of figure 1 will aid in an understanding of the following description of the path of the blood stream.

The heart is a tubular organ which lies on the ventral side of the animal, and extends for nearly two-thirds of its length. From its anterior or branchial end there arises the hypobranchial vessel. This is the great ventral channel which lies below the endostyle and through it the blood from the heart goes to the branchial sac. Before breaking up

into its smaller divisions, which communicate directly with the transverse vessels of the branchial sac, the hypobranchial vessel gives off a branch. This and an accompanying vessel (stippled in the figure) from the branchial sac alternately furnish the blood to the test. The double vessel thus formed constitutes the sole vascular connection between the body of Ascidia and its test.

From the posterior or visceral end of the heart is given off the visceral vessel. This at once breaks up into several branches which lead to the intestine, gonads and other body organs. From these regions the blood is collected by means of small channels into larger vessels, two of which are usually clearly visible on the left side of the body.

The blood from these two paths is led into the dorsal vessel. This is a spacious channel situated dorsally and like the hypobranchial vessel it gives off numerous branches into the transverse vessels of the branchial sac.

The blood which moves in the transverse vessels is in osmotic contact with the seawater which the cilia of the stigmata are constantly driving through the branchial sac. Here the respiratory exchange is accomplished and here most probably occurs the absorption of the organic (Pütter, '07) and inorganic constituents of the seawater. When the heart is beating from the branchial toward the visceral end, the blood from the branchial sac, aerated and containing perhaps other absorbed constituents, is pumped to the visceral regions. Here its oxygen supply is utilized and it becomes charged with the digested products of the food, with the secretions of the nearby glands and with carbon dioxide.

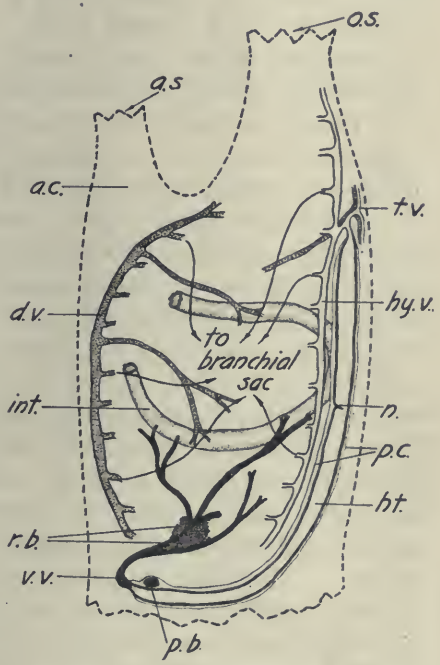

Fig. 1. Semi-diagrammatic representation of the heart and major blood vessels of Ascidia atra viewed from the right side; *a.c.*, atrial cavity; *a.s.*, atrial siphon; *d.v.*, dorsal vessel; *ht.*, heart; *hy.v.*, hypobranchial vessel; *int.*, intestine; *n.*, node; *o.s.*, oral siphon; *p.b.*, pericardial body; *p.c.*, pericardium; *r.b.*, renal body; *t.v.*, test vessel; *v.v.*, visceral vessel.

The blood is thence collected into the dorsal vessel to be further driven into the branchial sac, where the cycle begins all over again.

A phenomenon unique in tunicates is the reversal of the direction of the circulation. When this occurs in Ascidia atra so that the heart is beating from the visceral to the branchial end, it is the dorsal vessel which brings the aerated blood to the viscera. From here it flows through the visceral vessel to the heart and then to the branchial sac by way of the hypobranchial vessel. After being again aerated it is sent to the body through the dorsal vessel.

2. Plasma and cells. The blood of the intact animal is not directly subject to observation, because of the opacity of the test. However, the removal of a portion of the test is readily accomplished and apparently leaves unaffected the normal activities of the organism. The heart is an easily accessible portion of the circulatory system and being transparent, it offers a convenient location for the examination of the blood stream. By carefully slicing away the right ventral face of the test, the heart may be exposed for almost its entire length.

Such a preparation, freshly made, shows the blood as a vivid green, turbid fluid flowing through the heart and nearby vessels. This appearance, however, is entirely misleading. If the animal be allowed to remain undisturbed for a few minutes, the color and turbidity disappear, leaving the blood transparent and colorless. The conditions which cause the green and cloudy appearance will be considered subsequently. At present, however, it must be emphasized that the colorless state of the blood and its transparency are the normal characteristics of the animals of this species. They seem to be the general properties of tunicate blood (Herdman, '04).

The turgidity of the heart in an exposed preparation indicates that the blood is under an appreciable pressure. A similar interpretation is to be placed on the phenomena which are associated with a rupture of the heart or of a major vessel. Under favorable circumstances, a small puncture in the heart of an animal under water results in the blood being forced out to a distance of several centimeters. This appearance is striking because of the rapid color change which the blood undergoes as it leaves the point of injury: a streak of bright green marks the discharged stream.

The blood of Ascidians is distinguishable from that of Appendicularians by the presence of definite, regularly occurring blood-cells (Seeliger '93–11, p. 553). The medium in which these corpuscles are suspended and carried throughout the body is a balanced salt solution containing

protein to a small extent (Henze, '11; Cuénot, '91) and probably other organic substances. In Ascidia atra this plasma is colorless under all experimental conditions. The osmotic pressure of the blood fluid of Phallusia mammillata has been determined by Henze ('11), and it is practically isotonic with seawater: for the plasma $\Delta = 2.07°$, while for seawater $\Delta = 2.12°$. A similar relation undoubtedly exists in Ascidia atra because the blood cells will live and remain active in seawater for several days. Ascidians are thus "poikilosmotic" animals (Höber, '14, p. 37)—marine organisms the osmotic pressure of whose body fluids is isotonic with the seawater in which they live.

A piece of blue litmus paper inserted into a cut in the test of Ascidia rapidly changes to red. The same is true if a piece of the branchial sac or any other tissue containing blood be touched with blue litmus paper. Such a condition was observed for other Ascidians by Henze ('11) and he at first concluded that the blood was acid in reaction. Further study proved this incorrect. If freshly drawn blood of Ascidia be centrifuged, the clear plasma does not turn blue litmus red. It has the same reaction as seawater (cf. Henze, '12). The centrifuged corpuscles, however, are decidedly acid and turn litmus accordingly. In Phallusia the acid was identified by Henze as H_2SO_4. From his data I calculate a concentration of ca. 0.4 N H_2SO_4 within the blood cells. The small volume of blood obtainable from A. atra precluded a quantitative examination. The corpuscles, however, give an unforgotten sour taste.

An effective demonstration of the localization of the acid of the blood may be made by comparing the effect produced by a drop of dilute acid and by a drop of blood on a dry piece of blue litmus paper. The acid spreads out to form a circle several times the diameter of the original drop. As shown in figure 2, it is only at the periphery of the circle that the acid (HCl) fails to be adsorbed to the same extent as the water. The result is a moist circle of red surrounded by a narrow band of moist blue. A drop of blood produces an entirely different appearance. The plasma spreads and leaves a circle of moist blue paper. The corpuscles, however, remain within the confines of the original drop, and produce a small, intensely red space within the much larger one of the adsorbed plasma (cf. fig. 2).

The plasma is therefore alkaline, the corpuscles acid to litmus.

The acidity of the blood cells is of interest with regard to the theory of vital staining. On the assumption that basic dyes penetrate living cells because of the ordinarily alkaline reaction of protoplasm (Höber, '14, p. 432), acid dyes should penetrate easily the acid cells of the blood.

In a preliminary statement Bethe ('14) announced that such is the case. Using many acid stains he found that they entered Ascidian blood cells with great facility. Basic dyes, however, penetrated only feebly.

The blood cells of tunicates are mesenchyme cells and correspond more to the lymphatic cells than to the red corpuscles of vertebrate blood. In Ascidia mentula, Cuénot ('91) described four kinds of amebocytic cells, all of which are more or less related genetically. Like the blood cells of A. fumigata (Heller, '75), however, those of A. atra may best be divided into two classes: unpigmented and pigmented.

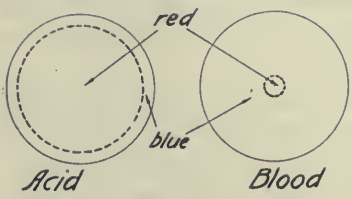

Fig. 2. Effects of a drop of acid (HCl) and of a drop of Ascidia blood on a dry piece of blue litmus paper.

The unpigmented cells are of two kinds at least. The first kind readily assumes an ameboid appearance (*c*, fig. 3) and continually changes shape when observed with the microscope. These cells are all nearly the same size, are quite numerous and, in the living condition, possess an apparently homogeneous protoplasm.

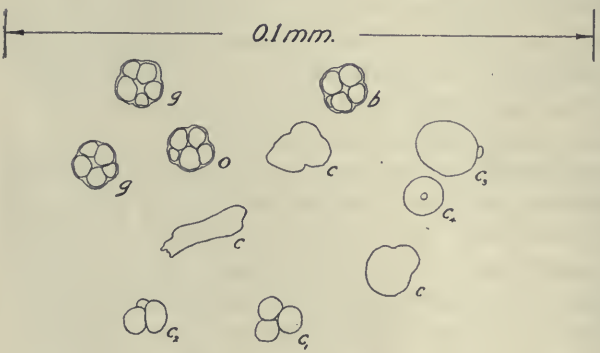

Fig. 3. Blood cells; *b*, blue cells; *c*, ameboid colorless cells; c_1–c_4 colorless cells of constant form; *g*, green cells; *o*, orange cells.

The second type of colorless blood cell retains its spherical form under the conditions of the examination. Its size is variable; often two or three are closely associated (c_1 to c_4). Undoubtedly further cytologic study would show that this type is composed of several classes of cells.

The pigmented corpuscles all appear to be alike in structure but they differ markedly in color and distribution. They are capable of a slight though definite ameboid movement. The most abundant of the pigmented cells, and indeed the most common of all the blood cells, are the green corpuscles (*g*), which occur in all parts of the circulatory system.

Their protoplasm is packed full of a number of spherical granules which are of a bright green color. According to Henze ('13) they are the cells which contain the acid of the blood; with methyl red they stained a deep red, whereas the plasma and seawater became yellow. This signifies a hydrogen-ion concentration of $10^{-4.2}$ to $10^{-6.3}$N within the cell.

The orange cells (*o*) are similar to the green cells in structure but the granules are of a rich orange color. As has already been observed in A. mentula (Cuénot, '91), these orange cells are distributed locally. They are very common in the branchial sac; in some of the papillae I have counted even more orange than green cells. But they are only rarely encountered in the general circulation. Blood drawn from the heart contains only an occasional orange corpuscle, frequently none at all.

The third type of colored cell has a deep blue color (*b*). Whereas the other pigmented cells are transparent, these are quite opaque. The blue cells are distributed in a manner complementary to the orange cells. They are not very common but in blood drawn from the heart they may be easily found. I have, however, seen but very few blue corpuscles in the branchial sac. Genetically they are related to the green cells, of which they probably represent the later stages of existence. It has been possible to observe the change from green to blue in the individual cells of drawn blood.

The localization of the blue and orange blood cells is of importance for an understanding of the functions of the blood and the blood cells. Cuénot concluded that the orange cells of A. mentula do not move with the blood stream. Although my observations on A. atra are not wholly in accord with the idea of this keen observer, the data are too meager for me to venture even a tentative hypothesis. However, the properties of the pigments of the blood cells may furnish a clue to the significance of their localization.

3. Pigment. Respiration is intimately connected with the movements and composition of the blood. The walls of the branchial sac are no more than a complex mass of interwoven blood vessels which, in this way, permit an exchange between the blood and the seawater passing through the sac. The respiratory activity of several tunicates has been measured and it was found that they maintain a definite, though small respiratory exchange (Pütter, '07; Vernon, '95).

The first investigator to study the relation of the blood to the respiration of Ascidians was Harless ('47). He reported that blood freshly drawn from "Ascidia mamillaris" (probably Phallusia mamillata) is a

"*wasserhelle Flüssigkeit,*" which turns blue after remaining exposed to the air for a few minutes. Fresh blood does not change color on contact with oxygen or nitrogen; carbon dioxide, however, at once turns it blue. Oxygen, bubbled through the blue blood makes it colorless, but not as colorless as it was originally. Essentially the same observations were reported by Krukenberg ('80 a), except that he denied the re-formation of colorless from blue blood by the addition of oxygen. It was Krukenberg who first noted that the pigment changes were localized in the cells of the blood.

Apparently the most spectacular contribution to the physiology of Ascidians is the reported presence of a colorless respiratory protein, γ-achroglobine, in the blood of Ascidia, Molgula and Cynthia. Griffiths ('92), its discoverer, assigned it a definite formula and stated that in its reduced state 100 grams would absorb 149 cc. of oxygen at standard pressure and temperature. It will be observed that this binding capacity is greater even than that of haemoglobin.

Using the species employed by the previous investigators, Winterstein ('09) failed to confirm their statements of the color changes and respiratory activities of the blood. He found that the blood of Ascidia certainly turns blue-black on standing, but it does so only after many hours. Moreover, CO_2 has no effect on the color change. Henze ('11) gives corroborative evidence, and my observations of Ascidia atra are also in complete accord with Winterstein's. Often the blood of A. atra requires more than a day to change completely to blue-black.

Winterstein determined the binding power of Phallusia blood, using a modern technic. One hundred cubic centimeters of blood saturated with air contains 0.24 cc. of CO_2, 0.38 cc. of O_2 and 1.33 cc. of N_2. This shows an absorption of these gases by the blood almost identical with their solubility in seawater. The small oxygen binding capacity is evidently a general characteristic of invertebrate blood (Winterstein, '09). It must therefore be concluded that, like the haemocyanin of Limulus (Alsberg and Clark, '14), the blood pigments of Ascidia have a function other than that of absorbing oxygen and carbon dioxide, or perhaps that their method of doing so is different from that of haemoglobin.

The pigment of Ascidian blood is exceptional in its possession of a remarkable chemical element. Henze ('11) describes the chromogen of the blood cells of Phallusia as a protein in combination with the rare element vanadium. The green blood cells of Ascidia atra furnish a pigment which is also a compound containing vanadium. The mass of dried and ignited blood cells, fused with KNO_3 and $NaCO_3$ forms an

alkali vanadate which gives the characteristic color reactions denoting the presence of vanadium.[1]

Chemically, the occurrence of vanadium and its compounds in the blood is important because they possess marked catalytic properties. For example, the oxidation of anilin hydrochloride to anilin black is catalyzed by the presence of one part of V_2O_5 to 100,000 parts of anilin (Witz,'76). Probably it is in the *rôle* of catalyst that the blood pigment is of service in the respiratory activity of ascidians

This element is significant, moreover, from the aspect of its physical properties. Ascidians possess a great variety of colors and color changes in the blood and tissues. Vanadium exists in five stages of oxidation, each showing a series of colors among its compounds. The oxides, for example, are V_2O, V_2O_2 and V_2O_3 which are basic, and V_2O_4 and V_2O_5 which are acid. V_2O_5 forms orange-red crystals, a yellow solution in water and a red soultion with acids and hydrogen peroxide. V_2O_4 is blue, its hydroxide, $V_2O_2(OH)_2$ is brown in alkaline solution. V_2O_3 is green. Such examples may be multiplied. Combinations with organic substances would give these compounds a still greater range of color.

Very probably the green pigment of the blood cells of Ascidia contains vanadium in the stage of oxidation corresponding to V_2O_3 (cf. especially, Henze,'12). That it is present in the green cells is undoubted. A water extract of the pigment turns black on the addition of osmic acid due to the precipitation of a lower oxide of osmium The careful addition of osmic acid to a blood preparation on a slide results in the blackening of the green cells.[2] If the orange cells contain vanadium in its pentavalent state, it is clear why they are not blackened by osmic acid.

4. Coagulation. The clotting of the blood of A. atra is solely an agglutination of the corpuscles,—the so-called first coagulation of invertebrate blood (L. Loeb, '10). The second coagulation, that is, the second-

[1] An example is the following. The fused mass is treated with water. This dissolves the vanadate, giving a colorless solution. To such a solution is then added a crystal of tartaric acid; the solution turns yellow-red at once. On boiling, the color changes to blue, due to the reduction to V_2O_4. Another color test is to acidify the alkali vanadate, and then to add hydrogen peroxide. The solution turns a rich orange color.

[2] The notion once prevalent among cytologists that the blackening of a cell by osmic acid invariably indicates the presence of fat, has led to confusion here as well as elsewhere. Even so careful a student as Cuénot ('91), observing the blackening of the green blood cells of Ascidians, concluded that they were cells which contained a reserve store of fat. This error has been perpetuated in the literature (Von Fürth, '03, '99).

ary clotting of the plasma, occurring in Limulus, for example (L. Loeb, '10), is not encountered in ascidian blood. The cells, pigmented and unpigmented, stick together to form sheets and threads. The strings of clotted blood indicate their composition by their vivid green color. Viewed with the microscope they show the preponderating presence of innumerable green blood cells, which change their form but slightly. The unpigmented cells maintain a slow and continued ameboid activity.

The blood cells will agglutinate on contact with seawater. It should however be pointed out that in a puncture of the circulatory system the blood is already clotted *just before* it leaves the vessels. Moreover, the blood can be made to coagulate within the closed and apparently uninjured blood system. The surface change which makes the cells stick to one another may therefore come about not only through contact with a foreign body (Drew, '10), but also by means of a change in the composition of the plasma.

In a consideration of the factors of coagulation, the behavior of the blood cells outside of the body is very suggestive. The corpuscles may be washed in seawater and stirred up in a watch glass. They then settle and form a thin layer on the bottom of the dish. In several hours the movements of the cells have resulted in the formation of isolated balls of cells a few millimeters in diameter. They remain in this condition for a day or two and then begin to flatten out into discs having thin, irregular margins. The cells may continue alive as long as a week; in my experiments they have always died from neglect. Most of the cells retain a green color, but of a darker shade than in the fresh condition. After the discs have flattened out, there is present in the middle of the plate a little peak of blue cells. Although chiefly located in this central mound, many blue cells are still to be found throughout the mass and especially on the surface of the disc.

I have not observed the formation of these cell masses with sufficient care to be certain whether the coalescence of the cells is wholly fortuitous or not. The entire process, however, possesses a striking resemblance to the formation of restitution masses by the isolated tissue cells of sponges and hydroids (Wilson, '11). The further similarity in the behavior of the smaller groups of blood cells to the appearances which Roux ('96) has called "Cytotaxis," suggests that the agglutination of the blood cells may involve something more than an accidental collision of cells whose surfaces have become sticky.

There remains to be considered still another aspect of the coagulation of the blood. At the beginning of the description of the blood it was

stated that a freshly exposed preparation shows the blood in the heart and vessels to be a vivid green, turbid fluid. This appearance is due to a clotting of the blood within the closed circulatory system due to the mechanical stimulation incident to the operation. After fifteen or twenty minutes, if the animal has been undisturbed, all traces of this green turbidity have disappeared and the blood has become so transparent and colorless that the heart is visible only when it contracts. The green, turbid condition of the blood may be induced again by merely handling the individual roughly: squeezing or bending it for a few seconds. After being replaced into seawater, the heart begins to beat in a jerky manner. This indicates an obstruction due to the clotting within the capillaries and smaller vessels, following which the blood in the heart turns green and turbid.

The maximum of turbidity and color is reached within a minute or two. After a short period, depending on the intensity and duration of the rough handling, the blood begins to revert to the normal condition. The disturbed state of the animals and the slow recovery which follow their collection and transportation to the laboratory are probably due to the effects of a prolonged clotting within the circulatory system.

In demonstrating this internal clotting to my associates in the laboratory, I soon found that the same animal could not be employed for more than four or five times in immediate succession. At the end of such a period the color and turbidity produced by rough handling were slightly different from the normal appearance.

An explanation of this may be that the clotting is caused by the secretion of a substance into the blood. The failure to coagulate after a few stimulations might then be accounted for by the rapid exhaustion of the secretory activity. It is necessary only to call attention to the well studied instance of adrenalin secretion (Cannon, '15) and its effects on body conditions and on blood coagulation, to see the possibilities of an extended investigation of this phenomenon in Ascidia.

III. HEART

The observations on the blood stream of Ascidia atra were made on animals the tests of which had been removed in the region of the heart. The heart itself may be readily exposed for its entire length by the removal of a narrow strip of the test. As long as care is taken not to cut the nearby double blood vessel which goes to the test, such a preparation involves almost no loss of blood and behaves in all other respects normally

1. Structure. The cardiac apparatus of ascidians consists of a double walled tube, the inner wall of which is formed in the embryo by an invagination along the entire extent of the outer wall. The two margins of the invagination have completely fused in A. atra, and the suture is an evident structure of the adult heart. The outer wall remains to form the pericardium, while the inner wall becomes the myocardium or heart proper.

The position of the heart is shown in figure 1. About two-thirds of it lies in an almost straight line along the ventral edge of the right side of the body, with the branchial end well anterior. An abrupt turn in the posterior region of the heart carries the remaining one-third along the posterior edge of the left side, the visceral end terminating close to the dorsal edge of the body. The heart is comparatively long. In a medium sized specimen of about 90 grams body weight, it is 4 or 5 cm. in length. Larger individuals may have a heart as long as 6 or even 7 cm.

Approximately in the middle of that portion of the heart which lies on the right side there is to be observed a definite, though slight constriction. A node similar to this has been described in the hearts of other tunicates, especially in Salpa (e.g., Schultze, '01, p. 250). Unlike the one in Salpa, however, the constriction in Ascidia atra is not the result of an arrested muscular contraction of the quiescent heart. It is always present, whether there is a contraction wave passing over the heart or not. Nor is it caused, as Nicolai ('08, p. 94) supposes, by the bending of a thin-walled, fluid-filled tube. In A. atra the heart makes no bend here; and where it does make a sharp bend toward the dorsal edge no such node is produced. Moreover, the pericardium is also a fluid-filled tube having even a much thinner wall and running exactly parallel to the heart. It should therefore also show a constriction, if Nicolai's idea were correct. But such is not the case. The heart node must consequently be regarded as an anatomically significant structure.

The node shows its presence physiologically as well as structurally. In Ascidia it may well be due to a shifting ventrally of the suture between the pericardium and the heart. An evidence of this is the behavior of the beat in its passage along the heart. This contraction wave is a constriction which runs almost completely around the circumference of the heart, but does not include the suture. In some animals it is easy to see the suture on the dorsal side unaffected as the contraction wave passes by. In such hearts, beating from the visceral toward the branchial end, the wave of constriction is clearly ventral as far as the node.

There it suddenly shifts, and the constriction wave may pass along the right side of the heart or it may even shift until it is visible on the dorsal side.

Another indication of the physiologic presence of the node is a change which may occasionally be observed in the velocity of the contraction wave as it passes the node. In instances of this nature the velocity of the beat in its passage from the visceral end to the node is noticeably greater than from the node to the branchial end.

The influence of the heart node is evidenced in still another way in animals which have suffered a loss of blood. In such a heart, beating from the visceral to the branchial end, the pre-nodal portion may continue to remain contracted as the wave progresses. When, however, the wave has passed the node, the pre-nodal region assumes the relaxed condition and the remainder of the wave is perfectly normal.

It seems quite clear therefore that the node is a real landmark in the structure of the heart of Ascidia atra. Of its functional significance, however, I am in complete ignorance.

The space between the pericardium and the heart—the pericardial cavity—is filled with a transparent fluid which contains, besides a protein substance in solution (Cuénot, '91), a variable quantity of cellular elements. According to Fernandez ('06) these elements are derived from the blood cells, and in the young of several species of ascidians they undoubtedly resemble blood cells closely.

In the pericardial cavity of A. atra there is present, in addition to what has already been mentioned, a grayish, spherical mass which, though unattached, is almost always found at the visceral end of the heart. This localization is due to a slight enlargement of the cavity at this end, into which this pericardial body fits snugly. Occasionally it is dislodged by the contraction of the heart and jerked into the general region of the node, where it is moved now this way, now that, depending on the direction of the heart beat. All the individuals which I examined contained this body in the pericardium. It therefore agrees in its position with the mass described by Fernandez ('06) in the pericardium of A. mentula and A. fumigata, and not with the alleged situation within the heart proper as stated by Heller ('75) for these species.

The size of the pericardial body of A. atra varies with the size of the individual. In animals 3 cm. long it is between 1 and 2 mm. in diameter, while it may reach a diameter of 4 mm. or more in the larger animals. It is composed of concentric layers of material which can be taken off in shreds similar to the layers of an onion. Fernandez ('06) has studied

the pericardial mass of several species of ascidians and finds that it is built up by the continuous deposition of the elements found in the pericardial fluid. To these are added the broken remains of the muscle cells of the heart. The presence of the latter furnishes the explanation (Fernandez,'06) of the occurrence of metamorphosed blood cells in the pericardial cavity and in the pericardial body. A tiny rupture of the wall of the heart results in the passage of a few blood cells, together with the torn muscular region, into the pericardial cavity. This idea is more in harmony with the histological findings than that of van Gaver and Stephen ('07), who concluded that the formation of the pericardial body was the result of the activity of a specific sporozoan parasite.

The tunicate heart proper is described as a single layer of cells the inner ends of which have developed muscle fibrils (Herdman, '04, p. 49). Although Roule ('84) has figured the presence of a delicate endothelial layer on the inner face of the heart of Ciona, Seeliger ('93–11, p. 515) has failed to confirm its existence there. However, Hunter ('02) reported its occurence in Molgula, and Schultze ('10) in several species of Salpa.

In spite of a considerable amount of investigation to determine the presence of nerve cells in the tunicate heart, they were not demonstrated until the publication in 1902 of Hunter's work on Molgula. Hunter succeeded in finding not only bipolar nerve cells but also nerve fibers which run spirally around the heart. The cells are grouped into a ganglion at each end of the heart, and Hunter ('03 a) presents histologic evidence for a connection between them and the central nervous system. Quite recently nerve cells and fibers were also demonstrated by Alexandrowicz ('13) in the heart of Ciona and of A. mentula by the use of methylene blue staining.

The entire wall of the heart is so thin that it precludes the presence of a coronary system. The various elements of the pulsating mechanism must therefore, derive their nourishment directly from the blood which passes through it.

2. Heart beat. The most characteristic feature of the physiology of the heart in tunicates is the periodic reversal of the direction of its beat—a fact first noted by Van Hasselt ('24) in 1821, and since then found to be true for all tunicates. Using the nomenclature suggested by Krukenberg ('80 b), the heart is said to beat in an *advisceral* direction when the contraction wave passes from the branchial end to the visceral end, and in an *abvisceral* direction when the wave passes from the visceral to the branchial end. After beating for a while in one direction the heart

sends out a series of beats in the other direction; the combined pulsations make up a *pulsation series* (Shultze, '01, p. 224).

It is frequently stated, especially in textbooks, that the reversal occurs in such a way that the number of pulsations is the same in each direction (cf. Hunter, '03 b). In Ascidia atra such a state of affairs is not altogether infrequent. An example is given in the following record of the heart beat.

Advisceral	16	20	19	14	
Abvisceral	18	21	18	15	

This, however, is not the normal condition. Most individuals show a marked preponderance in the number of advisceral beats. A truly typical example of this is the heart beat of which the following is the record.

Advisceral	20	44	51	65	
Abvisceral	17	15	31	36	

Here, the number of advisceral beats is nearly twice as great as the number of abvisceral ones. The sum of the beats of the twenty-odd animals which were recorded in my notebook, gives the ratio of advisceral to abvisceral beats as 1.6:1. This is clearly a predominance of the number of advisceral pulsations. The ratio would be even greater were it not that at first I thought the cases of unequal number of beats were abnormal. Consequently, I kept the records of a disproportionately larger number of examples showing equal beats.

The actual number of pulsations which takes place in one direction before reversal occurs, varies with the individual animals. An average of nine measurements on fresh animals gave 23.6 beats in the abvisceral direction and 37.0 beats in the advisceral direction. The abvisceral number varied from 14 to 43 beats and the advisceral from 15 to 83 beats.

Unfortunately A. atra did not prove to be a good laboratory animal. Under the most propitious laboratory conditions, with an abundance of running water, the animals failed to remain normal. For instance, after an individual had been in the laboratory twelve hours or even less, a change was evident in the number of beats in a pulsation cycle. An

average of ten counts on animals which had been in the laboratory for one day or a little longer, gave the number of abvisceral beats as 69.0 and the number of advisceral beats before reversal as 145.9. This is an unmistakable increase over the condition soon after collection.

The only difference of any magnitude between the water in the sea and the water furnished in the laboratory of the Bermuda Biological Station is the complete absence of plankton organisms in the latter. These are precisely what Ascidia uses for food. It would seem, therefore, that the increase in the number of beats as well as the general inability of Ascidia to live in the laboratory is probably due to the immediate starvation to which it is subjected. That this factor may become effective within a relatively short time merely indicates that this species leads a rather "hand-to-mouth" existence.

The literature pertaining to the physiology of the tunicate heart is meagre, but an examination of even these few papers reveals well recorded examples of a preponderance of the advisceral over the abvisceral beats, similar to those which I have presented for A. atra. Roule ('84), for example, showed that the advisceral beats in Ciona intestinalis are three times as numerous as the abvisceral beats. Lahille ('90) found a similar condition in Phallusia mammillata. Here the advisceral pulsations are approximately twice the number of the abvisceral ones.

It might perhaps be supposed that the predominance of advisceral beats is compensated by a difference in the intensity and frequency of the beats in the two directions. Such however is not the case. I could observe no dissimilarity in the intensity of the two beats in any of the individuals of the species. Moreover, although the pulse rate of the advisceral phase is slightly greater than that of the abvisceral, as will be shown presently (see fig. 4), the time occupied by the advisceral phase is still practically twice that required by the abvisceral beats. Similar time relations are true for the two phases of the heart beat in Ciona (Roule, '84).

By the use of drugs and adverse conditions it has been possible to affect the time relations of the two phases of a pulsation series in Salpa (Schultze, '01). In order to avoid the possibility of such effects, I have recorded the pulsation series of animals in their natural locations immediately on collection. There was present the usual preponderance of advisceral beats characteristic of animals which had been taken to the laboratory.

It is, therefore, quite certain that in Ascidia atra, and in at least two other species of ascidians, the quantity of blood which is pumped by the

heart during the advisceral phase of a pulsation series is greater than that pumped during the abvisceral phase of the same series.

3. Pulse rate. The pulse rate is one of the factors which determine the quantity of energy that is expended by an animal in the maintenance of its blood supply. Among various influences, the volume of blood moved in a single pulsation and its respiratory efficiency are perhaps the most important. With regard to the respiratory activity of Ascidia atra, as evidenced by the extent of the water current, it has been shown (Hecht, '16) that the energy expended per unit body weight falls off with an increase in the size of the individual. The volume of blood moved in one pulsation depends on the size of the heart. This has not been determined systematically. The frequency of pulsation and its

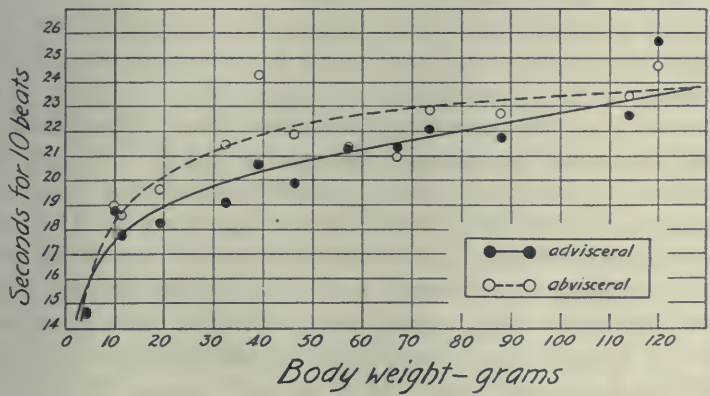

Fig. 4. Relation of pulse rate to weight of animal.

relation to the size of the body have, however, been studied. In figure 4 are plotted the data secured from the hearts of thirteen individuals of varying weight. Altogether, seventy-eight determinations were made, thirty-nine in each phase of pulsation cycle. Three measurements were taken of the time required for ten pulsations to occur in each direction for every animal. The average of the three readings is represented by a circlet in the figure. The smoothed curves in general parallel that obtained for the relation between the extent of the water current and the weight (Hecht, '16). The significance of this is that the pulse rate, which is the reciprocal of the ordinates, varies inversely with the size of the individual. Therefore, in so far as the frequency factor is indicative of the general blood supply, we may conclude that there is relatively less energy devoted to that activity in the larger animals than

in the smaller ones. This conclusion is in harmony with the findings of other investigations of rhythmic activities, such as the respiratory movements of the cloaca of Holothurians (Crozier, '16).

For an understanding of the physiology of the heart beat, however, the most significant feature of the data is the difference in pulse rate between the two phases of a pulsation series. The average of all the readings taken gives the advisceral pulse rate as 29.3 beats per minute and the abvisceral at 28.1 beats per minute. This difference of about 5 per cent in favor of the advisceral phase is not great. But its constancy and regularity, as shown by the two curves in figure 4, serve to emphasize the greater activity of the heart during the advisceral beat. This was already evident in the study of the number of pulsations emitted during the two phases of a pulsation series.

4. Velocity of pulsation wave. The exceptionally long heart of A. atra makes it a convenient object in which to determine the velocity of the pulsation wave. The time taken by a wave to pass over a given distance was determined in three animals of different sizes. The animals were prepared in the usual way, so as to expose only that portion of the heart which lies in a nearly straight line on the right side of the body. The beginning and end of the distance used were sharply marked off by the cut edges of the opaque test. The actual distance was determined at the end of the experiment by placing a piece of fine wire in contact with the heart and bending it into a curve corresponding to that of the organ. The piece of wire was then straightened out and measured. The time interval was determined with a stop-watch.

In all, thirty-eight determinations were made at a room temperature of 27.0°C; nineteen were of the advisceral and nineteen of the abvisceral wave. An equal number of readings in both directions were made on each animal. The figures agreed closely with one another, as shown in the following record:

Experiment I.18. Temperature, 27.0°. Distance of heart exposed 4.4 cm.

Seconds for wave to pass

ADVISCERAL	ABVISCERAL
1.9	2.2
1.9	2.3
1.8	2.2
1.9	2.1
1.9	2.3

An average of the nineteen determinations in each direction gave the velocity of the pulsation wave at 27.0°C. as 2.12 cm. per second in the advisceral direction, and 1.76 cm. per second in the abvisceral direction. This represents a difference of nearly 20 per cent in the velocity of the beat in the two directions, and points again toward the greater activity of the heart while it is beating in the advisceral phase.

5. Significance of unequal phases. All the evidence which has been accumulated in the study of the tunicate heart points unmistakably toward the existence of two centers situated one at each end of the normally acting heart. Moreover, all recent investigators are agreed that the reversal of the direction of the heart beat depends on the alternating dominance in the activity of these two centers.

To show how such a result could be produced on the basis of this assumption, Garrey ('11) has converted the turtle heart into an "Ascidian preparation," whose structure and method of pulsation possess many resemblances to the tunicate heart. By splitting the turtle heart sagittally and keeping the halves united at the apex of the ventricle, a preparation may be made which beats in one direction—from the right heart to the left. This is because of the greater rhythmicity of the right vein, which in the normal heart serves as the pace-maker. If now the rhythmicity of the right vein and the left vein be alternately decreased by vagus stimulation or increased by a local rise in temperature, a periodic reversal in the direction of the pulse wave may be obtained, simulating the normal reversal of the tunicate heart.

In the four aspects of the *normal* heart beat of Ascidia atra which I have just considered, the advisceral phase of a pulsation series shows a decidely greater activity than the abvisceral phase. In terms of the accepted concept of the heart beat this, therefore, means that the advisceral center situated at the branchial end is more powerful than the center situated at the visceral end. Not only does the branchial center initiate pulsations of greater number and velocity, but it maintains this process for a longer time and at a greater rate than does the visceral center.

Aside from the support which this conclusion receives from the data of the normal heart beat, there are other phenomena exhibited by the heart under experimental conditions which also point toward the greater potency of the branchial end. It frequently happens under certain circumstances that another type of beat may be produced in the heart of Ascidia atra. This may be called a central beat because it consists of a series of pulsations originating in the region between the two ends that

normally originate the beats. From this new point of origin two waves proceed simultaneously, one in the advisceral and the other in the abvisceral direction. If such a central beat persists for any length of time, it almost invariably displaces the *abvisceral* phase of the normal pulsation series. For example, the temperature of an animal (I. 23) had been raised from 27° to 30° in ten minutes. At this juncture the central beat appeared. During the half hour in which I observed the animal, not a single abvisceral beat made its appearance. The series of central beats alternated regularly with the advisceral series in a manner resembling the two normal phases of a pulsation cycle.

Not only is it possible to replace the beat with another type of pulsation, but under certain conditions a complete suppression of everything but the advisceral beat may be secured, resulting in a continuous circulation in the advisceral direction.

Such a condition is most readily produced in Ascidia by subjecting the animal to a temperature about 8° higher than that of the room. Another method of accomplishing the same result is to place the individual in a solution that is slightly toxic, such as diluted seawater or $\frac{5}{8}$ M NaCl. I made no attempt to see how long the advisceral circulation could be maintained, but in one experiment (I. 21) it continued for over twenty-one minutes while the animal was kept at a temperature of 35°C.

The difference in potency and viability of the two centers in Ascidia finds its parallel in the differential resistance to drugs of the two centers in the Salpa heart. For example, helleborein reduces the activity of the abvisceral phase to almost nothing, whereas it affects the number of advisceral beats only slightly (Schultze '01, table 27). There are also similar variations in the rhythmicity of different portions of the vertebrate heart. Not only do the different chambers of the vertebrate heart exhibit a difference in the frequency of pulsation, but they also show a differential resistance to various external conditions, notably the hydrogen-ion concentration (Dale and Thacker, '14). Perhaps the example most comparable to the ascidian heart is the variation in activity of two such symmetrical portions of the heart as the two veins of the turtle's heart, the right vein having a greater rhythmicity than the left vein (Garrey, '11).

The greater potency of the branchial end of the heart of A. atra and of the two other species of ascidians previously mentioned is, to my mind, of prime importance for understanding the physiology of the blood supply. The commonly accepted idea is that the heart pumps blood in one direction, and then reverses and pumps an equal quantity of blood—

the same blood, in fact—in the opposite direction. The thesis which I wish to maintain is that, in spite of the periodic reversal of the direction of the beat, there is, in these three species at least, a definite one-way *circulation* of the blood.

It has been pointed out that the quantity of blood which the heart moves in the advisceral direction is about twice the amount which it pumps in the abvisceral direction. The other aspects of the beat, showing the superior potency of the branchial end, merely serve to emphasize this important fact. The blood cells of Ascidia, therefore, *circulate* in the direction of the advisceral beat, except that during the abvisceral phase they are put back in their progress to the extent of one half the distance which they have travelled during the advisceral phase. This corresponds exactly to the kind of progress a man would make who walked along the circumference of a large circle, taking ten steps forward and five steps backward, then ten more forward and five backward and so forth. The resultant of this type of activity would be a forward locomotion leading him around and around the circle in a single direction. Similarly, the greater heart activity during the advisceral phase results in a one-directional circulation in these Ascidians.

An attempt to determine the circulation time of the blood met with no success. Consequently, it is not possible to state how many pulsation series are required for the blood to complete one circulation in the predominating direction.

The existence of this circulation is of interest in an evolutionary connection. The idea that vertebrates arose directly from Ascidians does not receive support from the investigation of the direction of the blood flow. In Ascidia the path of the blood from the heart is away from the gill; in the lower aquatic vertebrates it is toward the gill.

The dominance in the activity of one phase of the pulsation cycle has a distinct bearing on the cause of the reversal of the heart beat. The ideas of many writers on the alternating character of the direction of the blood flow in the tunicates is fairly expressed by the following quotation.

> It has been suggested that the cause of this remarkable reversal may possibly be that the heart being on the ventral vessel, which is wider than the corresponding dorsal trunk, pumps the blood into either the lacunae of the branchial sac or those of the viscera in greater volume than can possibly get out through the smaller branchio-visceral vessel in the same time, the result being that the lacunae in question soon become engorged, and by back pressure cause the stoppage, and then reversal of the heart. The absence of any valves in the heart to regulate the direction of flow obviously facilitates this alternation of the current (Herdman, '04, p. 50).

Somewhat the same idea is at the bottom of the explanation of the change in direction which its discoverer, Van Hasslet ('24), gave. Among others, Todaro ('85), Ritter ('93) and Lahille ('90) have suggested explanations, all based on the same "back pressure" assumption. Lahille ('90) in particular has gone into great detail and has even attempted an experimental proof of this conception.

As Schultze ('01) has already pointed out, this idea would be excellent if there were no *active* contraction of the heart in the reversed direction. On the assumption of the existence of such "back pressure," it would furnish a good explanation if the blood system were a mechanical pumping arrangement run by a physiologically indifferent mainspring. The direction and rhythm would then be dependent on the volume of the containing vessels and on the velocity and quantity of the fluid moving in the apparatus. This, the blood system is not. The heart is an *active* pump. The fact that it may be isolated from the rest of the system—even removed from the body—and still maintain the reversal of its pulsations, shows that the cause of the change in direction of the beat must lie within the heart itself.

Aside, however, from these objections to the back-pressure hypothesis, it seems to me that the assumption on which it is based does not fit the facts which I have summarized with regard to the direction of the circulation. In tunicates there are no blood *vessels* in the ordinary sense of the term. The blood spaces are continuous, and their bore does not vary from pulsation to pulsation as in the blood vessels of the vertebrates. Thus the volume of the blood and the capacity of the blood spaces are constant quantities. Consequently, if any back pressure were to develop due to an inability on the part of certain spaces to transmit the blood supplied by the heart, this back pressure would have to be the same no matter in which direction the heart was beating. Moreover, if a congestion were to result, the quantity of blood required, and therefore the number of pulsations, would have to be equal in both directions before the occurrence of a reversal. Such, however, is not the case, since the quantity of blood and the number of pulsations in the advisceral phase is much more than in the opposite phase.

That the blood is under a definite pressure is undoubted, as I have previously described. But that there is a gradual increase in the blood pressure until congestion sets in, with the resulting reversal to relieve the situation, seems at variance with the findings which these investigations have brought to light. One may as well expect the vertebrate heart to reverse because of the resistance in the capillaries.

6. Nature and origin of contraction wave. In attempting to understand the nature of the heart beat of Ascidia, it is well to keep in mind the distinction between the origin of the contraction wave and its propagation along the heart. The latter phase is the simpler, and a discussion of it will serve to introduce the problem of the basis of the heart activity.

The velocity of the beat along the heart of A. atra is 1.76 cm. per second in the abvisceral, and 2.12 cm. per second in the advisceral direction. The order of magnitude of this velocity is of significance in determining the pathway of the contraction wave.

It is out of the question to compare this velocity with that of the passage of an impulse along well-defined nerve trunks like those found in vertebrates. Such structures certainly do not exist in the heart. The only demonstration of nerve tissue at all in the heart of an Ascidian is the work of Hunter ('02). Alexandrowicz ('13) merely states that he saw nerve cells but gives no figures to show their distribution.

On the tentative assumption that there is a type of loose nerve-net in the heart, it is clarifying to examine the velocities that have been obtained for such a type of conduction. A well-defined case of nerve-net conduction is the passage of the wave in a ring of the disc of the medusa Cassiopea. At 25°C. the velocity of this conduction varies from 27.6 to 49.9 cm. per second (Harvey, '11). The slowest conduction obtained in nerve tissue by Jenkins and Carlson ('04) was in the ventral chain of Cerebratulus. This is a nerve-net, and the velocity of transmission along the chain varied from 5 to 9 cm. per second. The ventral chain of other worms showed a much greater velocity of transmission: Nereis gave 89 cm. per second and Bispira 694 cm. per second. In short, even the slowest velocities of nerve-net conduction are at least three times as great as that found in the heart of Ascidia.

The only case in which the nervous nature of the transmission of the contraction wave has been unmistakably demonstrated is the heart of Limulus (Carlson, '04). In this animal the velocity of propagation is so great that the heart, half a foot long, seems to contract as a single unit. Only under special circumstances is it possible to show that the beat arises in the posterior third of the heart and travels forward along a nerve-net which lies on the outside of the heart (Carlson '04, p. 70). The magnitude of the velocity of the heart beat of Ascidia would therefore seem to prove that we are dealing with a slow, muscular transmission, comparable to the wave of muscular contraction which causes the locomotion of the earthworm (Friedländer, '88).

The nature of the activity which underlies the *origin* of the beat is complicated by several factors. As already stated, the immediate cause of the curious behavior of the ascidian heart is most probably the alteration in the potency of its two ends (J. Loeb, '02, p. 29). The origin of a single beat is therefore due to the activity of the center situated at one of these ends. This idea was first expressed by J. Loeb ('02) on the basis of Lingle's work on Molgula (Bancroft and Esterly, '03). J. Loeb describes how the source of automatic activity is confined to a small region at each end of the heart. The discovery of a mass of nerve cells in these spots in the Molgula heart (Hunter, '02) seemed to prove that the initiation of the heart rhythm is due to the activity of the nerve cells. This neurogenic idea was emphasized by Lingle's (J. Loeb, '02) inability to cause the central portion of the heart to beat in seawater, and the comparative sparseness of nerve cells found by Hunter ('02) in this region.

Further investigation, however, showed that some of this work was in error. For example, both Schultze ('01) and Bancroft and Esterly ('03) found no difficulty in securing an automatic rhythmicity of the isolated central part of the heart in ordinary seawater. Later, Hunter found the same to be true for the species with which Lingle had first worked (Hunter, '03b).

Similarly, the isolated central portion of the heart of Ascidia atra beats in seawater. Moreover, not only can the isolated central part of the heart initiate automatic activity, but this portion may produce beats even when the heart is in its normal position in the intact animal. Careful observation has convinced me that this central beat may originate in practically any place between the two ends, although it frequently occurs at the node. This may happen not merely in hearts which have been subject to slightly toxic conditions but is frequently to be observed in freshly captured animals.

In terms of a neurogenic conception of the heart beat, these facts have no significance. If, however, we adopt the myogenic conception, viz., that all the heart muscle cells are capable of an automatic rhythm, the situation at once becomes clear. The ability of any portion to initiate pulsations for the entire heart depends upon its rate of pulsation and upon the refractive properties of heart muscle. In the normal Ascidia heart the two ends alternately act as pace-makers, due to the alternating superiority of their pulse rates. The balance in their favor over the central portion of the heart is, however, so delicate that it requires only a slight change in metabolic activity of the heart to upset it. The

branchial end is normally the most potent part of the heart, as is evidenced by the much greater duration and rate of its activity. This probably signifies so large a balance in its favor that, unlike the visceral end, it is never replaced by a central beat in the regular pulsation cycle.

The fact that external stimulation may change the rhythm and intensity of the heart beat of Ascidia and of Salpa (Nicolai, '08), is no argument for the nervous nature of the origin of the beat. Such changes are entirely comparable to the effect of vagus stimulation of the vertebrate heart, the beat of which has been shown by the experiments of Burrows ('12) and of Hooker ('11) to be undoubtedly of muscular origin.

It is possible, therefore, to summarize the discussion in this section by the following conclusion. The heart beat of Ascidia atra originates within the muscle cells, and the conduction of the pulsation wave is accomplished by the passage of the impulse across a continuous muscular pathway.

7. Temperature and heart activity. The general effect of a change in temperature on the cardiac activity of Ascidia atra serves as a demonstration of the balance maintained by the different parts of the heart. The precision of this balance undoubtedly varies among individuals and it is therefore not surprising that the degree of disturbance produced by a change of temperature should be different in individual cases. In some animals, for example, a change in temperature may produce no other than an accelerating or retarding effect depending on the direction of the change; in others additional phenomena may make their appearance.

Some reference has already been made to a central beat which appears under these conditions. If this beat is only occasional, it may occur between the usual phases of a pulsation cycle. If it persists, it will often supplant the abvisceral beat completely. These results can be obtained with a decrease in temperature as well as with an increase, although the latter is a more efficient means.

An effect on the color of the blood, not directly connected with heart activity, is produced by a change of temperature. The clotting of the blood of Ascidia within the intact blood system has already been described: One way of producing this agglutination is to raise the temperature of the animal several degrees. The blood becomes green and to all appearances behaves as if it had been clotted by a vigorous external stimulation of the animal. The color change occurs at a point (32.0°) well below the death temperature of the animal, and consequently is not due to a heat coagulation of either the plasma or the corpuscles.

This is further assured by the reassumption of the usual transparency with continued warming.

The temperature limits of heart activity show a marked individual variation which in all probability depends upon the rate of the temperature change and the duration of the exposure. For example, in one experiment the cooling was performed at the rate of about one degree for every ten minutes. The lowest temperature at which the heart maintained its beat was 13.0°C. In a similar experiment the cooling was accomplished twice as rapidly, and then the lowest temperature at which the heart was active was 17.1°C. These experiments were performed on two occasions only, and are consequently merely suggestive of the imperative consideration to be given to the time factor in determinations of temperature limits. As has been pointed out by Crozier ('16, p. 329), attention to such sources of error is, however, all too uncommon in biologic investigations.

Bearing this in mind, we may say that the range of temperature over which the heart of Ascidia is active is about 20°. The lower limit varies around 15°; the upper limit is near 36°C.

The effect of the temperature is evident in a change in the pulsation rate and in the velocity of the contraction wave. Both of these aspects of the cardiac activity admit of such accurate measurement that it seemed desirable to investigate quantitatively their relation to the temperature.

The method adopted was simple, uniform and avoided certain sources of error. The animal was in a beaker of seawater, which in turn was suspended in a larger dish of water. The large dish received the brunt of the warming and cooling, the temperature being equalized in the system by constant stirring. At selected temperatures measurements were made in each direction of the beat: five of the velocity or three of the pulsation rate, depending on the experiment. The actual procedure was to make determinations at room temperature, then to cool the animal about three degrees in ten minutes, and to maintain it at this temperature for ten minutes. During the last two minutes of this period readings were again made, after which the cooling continued until the next selected temperature was reached. This was again maintained for ten minutes, and so on, the process being repeated until the lowest temperature was recorded. Then the animal was allowed to return slowly to room temperature. After a few hours the warming was begun, the experiment proceeding along the same lines as before.

The relation of the temperature to the time required for ten beats is

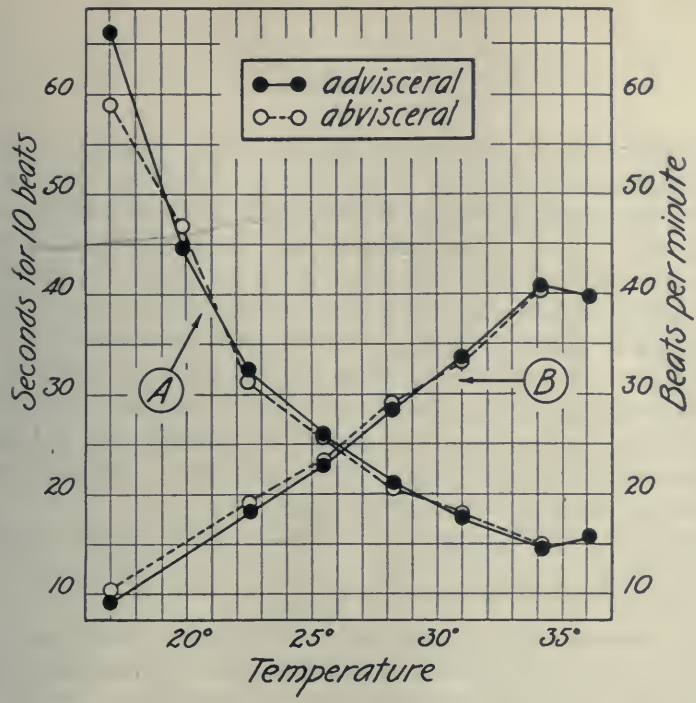

Fig. 5. Relation of pulse rate to temperature. *A*, number of seconds required for ten beats; *B*, number of beats per minute.

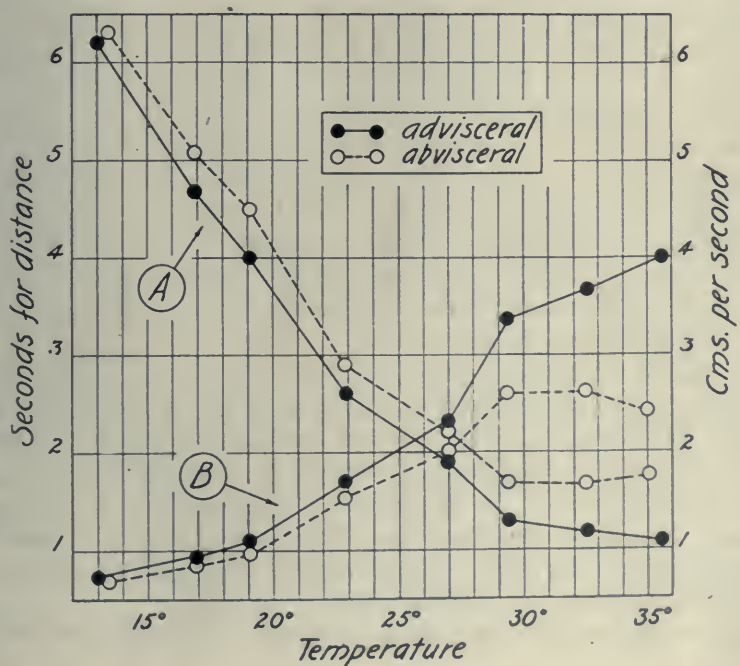

Fig. 6. Relation between temperature and velocity of contraction wave of heart of Ascidia. *A*, number of seconds required for a wave to pass across a given distance; *B*, velocity of the wave in centimeters per second.

given for one animal in curve A of figure 5. It is entirely typical of the other experiments. Curve A of figure 6 expresses similarly the average results of an experiment on the relation of temperature to the velocity of pulsation. This may best be shown as the time required for a contraction wave to pass over a given distance along the heart. The two curves resemble the numerous figures that have already been drawn to show the relation of temperature to various life processes (Kanitz, '15).

Although there has been much controversy about the exact shape of such curves in general, the matter is far from settled. Snyder ('13), among others, has consistently supported the idea that they are exponential in character, whereas Krogh ('14), for example, has interpreted similar results as linear functions of the temperature.

A minor source of confusion in this connection is the difference in the methods of recording data. It will be remembered that $y = \frac{1}{x}$ represents the equation for an hyperbola referred to its asymptotes as axes, and that $y = x$ is the equation of a straight line. Consequently, much depends on whether the experimental value itself or its reciprocal is used in plotting the results. Figure 5 represents just such a situation. Curves A and B have the same abscissas, whereas the ordinates of one are the reciprocals of those of the other. In the one case the curves resemble an hyperbola; in the other, a straight line.

Only a few of the discrepancies (cf. Krogh, '14, p. 170), however, are to be explained on the basis of this confusion. In Ascidia, for example, two phases of the activity of the same organ show the two types of results illustrative of the effect of temperature. Curves B of figure 5 show that the pulse rate is clearly a linear function of the temperature. Its temperature coefficient is therefore constant for the complete range of its normal activity. The velocity of the pulsation wave, however, is an exponential function of the temperature (B, fig. 6), and its temperature coefficient varies from 2.1 to 2.9.

In spite of the heated controversy (Snyder, '13, p. 77, footnote), it is difficult to see wherein either result is possessed of any great significance. The earlier investigators were much impressed by the resemblance between the temperature coefficient of protoplasmic activity and of chemical reactions. On this similarity was based the conclusion, for example, that the underlying process of the heart beat is a single chemical reaction (Harvey, '11). It must, however, be clear at present that this is much too simple an explanation.

Any protoplasmic movement is composed of several chemical and

physical reactions, which may differ in direction, velocity and intensity (cf. especially, Pütter, '14). A few of the more obvious physiologic factors which are concerned in the heart beat of Ascidia atra are: the origin of the internal stimulus, its propagation through the cell, the refractory period of the muscle and the degree of its irritability. Each of these is undoubtedly conditioned by several reactions which result in an interplay of several kinds of energy. A change of temperature has an individual effect on these numberless reactions, and it is their resultant which is expressed by the relation of the temperature to the frequency of the pulsation.

The remarkable thing, to my mind, is not whether the relation between temperature and pulse rate is linear or exponential, but that there exists any definite relation at all. The real significance of temperature effects will not become clear from a superficial comparison with simple chemical reactions. Their interpretation will come only after an analysis of the principal reactions concerned in protoplasmic activity, much as the understanding of the timbre of a musical sound is the result of its resolution into the tones and overtones combined in its production.

IV. SUMMARY

1. The blood of Ascidia atra is colorless and transparent. It flows through the body under an appreciable pressure.

2. The blood plasma is isotonic with seawater.

3. The blood has an acid reaction; the acidity is resident in the green corpuscles and not in the plasma.

4. There are at least two kinds of cells in the blood: pigmented and unpigmented. Of the pigmented cells, the green are distributed all over the body; the orange are localized in the branchial sac; and the blue are found in the regions of the viscera, etc. Some of the unpigmented cells are ameboid; others are not.

5. In the green cells, the pigment is a compound containing vanadium, probably in a stage of oxidation corresponding to V_2O_3. It is not a respiratory pigment but is most likely of value as a catalytic agent.

6. The coagulation of the blood depends on the agglutination of its cells. Clotting often occurs within the intact blood system as a result of vigorous external stimulation.

7. The heart of Ascidia atra is long and has a node which divides it unequally. The presence of the node may be demonstrated physiologically as well as anatomically.

8. A pericardial body is present.

9. In most of the animals a pulsation series shows about twice as many advisceral beats as abvisceral beats.

10. The pulse rate decreases as the size of the animal increases. It is greater in the advisceral phase than in the abvisceral phase.

11. The velocity of the contraction wave is greater in the advisceral direction than in the abvisceral direction.

12. The greater activity of the heart during the abvisceral phase of a pulsation cycle indicates that, in spite of the periodic reversal of direction, there is a resultant *circulation* of the blood in the advisceral direction.

13. These facts are incompatible with the "back pressure" explanation of the periodic reversal of the heart beat. They are, however, in harmony with the idea that the reversal is due to the alternating dominance as pacemakers of the two ends of the heart.

14. The presence of a central beat, the suppression of the abvisceral beat and the magnitude of the velocity of the pulsation wave indicate that the heart beat is myogenic, and that the contraction wave passes along the muscular elements across the heart.

15. The pulsation rate is a linear function of the temperature, whereas the velocity of the pulsation wave is an exponential function of the temperature. Neither relation is shown to be of real significance in view of the complexity of reactions involved in the heart beat.

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EVIDENCE FOR THE ENZYMATIC BASIS OF HEART-BEAT[1]

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Received for publication December 6, 1917

Among the many investigators who studied the effect of temperature on the heart-beat, Cyon (1) did the most conclusive of early work. He studied the effect of changes of temperature on the number, duration and strength of the beats of the excised frog heart. He found that the rate increased regularly with the temperature until the number of beats reached a maximum. After reaching this point the rate became irregular and slowed rapidly so that the heart came to rest only a few degrees above the maximum, between 30° and 40°. These observations were extended and confirmed for the mammalian heart by Newell Martin (2) and by Langendorff (3). G. N. Stewart (4) found that extreme high temperatures cause first an increase in rate to a maximum and later a decrease followed by standstill in the hearts of the frog, toad and turtle. The same was found to be true of the terrapin heart by E. G. Martin (5).

The problem took on a new aspect with the application of the law of van't Hoff and Arrhenius to biological reactions. This law applies to chemical reactions and states that for every increase of ten degrees in temperature the rate of reaction is doubled or trebled. A great number of investigators have established the fact that metabolic processes are influenced by heat in the same direction and degree as chemical reactions, and they have shown that the law holds for medium temperatures (10° to 27°). The conclusion arrived at is that life activities are at bottom chemical.

The question at issue is, Does the law hold for higher temperatures, and in case of deviation, what are the modifying factors? The heart-beat of crustacea (6), of cold-blooded (7) and warm-blooded vertebrates (8) has been shown to obey this law within the medium range. If the law holds for higher temperatures, the heart ought to beat with

[1] Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Rutgers College.

increasing velocity as the temperature is raised until it abruptly stops at the critical coagulation point.

Loeb (9) long ago suggested that the heart owes its rhythmicity to the "constant fermentative production of certain compounds in the automatically active cell." If this idea is correct, the curve of the rate of heart-beat plotted against temperature should be a typical enzyme curve and the temperature coefficient of the rate of heart-beat should show the same variations which that of enzyme reactions do, i.e., become smaller with increasing temperature. The rate of reaction should increase more rapidly at low temperature than at medium temperature; should then obey the law of van't Hoff and Arrhenius for the middle range; should reach a maximum and finally fall off to zero with further rise in temperature. In the last few degrees in which any reaction is detected, there should be a negative temperature coefficient. It is known that the rule of van't Hoff holds for enzymes generally between 15° and 35°. Above 35° and below 45° the increase in velocity of reaction is greater than the law predicts, while above 45° there is a rapid fall possibly due to the destruction of the enzyme at the high temperature (10). The position of the maximum and the killing temperature varies a great deal with different methods and different ferments but the characteristic effects of temperature on the rate of the reaction is the same in all cases.

THE EFFECT OF TEMPERATURE ON THE RATE

Dr. Loeb suggested to me the possibility of determining whether the law of van't Hoff and Arrhenius holds for the heart-beat at high temperatures or whether the course of the reaction is like that of an enzyme. Accordingly, in the summer of 1917, I carried out a series of experiments on Fundulus embryo-hearts.

Fundulus heteroclitus embryos were used from the time the heart had begun to beat regularly until it was obscured by the developing pigment, i.e., when the embryos were from seven to fourteen days old. The embryo was placed in a small Petri dish holding 10 cc. of seawater, the temperature of which was varied by means of a surrounding water-bath. The bulb of a thermometer was placed in the small Petri dish as near as possible to the embryo. The time for twenty beats of the auricle was taken with a stop-watch after the mercury had registered a convenient temperature continuously for about two minutes. The embryo was kept under observation during the entire course of the experiment, the temperature being gradually but steadily raised from

room temperature or a little below, to the point at which the heart ceased beating altogether. From 32° to 46° the readings were taken as rapidly as possible.

The results of these experiments are illustrated by the experiments and their corresponding graphs given below. The rate, calculated as $\frac{100}{\text{Time for 20 beats,}}$ is plotted against temperature (see tables and figures 1 and 2).

The rate increases quite regularly with the temperature from 10° to 40°. Above 40° the rate suddenly increases more rapidly than before, and above 41° to 43°, the curve falls to zero. The exact position of the maximum depends upon the length of time exposed to the high temperature, and upon the length of time taken to raise the temperature to a high level. This time factor will be discussed later. The shorter the time, the higher the maximum. As nearly as was possible the length of time taken up by each experiment was kept at about fifteen minutes in all.

THE TIME FACTOR

Some experiments were made in which the temperature of the water-bath was lowered from 46° to room temperature, instead of raised from a lower to a higher temperature. One of these experiments, number 9, is quoted below (see table 3).

It is evident that in this experiment the influence of length of exposure is shown. At 45° and 46° the rate steadily decreases. When the temperature is lowered, the rate increases to a maximum and the temperature coefficient becomes positive. The factor of length of exposure must be important at temperatures near the death point of the organism; but it is negligible at medium or normal temperatures since the animal spends a lifetime at such temperatures. Using the method described by Loeb and Ewald (11) in their experiments on the effect of temperature on the heart-beat of Fundulus embryos, I determined the temperature at which the rate of heart-beat was greatest after from fifteen to twenty minutes in a thermostat. The maximum rate was found at 32° or 33°. The maximum is much higher in the above results because the time of exposure has been greatly lessened. At temperatures higher than the optimum the time factor becomes greater with each degree. If the injury due to length of exposure were zero, as at temperatures below 32°, the rate would continue to rise with the temperature until the coagulation point of the tissues is reached.

As a matter of fact, injury by exposure intervenes, just as in an enzyme reaction, and slows the rate before an observation can be made, since the time factor cannot be completely eliminated. The slowing of the

TABLE 1

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
10.0	33.2	3.0
12.5	27.0	3.7
16.0	17.6	5.6
18.0	14.8	6.7
20.0	12.0	8.3
24.0	9.0	11.1
27.0	7.5	13.3
29.0	6.6	15.1
32.0	5.6	17.8
36.0	5.2	19.2
39.0	4.0	25.0
42.0	3.6	27.7
43.0	3.0	33.3
44.0	2.8	35.7
45.0	3.2	31.2
46.0	4.0	25.0
47.0	4.2 stopped	23.8

TABLE 2

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
11	27.4	3.6
15	15.4	6.5
20	10.0	10.0
24	7.5	13.3
28	6.4	15.6
33	5.0	20.0
35	4.8	20.8
38	4.2	23.8
40	3.6	27.7
42	3.0	33.3
43	2.6	38.4
44	3.2	31.2
45	4.0	25.0
46	stopped	

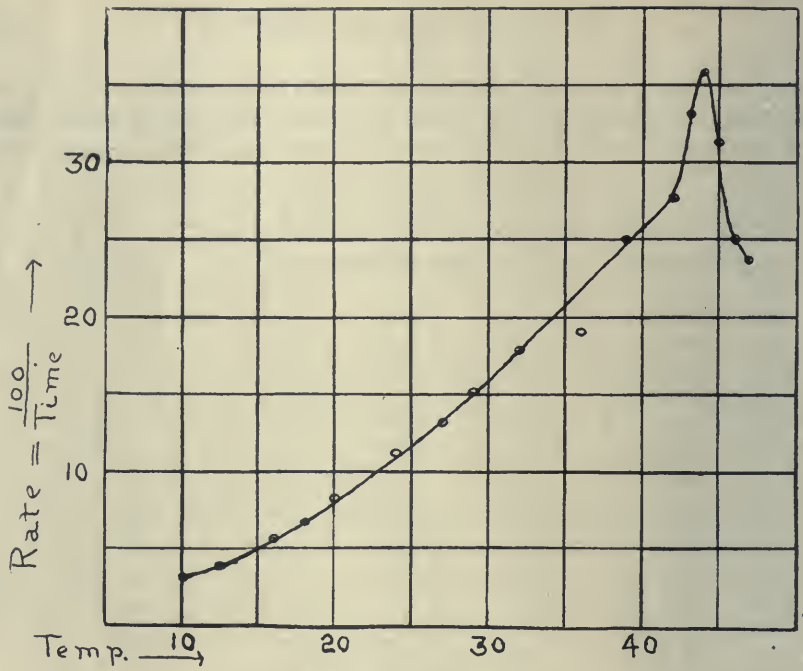

Figure 1 from table 1

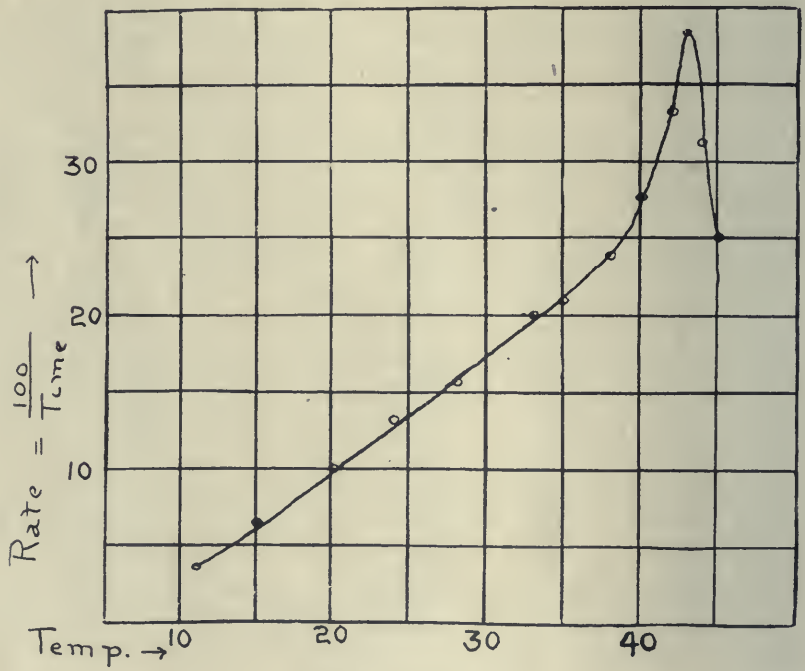

Figure 2 from table 2

rate comes before the coagulation process has become irreversible, or possibly before any coagulation has occurred. The heat may partly destroy the enzyme before it has killed the cells, in which case complete recovery of the heart should occur when the temperature is lowered even if the heart has altogether ceased to beat. This in fact is the case and can be observed with any embryo.

If the embryo is kept at a constant high temperature and observed continuously, the rate of heart-beat is seen to increase at first, then to decrease and finally to fall to zero. This is shown by the following experiment (see table 4).

TABLE 3

Experiment 9

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
46 (1)	6.0	16.6
(2)	7.5	13.3
45	8.0	12.5
43	4.0	25.0
42	3.8	26.3
41	3.2	31.2
40	4.0	25.0
39	4.0	25.0
36	4.4	22.7
34	5.0	20.0
32	5.0	20.0
30	5.2	19.2
28	5.8	17.2
26	8.0	12.5
25	8.8	11.3
23	9.8	10.2
19	15.4	6.5

THE TEMPERATURE COEFFICIENT

C. G. Rogers (12) has shown that the temperature coefficient of the rate of heart-beat of the Fundulus embryo is of the order of magnitude of a chemical reaction. Loeb and Chamberlain (13) pointed out that the temperature coefficient of the rate of heart-beat agrees with the supposition that the heart-beat is determined by the velocity of an enzyme reaction. The temperature coefficients of various enzyme reactions have been determined for the entire temperature range of

their action (14). The values of Q_{10} in all these cases are higher for temperatures near zero and decrease as the temperature is raised.

The following table (table 5) gives in terms of beats per minute the average rates of heart-beat from a large number of observations. Previous to making the observations the embryos were kept in a thermostat at the desired temperature for from fifteen to twenty minutes.

TABLE 4

TEMPERATURE	MINUTES AFTER BEGINNING EXPERIMENT	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees		*seconds*	
44		3.8	26.3
45	1	5.0	20.0
45		6.6	15.1
45	2	7.0	14.2
45		7.2	13.8
45		7.5	13.3
45	3	7.7	13.0
45	4	7.8	12.8
45	5	8.0	12.5
	6	stopped	

TABLE 5

TEMPERATURE	BEATS PER MINUTE	Q_{10}
2.5–12.5	53.2/ 7.0	7.6
5.0–15.0	69.0/ 19.0	3.6
7.5–17.5	86.3/ 29.7	2.9
10.0–20.0	100.0/ 41.1	2.4
12.5–22.5	122.5/ 53.2	2.3
15.0–25.0	139.0/ 69.0	2.0
17.5–27.5	180.9/ 86.3	2.0
20.0–30.0	200.0/100.0	2.0
22.5–32.5	223.5/122.5	1.9
25.0–35.0	200.0/139.0	1.4

The temperature coefficients are calculated for 10 degree intervals. The resemblance of these values to the temperature coefficients of enzyme action is obvious. In this connection it is interesting that E. N. Harvey (15) determined the effect of temperature on the pulsations of the medusa Cassiopea xamachana, and found that the temperature coefficient resembles closely that of an enzyme action.

HEART-BLOCK

If the temperature of the heart is gradually raised, a point is reached at which the normal rhythmical sequence of the parts no longer occurs. G. N. Stewart (16) observed heart-block as the result of heat in the case of the frog. With Fundulus embryos a block develops so that regularly not all of the beats of the auricle are followed by ventricular contractions. If the heart is not heated too suddenly, the block develops in well-defined steps. The ratio of auricular to ventricular beats may be successively, 6/5, 5/4, 4/3, 3/2, 2/1, 3/1, etc., until the ventricle stops beating altogether. If the temperature is then slowly lowered, the reverse changes in the ratios take place until the normal sequence is resumed. The temperature at which the block appears is two or three degrees above that at which the block disappears upon lowering the temperature. The exact point at which the block appears depends upon the former treatment of the embryo. Embryos which had been kept in a refrigerator with a temperature of from 8° to 10° for several days, when gradually heated developed heart-block at from 28° to 29° in every instance. Embryos which had been kept at room temperature developed block at from 34° to 35°, while embryos which had been kept at 34° to 35° for an hour and had recovered from the block at that temperature, had normally beating hearts up to 41° or 42°. If the embryo is kept for some few minutes at the temperature at which the block appears, the heart recovers its normal beat, and in order to produce block again, the temperature must be further raised. I never observed a recovery above 39°, however. One embryo was kept at 39° without affecting the block, for an hour and a quarter, when the experiment was discontinued. After a half hour, during which the temperature had fallen to that of the room, the heart had completely recovered its normal beat.

The ventricle is unable to beat at temperatures above 42°. The auricle stops beating at from 44° to 46°, according to the length of time exposed, and the sinus stops immediately after the auricle. It was difficult to determine this point exactly since the beats of the sinus, besides being very rapid at high temperatures, become weak and almost indistinguishable. In several experiments the sinus was seen to beat two or three times immediately after the auricle had ceased beating, and then to come to rest, relax and fill with blood from the auricle and ventricle. In every case after standstill of the heart, the sinus was full of blood so that the spot was visible to the naked eye.

CONCLUSIONS

1. The changes in the rate of the heart-beat of the Fundulus embryo at high temperatures are such as would be expected in case the rhythmical contractions of the heart depend upon the velocity of an enzyme reaction.

2. With high temperatures the length of time exposed is an important factor. The longer the time exposed, the lower the temperature necessary to bring about standstill of the heart, indicating a temperature coefficient of the destruction of the enzyme.

3. The values of Q_{10} are shown to be higher at temperatures near zero and to decrease, as is the case with enzymes, when the temperature is raised.

4. Auriculo-ventricular block was observed as a result of high temperature. The ventricle is first affected by the high temperature, and finally the auricle and sinus.

I wish to thank Professor A. R. Moore for constant encouragement and many helpful suggestions in carrying out this work.

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VASODILATOR REACTIONS. I

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Received for publication December 7, 1917

The experiments described in the following resulted from an endeavor to discover the cause of the marked fall of blood pressure following the administration of certain drugs; they have led to the conclusion that there is present in mammals (and perhaps other animals) a vasodilator mechanism not recognized, or only vaguely recognized, capable of responding with more intense reactions than any of the mechanisms generally recognized and one which may be more perfectly controlled than the latter.

The present work began with the discovery by Taveau and myself in 1906 of the intense blood pressure lowering action of acetyl-cholin.[1] In our first publication (1) on this subject I said,

> I think it safe to state that, as regards its effect upon the circulation it (acetyl-cholin) is the most powerful substance known. It is one hundred thousand times more active than cholin, and hundreds of times more active than nitroglycerin; it is a hundred times more active in causing a fall of blood pressure than is adrenalin in causing a rise.

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[1] In our paper in 1906 Taveau and I described a physiological test for cholin, based upon its conversion into the acetyl compound, by means of which 0.0001 mgm. or less of cholin could be detected. This method was later elaborated (2) using the isolated frog heart as a test object so that 0.00001 mgm., and probably less, cholin could be detected; a number of applications of the method were described. Ten years after our first description of this method Guggenheim and Loeffler (3) without knowing of my work (as stated in a letter from Doctor Loeffler) published a similar method making use of the guinea pig intestine as a test object. This method seems (I have not seen the original paper) to be far less sensitive than mine; but although these authors do not seem to have taken the precautions I did to avoid splitting off cholin from the lecithin of the serum the figures given for the cholin content of the blood serum (from 0.2 to 2.0 mgm. per 100 cc.) are similar to those I found. Their observations upon the rapid disappearance of cholin from the blood and upon the amount present in the urine also agree with mine. Führer (4) is quoted as having emphasized the superiority of the frog heart as a test object.

These results were fully substantiated in subsequent work and were confirmed eight years later by Dale (13). Figure 1 shows the effect of 0.000,000,002,4 mgm. of acetyl-cholin per K upon the blood pressure when injected intravenously into a cat. The response was, I believe, more active in this experiment than in any other; the stated dose caused the same fall of pressure repeatedly. Injections of equal amounts of the 0.9 per cent sodium chloride solution used in making the acetyl-cholin solution, had no effect. In order to avoid the possibility of an error in the dilution a fresh series of dilutions was made; the results were the same.

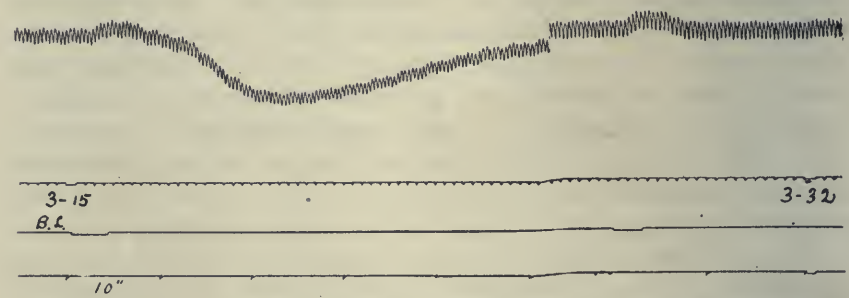

Fig. 1. Experiment 359. Cat, 4.16 K; paraldehyde; vagi cut. Blood pressure from left femoral artery; injections into right saphenous vein. *3–15*, 1 cc. of acetyl-cholin 1:100,000,000,000. *3–32*, 1 cc. 0.9 per cent sodium chloride solution.

Taveau and I also described in 1906 a chemical test for cholin based upon its conversion into the benzoyl compound and the precipitation of the latter with platinum chloride; this test seemed to have advantages over the tests then in use (cf. 5).

My work on the cholin esters was suggested by some work I had done previously which had resulted in the isolation of cholin from adrenal extracts and the identification of it as the chief substance causing the fall of blood pressure of such extracts after the removal of the epinephrin (6)—an observation frequently ascribed to Lohmann, but the publication of my work antedated that of Lohmann by seven years. At that time no one, apparently, except Marino-Zucco (who had reported finding "neurin," evidently cholin, in the adrenal glands) had isolated cholin from any organ extract and the thought at once occurred to me that possibly this substance might represent an internal secretion of the cortex in the same way that epinephrin was supposed to be an internal secretion of the medulla. Further work (7) (1) suggested that there may be present in the adrenal glands compounds of cholin much more active than cholin itself and this led me to prepare and study pharmacologically a few cholin esters already known and later, in collaboration with Taveau and Menge to prepare and study a large number of new cholin and analogous compounds (1), (8), (9), (10), (11), (12).

Acetyl-cholin was but one of nearly a hundred compounds (none of which at that time had been studied pharmacologically and most of which had not previously been made) tested by Taveau and myself and my physiological investigations were necessarily of a preliminary nature and I (incorrectly as I now know) attributed the fall of blood pressure resulting from acetyl-cholin largely to an action upon the heart ("negative inotropic action"). I based this preliminary conclusion upon the following experiments: comparatively large doses cause a marked slowing of the heart evidently from a stimulation of vagus endings; concentrations of acetyl-cholin which had a pronounced weakening effect upon the frog's heart had no effect upon the outflow when perfused through the vessels of the frog; with the animals (dogs) and anaesthetics employed, the myocardiograph showed a marked weakening of the auricle and definite inhibitory changes in the ventricle (which will be described later) when amounts of acetyl-cholin having a minimal effect upon the blood pressure and no effect upon the heart rate, were injected; in two or three experiments in which the *hind*leg of the animal was placed in a plethysmograph acetyl-cholin caused sometimes no change in the volume, sometimes a diminution (evidently passive; see figs. 6 and 14, pp. 206 and 215) or a dilatation which occurred after the blood pressure had returned to normal and which might possibly have been attributed to a rise of vena cava pressure or interpreted as "a reaction to diminished tension" (14); in what seemed to be satisfactory perfusion experiments upon three rabbit ears acetyl-cholin either had no effect on the outflow or caused a diminution (vasoconstriction). Similar results have frequently since been obtained but I realize that they are not sufficient to disprove the contention of Dale that vasodilation is a large factor in the fall of blood pressure from acetyl-cholin.

Another fact which inclined me to the view that the fall of blood pressure was of cardiac origin was the observation made by Taveau and myself in 1906 that the fall was prevented by small amounts of atropine. No one had at that time, so far as I am aware, shown that atropine has a paralyzing action upon vasodilator nerves; in fact it had long been a common physiological demonstration that atropine does not paralyze the most frequently studied of these nerves (chorda tympani). And, of course, it has long been known that atropine does not prevent the action of drugs (nitrites, for example) which are believed to act directly upon the muscle of the vessels.

On the other hand, as is well known, atropine does paralyze all of

the endings of the inhibitory nerves to the heart and it abolishes all of the effects of acetyl-cholin upon the heart; there is not, however, as was shown in one of the earlier papers, a strict parallelism between the action of atropine and of various members of the atropine series in paralyzing the cardio-inhibitory nerves and their action in preventing the fall of blood pressure from acetyl-cholin. I had also observed that acetyl-cholin, and especially some of its homologues, has a stimulating action upon other organs innervated by the parasympathetic nervous system (salivary glands, intestines and eye). Hence the hypothesis that the blood pressure lowering action of acetyl-cholin was but a part of its stimulating action upon the parasympathetic nervous system seemed reasonable; and as the only part of this system the stimulation of which could be expected to cause a fall of blood pressure and which is also paralyzed by small doses of atropine is the cardio-inhibitory mechanism, I was led to the view that this was probably the explanation.

It was, however, easy to confirm the work of Dale that acetyl-cholin does have a pronounced vasodilator action, but there were a number of points which seemed worthy of further investigation. Thus the question, through what mechanism does the drug act, was unanswered. There were, as already intimated, good reasons for believing that the vasodilation was not due to any considerable degree to an action upon the endings of known "parasympathetic vasodilators;" the possibility that it was exerted through the posterior root or the so-called "sympathetic" vasodilators remained to be considered. Also the questions arose whether the mechanism through which acetyl-cholin acts (which is by far the most powerful vasodilator reaction known) is involved in the action of other drugs or in that of the depressor and other nerves causing reflex changes in the blood pressure. These and some other questions will be considered in a subsequent communication. It seemed desirable to first determine more fully in what organs, or vascular areas, the acetyl-cholin vasodilation occurs; this is the chief purpose of the present communication.

THE VASCULAR AREAS INVOLVED IN THE VASODILATION CAUSED BY ACETYL-CHOLIN

The only author who has discussed this subject is Dale; reference will be made to his results in connection with individual organs.

Ligation of arteries to various areas. Before considering the action

of acetyl-cholin[2] upon individual organs brief reference may be made to the results of a few experiments in which the effect of acetyl-cholin upon the blood pressure was determined after the elimination of extensive vascular areas by the ligation of their arteries. The results of such experiments were obviously complicated by the fact that the drug reached the remaining tissues in greater concentration after the elimination of large vascular areas. Thus, with rather high dosage, slowing of the heart (action upon the terminations of the cardio-inhibitory nerves) was more marked after the ligation of a number of arteries.

When the abdominal aorta was clamped just below the diaphragm the absolute fall of blood pressure from a given dose of acetyl-cholin injected into the jugular vein was greatly increased; doses which had previously been ineffective became effective (exps. 349, 350). The percentile fall was usually but not always increased also. Such results show that the splanchnic area is not essential for the vasodilator action of acetyl-cholin. The results were different with nitroglycerin: after clamping the abdominal aorta not only the percentile but the absolute fall was less (exp. 350). These experiments suggest that in the fall of blood pressure from acetyl-cholin the splanchnic area is involved to a less extent than it is in the fall of pressure from nitroglycerin.

Ligation of the main vessels to the splanchnic area (the coeliac axis, the superior and inferior mesenteric and, in some cases, the renal arteries) increased both the absolute and usually the percentile fall of blood pressure (exps. 363, 432, 435, 478, 480, 488); the fall from nitroglycerin was diminished (exp. 480). Ligation of the carotids, subclavians and abdominal aorta (just above the iliacs) diminished both the absolute and percentile fall of blood pressure from acetyl-cholin (exp. 432). Such experiments at least suggest that in the fall of blood pressure from acetyl-cholin the "peripheral" blood-vessels (i.e., those of the skin and muscles) are involved to a greater degree than are those of the "splanchnic" area. It may be mentioned incidentall that (confirmatory of Hartman (15)) in some of these experiments doses of epinephrin which had caused a rise of pressure before the splanchnic arteries were ligated had no effect or caused a fall after their ligation; that doses which had caused a fall of pressure caused a rise after ligation of the "peripheral" arteries. On the other hand, at times, with

[2] By "action of acetyl-cholin" I refer in this paper, unless otherwise stated, to the vasodilator action of small doses which is prevented by atropine; the drug has, under certain conditions, vasoconstrictor and other vascular actions also.

large doses of epinephrin ligation of the coeliac axis increased the rise of pressure; this probably resulted from the increased concentration of the epinephrin in the restricted vascular area (exp. 488).

A number of organs were next examined to determine whether vasodilation occurred in them after the administration of acetyl-cholin. The methods consisted in the use of plethysmographs, the recording of the outflow from a vein and the perfusion of isolated organs with Ringer's solution containing acetyl-cholin. Air plethysmographs were used; the recording apparatus was either a tambour or a form of water manometer the distal limb of which was somewhat enlarged and in which was a float moving in a layer of liquid petrolatum, which transmitted the volume changes to a delicate lever. The apparatus was somewhat similar to that recently described by Hoskins, Gunning and Berry (16). A record of the blood pressure was always taken in the plethysmograph and venous outflow experiments; this often helped in determining whether a given change in volume or outflow was passive or was due to active changes in the vascular areas. In many cases, however, the blood pressure record and the examination of a single organ were not sufficient; I have frequently seen a pronounced change in the volume of an organ or in the venous outflow from it without any change in the blood pressure, but when another organ was simultaneously examined it was found that this was undergoing changes the reverse of those in the other organ; that is, the dilatation or constriction in one area was so nearly equalized by the opposite changes elsewhere that the general blood pressure was unchanged. Accordingly two organs were usually examined at the same time as well as the blood pressure; even then, however, the results of an experiment were sometimes difficult to interpret.

The figures for the doses of acetyl-cholin given in the protocols of the experiments are often far from accurate; they are almost invariably far (often many times) too great. Acetyl-cholin undergoes rather rapid deterioration both in solution and in the dry state. As in most of these experiments I was not especially interested in quantitative results I usually used the same preparation for several days although I knew it to have lost much of its activity; control experiments, however, showed that the changes were purely quantitative, not qualitative, in character. Unless otherwise stated the injections were made intravenously, usually into a saphenous vein.

Limbs; skin; muscle. Dale found, by the plethysmograph method, a dilatation of the cat's leg to result from the intravenous injection of

acetyl-cholin; he does not state whether he considered the dilatation to occur in the skin or muscle or both.

Before describing the effects of acetyl-cholin upon the volume of the limbs, as determined by the plethysmograph, a word may be said as to the effect of this drug upon the vena cava pressure; an objection sometimes made to the interpretation of plethysmograph records is

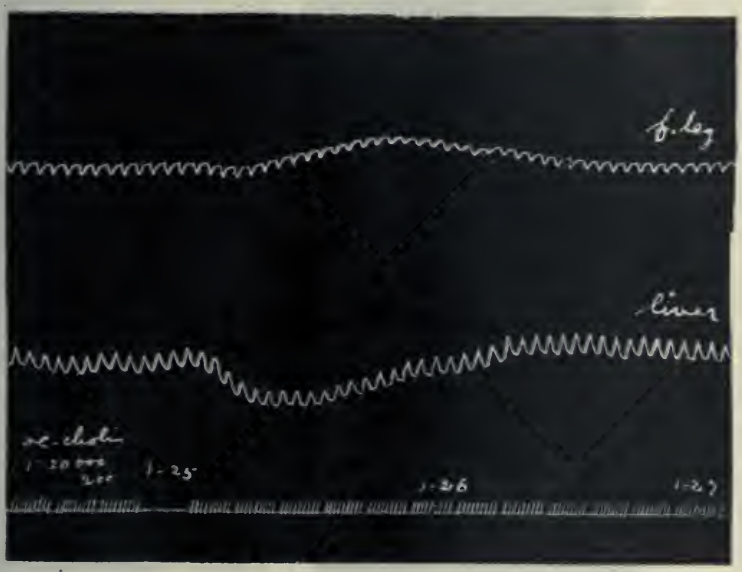

Fig. 2. Experiment 481. Bitch, 6.7 K; morphine; ether; curare; vagi cut. Plethysmograph records from foreleg (above) and left lobe of liver (below). Up = expansion. At *1–25* acetyl-cholin (< 0.1 mg.) injected into saphenous vein. Blood pressure fell 32 mm. Hg.; 0.2 mgm. nitroglycerin caused almost identical effects upon the blood pressure and the volume of the leg and liver; 2 mgm. of atropine sulphate prevented the action of acetyl-cholin but not that of nitroglycerin.

that a rise of vena cava pressure (caused perhaps by an interference with the return of the blood to the heart by some change in the latter) may lead to a passive dilatation of the limbs. However, a number of determinations of the vena cava pressure (made by connecting the central end of a renal vein with a water manometer) showed only a fall when acetyl-cholin was injected intravenously. This fall of vena cava pressure doubtless resulted from an increase in the total vascular area caused by the vasodilator action of acetyl-cholin.

Fig. 3. Experiment 488. Cat, 3.4 K; paraldehyde; vagi cut. Plethysmograph records of foreleg (above) and left lobe of liver (below). Up = expansion. (1) *1–44*, 0.05 mgm. pilocarpine injected into saphenous vein; blood pressure fell 29 mm. Hg. (from 83 to 54). (2) *1–47–*, 0.0025 mgm. acetyl-cholin; blood pressure fell 22 mm. Hg. (71 to 49). When a clamp was placed on the coeliac axis acetyl-cholin caused a greater fall of blood pressure and about the same change in the leg but had no effect on the liver volume. (3) *1–58+*, 0.02 mgm. epinephrin, blood pressure rose 29 mm. (57 to 86).

The cat had an abscess on the nose and had received an overdose of paraldehyde; the blood pressure fell from the beginning.

Fig. 4. Experiment 496. Bitch, 8.2 K; morphine; ether. Plethysmograph records from foreleg; down = expansion. (1) *11–56* + < 0.01 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell from 74 to 52 mm. Hg. (2) *12–02* +, 0.05 mgm. pilocarpine hydrochloride; blood pressure fell from 77 to 56 mm. Hg. (3) *12*–15, 0.1 mgm. nitroglycerin; blood pressure fell from 69 to 48 mm. Hg.

Fig. 5. Experiment 471. Cat, 1.88 K; paraldehyde. Plethysmograph records from nasal cavity (above) and foreleg (below): up = expansion. (1) *11–48*, epinephrin 0.01 mgm. into saphenous vein; blood pressure fell 18 mm. (102 to 84). (2) *12–07*, 0.002 mgm. acetyl-cholin, blood pressure fell from 96 to 48 mm. Hg.; 9 mgm. atropine was now injected. (3) *1–38*+, 5 mgm. acetyl-cholin; blood pressure rose from 44 to 78 mm. Hg. (4) *2–02* 0.0025 mgm. epinephrin; blood pressure rose 23 mm.

I invariably found a dilatation when the foreleg of an animal (cat, rabbit, dog) was placed in a plethysmograph and acetyl-cholin, sufficient to cause a fall of blood pressure, was injected. Illustrations of this effect are shown in figures 2 to 7 and 19 (exps. 481, 488, 496, 471, 477, 479, 468). This result was obtained although the blood pressure might be very low (30 mm. Hg., for example). It was also often obtained with doses of acetyl-cholin sufficiently large to cause slowing of the heart; in this case the dilatation was often preceded by slight (passive) contraction (exp. 419). Acetyl-cholin caused an expansion

Fig. 6. Experiment 477. Bitch 8.2 K; morphine; ether. Plethysmograph on right hindleg (above) and right foreleg (below); up = expansion. At 2–47+, 0.2 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell from 106 to 60 mm. Hg.

of the foreleg in experiments in which stimulation of the depressor caused only a (passive) diminution (exp. 423).

When the dose of acetyl-cholin was small, a small dose of atropine abolished the effect upon the limb volume as well as that upon the blood pressure. After atropine, however, a much larger dose of acetyl-cholin caused the leg to expand; this often occurred when there was little or no fall of blood pressure. With still larger doses (and after much atropine) there was a rise of blood pressure or a fall followed by a rise and usually an expansion of the leg; the latter may have been largely passive, however, for with these large doses the curves were

very similar to those following the injection of epinephrin (fig. 5; exp. 471).

The effect of acetyl-cholin upon the volume of the hind limb was usually less marked than that upon the foreleg (fig. 6, exp. 477). Sometimes there was no change although there was a marked fall of blood pressure; not infrequently there was a diminution (evidently passive) although this might be followed by an expansion (fig. 14, exp. 480); frequently there was an expansion which began only when the blood pressure had returned to normal. Sometimes, however,

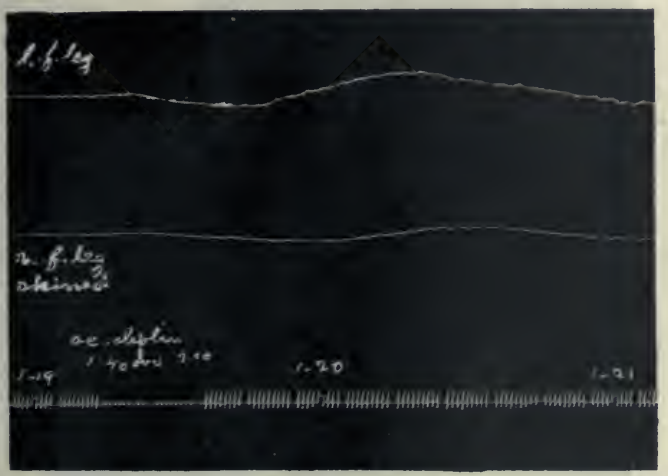

Fig. 7. Experiment 479. Dog, 6.4 K; morphine; ether, vagi cut. Right forepaw removed, leg skinned and in plethysmograph (below). Left foreleg in plethysmograph (above). Up = expansion. *1–19* +, 0.05 mgm. acetyl-cholin into saphenous vein; the blood pressure fell from 106 to 53 mm. Hg. The effects of nitroglycerin were similar.

there was an expansion which began during the fall of blood pressure (fig. 8, exp. 484). When acetyl-cholin was injected, peripherally, into the femoral artery there was a prompt and marked expansion of the limb (fig. 8); a much smaller dose sufficed to cause a given effect when administered in this way than when injected intravenously. There seems to be no reason for supposing that the vessels of the posterior extremity are less sensitive to acetyl-cholin than are those of the anterior extremities; the smaller reaction of the former probably results from the drug reaching them in a more dilute solution and after dilatation has commenced in areas nearer the heart. It is stated that

amyl nitrite has less effect upon the volume of the foot than upon that of the hand; a similar explanation may hold here also. In experiments in which there was a diminution of the volume of the leg the pulse waves often became more prominent, indicating a relaxation of the vessel walls.

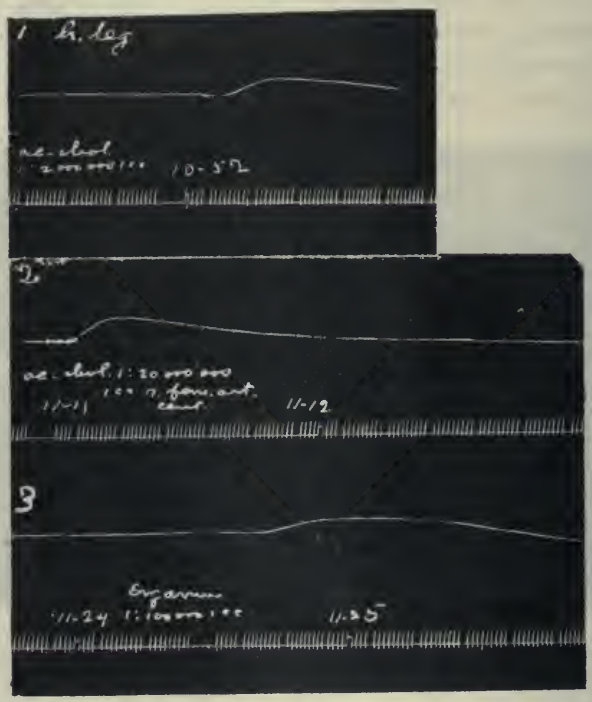

Fig. 8. Experiment 484. Cat, 2.77 K; paraldehyde. Plethysmograph record from left hindleg. Up = expansion. (1) *10–52*, 0.0005 mgm. acetyl-cholin injected into right saphenous vein; blood pressure fell from 133 to 100 mm. Hg. (2) *11–11*, 0.00005 mgm. acetyl-cholin (in 1 cc. normal saline) injected centrally into right femoral artery near its origin; blood pressure fell from 129 to 124 mm. Hg; a control injection of 1 cc. normal saline had no effect upon the volume of the limb. (3) *11–24* +, 0.01 mgm. histamin ("ergamine") intravenously; blood pressure fell from 121 to 84 mm. Hg.

In order to determine whether the dilatation in the limbs resulted chiefly from an effect upon the muscles or upon the skin (and its appendages) the experiment, the results of which are shown in figure 7, (exp. 479), was performed; the changes in the muscle were slight consisting usually of a slight diminution of volume (doubtless passive)

followed by a slight expansion. As the volume of tissue in the two plethsymographs was about equal it would seem that the greater part of the dilatation occurs in the skin or its appendages.

Epinephrin, causing a slight rise followed by a slight fall of blood pressure, caused in this experiment a slight expansion followed by a long continued contraction of the normal leg and only an expansion of the skinned leg; this is in agreement with the results of Hoskins, Gunning and Berry (16). In view of the important deduction these authors drew from their experiments, namely, that epinephrin causes an active dilatation of the vessels of the muscles, attention may be called to the fact that the contraction of the intact limb often continued long after the blood pressure had returned to normal; this seemed also to be the case in the tracing shown in figure 5 of these authors' paper. This continued contraction would seem to be a sufficient explanation of the expansion of the skinned leg which also continued after the blood pressure had returned to normal; hence these authors' statement that "a persistence of the limb expansion after the blood pressure had returned to normal indicates that the dilatation was not a passive process" is not a very strong argument for their view that epinephrin causes an active dilatation of the vessels of the muscles. I infer, however, that these authors obtained an expansion of the skinned leg with depressor doses of epinephrin; it is difficult to see how such a result could be explained except on the basis of active vasodilation in the muscles.

The vasodilator action of acetyl-cholin upon the blood vessels of the limbs (cats) was also shown by recording the drops of blood from veins; the coagulability of the blood was diminished by the injection of hirudin, or the defibrination of the blood and by the use of paraffined cannulas. An illustration of this effect is shown in figure 9 (exp. 483). The increased outflow was most marked toward the end of and after the injection; it often continued for some time after the blood pressure had returned to normal.

The effect of acetyl-cholin upon the outflow from muscle veins was determined in a number of cases (fig. 9); often there was no effect or a very slight, brief increase. Frequently there was a distinct increase for a few seconds, followed by a decrease. In one experiment the outflow from a vein coming from some of the extrinsic muscles of the larynx was determined; there was a marked increase but it was noticed soon afterwards that the animal was making swallowing movements and this may have accounted for the increased outflow.

Although the results of the experiments on the venous outflow from muscles were not entirely conclusive (especially as they were made chiefly upon muscles of the posterior extremities) we certainly seem justified in concluding that dilatation of the vessels of the muscles

can have at most only a minor part in causing the acetyl-cholin fall of blood pressure. The contrast between the constant, prompt and great increase in the outflow from the superficial veins and the, at most, slight and inconstant increase from the muscle veins was very striking.

In a number of these experiments I also tested the effects of epinephrin upon the outflow from superficial and muscle veins: there was a marked decrease in the former and an increase in the latter. The doses injected caused a rise of blood pressure although this was sometimes only a few (e.g., 6) millimeters Hg. This constriction of the superficial vessels was so marked and prolonged that the increased outflow from the muscle could readily be explained as merely a

Fig. 9. Experiment 483. Cat, 3.25 K; paraldehyde; hirudin. Drops of blood from muscle branch of left femoral vein (above) and from superficial vein of left forepaw (below). At *2–12* +, 0.002 mgm. acetyl-cholin injected into right saphenous vein; the blood pressure fell 17 mm. Hg.

passive shifting of the blood from one region to another; in other words, they did not necessarily indicate that epinephrin had a dilator action upon the muscle vessels. In one experiment, with a depressor dose of epinephrin, there was a marked increase in the outflow from the muscle but as the latter was occasionally twitching I did not consider the experiment of value. Hoskins, Gunning, and Berry (16), however, found an increased outflow from muscle veins after depressor doses of epinephrin but it may perhaps be questioned whether it is permissible to draw such far-reaching conclusions as these authors do (that epinephrin "exercises a selective vasodilator effect in skeletal muscle;" that the reduced arterial pressure, from depressor doses of epinephrin, is probably due very largely to the augmented outflow from the vessels of the muscles, etc.) from the type of experiments reported. Moreover, if vasodilation in the muscles is such an important action of epinephrin as these authors consider it to be it seems remarkable that it does not, at times at least, occur to such an extent as to lead to a fall of blood pressure in the rabbit; a depressor action of epinephrin does

not seem to have been obtained in this animal even after the pressor action had been prevented by ergotoxine (17) and the same was found for the fowl and the pig (18). Perhaps these doubts are not well founded but in view of the importance of the subject, involving as it does a vasodilator mechanism in the muscles not generally recognized, all of the evidence should be critically examined. It must be admitted, however, that this is the only probable explanation offered for the reported observations.

I may add that, with possibly one exception, I did not observe diminution in the muscle outflow even with doses of epinephrin which caused a doubling of the blood pressure; Gunning (19) has reported such a diminution from "massive" doses of epinephrin but his doses were apparently much greater than those I used. All of my experiments on venous outflow were performed upon cats, whereas Gunning's were performed upon dogs.

Perfusion of isolated muscles (dog, cat) with Ringer solution containing varying amounts of acetyl-cholin showed in one case a slightly increased outflow; in another, weak solutions had no effect but a relatively strong solution caused a marked diminution in outflow. (In some of these experiments I also tried the effects of epinephrin; like others I obtained no indication of a vasodilator action.)

The increased outflow from the superficial veins of the paw following the injection of acetyl-cholin, described above, may have resulted from a dilatation of the cutaneous vessels or of the vessels of the glands of the paws. Hence the outflow was determined from cutaneous vessels on the abdomen and from similar vessels in the legs after removal of the paws; acetyl-cholin caused a marked increase in the outflow, comparable with that from the paw. Areas of skin from the abdomen of cats and rabbits were perfused, some time after the death of the animals, with Ringer solution; a small dose of acetyl-cholin caused a distinct but not very marked increase in the outflow whereas large doses caused a marked diminution.

The effects of acetyl-cholin upon the vessels of the limbs, skin and muscle may be summarized by saying that this drug causes a great dilatation of the limbs and that the dilatation occurs chiefly in the cutaneous vessels.

Ear. Dale found a vasodilation from acetyl-cholin in a rabbit ear perfused with Ringer solution. I have often obtained the same effect. In the first three experiments I obtained no vasodilation; on looking over the records I find that these experiments were performed as follows: the ears were removed under ether anaesthesia, the cannulas were inserted and the perfusion and the injections of acetyl-cholin begun as quickly as possible. Increasing doses of acetyl-cholin were

injected at short intervals; there was no effect except with large doses, and then only a diminished outflow. In some of the later experiments, performed in exactly the same manner, the first injection of acetyl-cholin caused a marked increase in the outflow, but in others there was at first no effect and small doses of sodium nitrite also had little effect; but after some time the outflow increased spontaneously

Fig. 10. Experiment 494. Drops from perfused rabbit ear; time in 10 seconds and minutes. The amounts of acetyl-cholin were far less than those indicated. (1) *3–21*, < 0.1 mgm. acetyl-cholin injected; (2) *4–40* +, 0.1 mgm. pilocarpine (3) 2 mgm. atropine sulphate injected at *4–54*. (4) *5–17* + same dose of acetyl-cholin as in (1); the outflow had diminished at *5–21*. Between (4) and (5) 6 mgm. atropine injected. (5) *5–53* + same dose of acetyl-cholin as at (1) and (3). Ten times the amount of acetyl-cholin caused a slight acceleration of the outflow; fifty times a slight slowing.

and now acetyl-cholin was very active. It seems probable that in some cases the vessels are in such a condition of tonicity (perhaps as a result of irritation, change of temperature, etc.) that acetyl-cholin and also small amounts of sodium nitrite are unable to overcome it; later, as the spasmodic condition passes off, the vessels readily dilate under the influence of acetyl-cholin. A small dose of atropine sulphate

lessens this tonicity at once so that the vessels respond to acetyl-cholin; larger doses of atropine abolish the dilating action of acetyl-cholin. Figure 10 (exp. 494) illustrates some of these points. Acetyl-cholin was also frequently more active after small amounts of sodium nitrite had been perfused.

As stated in a previous paper acetyl-cholin in relatively extremely large doses causes a constriction of the vessels of the rabbit ear; I have shown above that it has the same action on cutaneous vessels. Dale found large doses of acetyl-cholin to cause a rise of blood pressure after atropine; I had observed the same but had misinterpreted it. Dale found that after a dose of nicotine sufficient to paralyze the ganglia of the involuntary nervous system acetyl-cholin no longer caused a rise of blood pressure; he attributed the rise of pressure observed before nicotine to a nicotine-like action of the acetyl-cholin (an action also shown by cholin itself but which had been overlooked by those who

Fig. 11. Experiment 497. Drops from perfused rabbit ear; time in 10 seconds and minutes. At *7–01*, 20 mgm. nicotine had been injected; this caused a long continued constriction. *7–46*, 5 mgm. acetyl-cholin injected.

had discussed whether the action of cholin was "depressor" or "pressor"). Although the rise of blood pressure from acetyl-cholin, in the intact animal, is doubtless largely due to this nicotine-like action the experiments on the perfused rabbit ear and cutaneous vessels show that acetyl-cholin also acts peripherally (i.e., beyond the ganglia cells) to cause a vasoconstriction. Atropine seems to be without action in either diminishing or intensifying this effect. Figure 11 (exp. 497) is of interest as showing that this peripheral vaso-constrictor action of acetyl-cholin is not prevented by the previous perfusion of nicotine; the antagonism between the action of nicotine and the pressor action of acetyl-cholin does not seem to extend to the peripheral action of the latter. Similar results were obtained after the perfusion of both atropine and nicotine.

The dilator action of acetyl-cholin upon the rabbit ear was also shown by plethysmographic records (fig. 12, exp. 465; fig. 13, exp. 497).

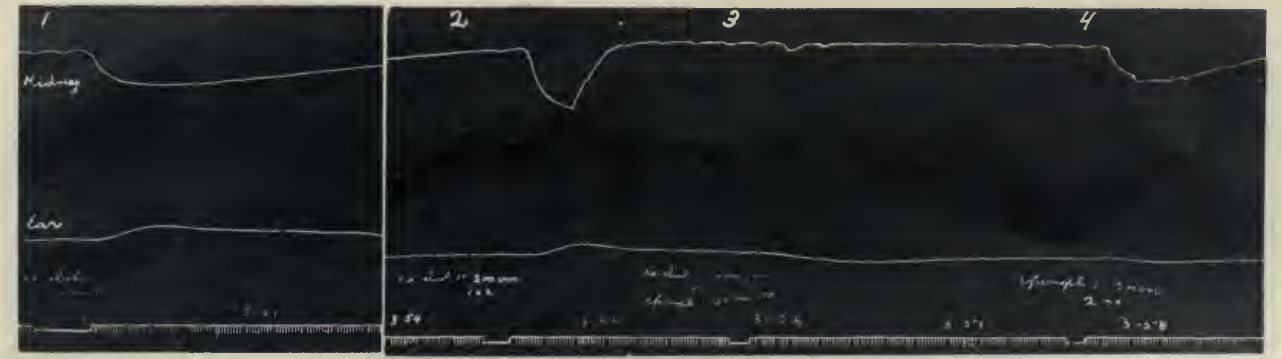

Fig. 12. Experiment 465. Rabbit, 2.05 K; chloral hydrate and ethyl carbamate; vagi cut. Plethysmograph records of left kidney (above) and right ear (below). Up = expansion. Time in seconds, 10 seconds and minutes (1) *2–20*, 0.002 mgm. acetyl-cholin into femoral vein; duration of injection 20 seconds; blood pressure fell 39 mm. Hg. (from 93 to 54). Small dose of atropine now injected. (2) *3–54* +, 0.005 mgm. acetyl-cholin injected; blood pressure fell 51 mm. (3) *3–55* +, 0.01 mgm. acetyl-cholin and 0.02 mgm. epinephrin (mixed) injected; blood pressure fell from 100 to 81 mm., then rose to 112 mm. (4) *3–58* −, 0.01 mgm. epinephrin; blood pressure rose 26 mm.

Fig. 13. Experiment 497. Rabbit, 1.7 K; ethyl carbamate; ether. Plethysmograph record of right ear. Down = expansion. (1) *11–47*, 0.5 mgm. pilocarpine hydrochlor. into saphena vein; blood pressure fell from 102 to 71 mm. Hg.; heart slowed from 42 to 31 in 10 seconds. (2) *11–49* +, < 0.0025 mgm. acetyl-cholin; blood pressure fell from 104 to 74 mm. (3) *1–05*, 0.005 mgm. epinephrin; blood pressure rose 26 mm.

(The usual effect of epinephrin upon the ear was to cause a constriction; fig. 13, exp. 497.)

Penis. Plethysmograph records showed a slight expansion of the penis after acetyl-cholin or a contraction (evidently passive). After ligation of the coeliac axis, the mesenteric and renal arteries, acetyl-cholin caused a pronounced expansion of the penis; this was abolished by atropine (fig. 14, exp. 480).

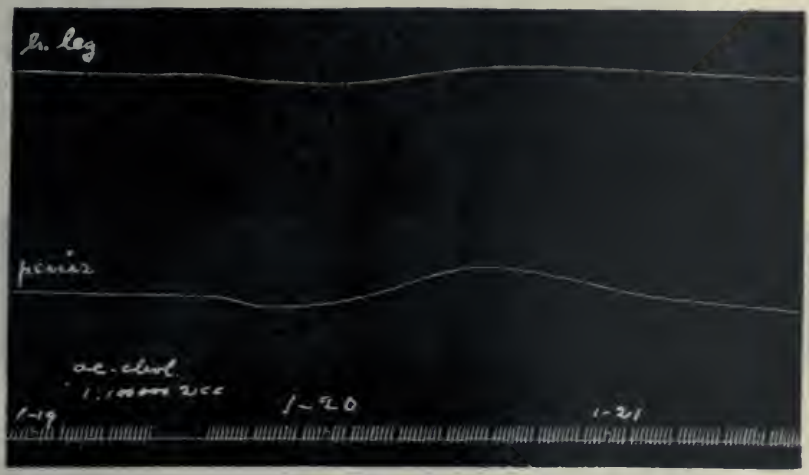

Fig. 14. Experiment 480. Dog, 7.1 K; morphine; ether. The coeliac axis, the renal and mesenteric arteries had been ligated. Plethysmograph records of hindleg (above) and penis (below). Up = expansion. *1–19* +, 0.02 mgm. acetyl-cholin into vein of forepaw; blood pressure fell from 104 to 46 mm. Hg. After 2 mgm. atropine sulphate 0.5 mgm. acetyl-cholin had no effect; nitroglycerin caused a slight expansion of leg and penis; a small amount of epinephrin caused a contraction of the leg and a very slight contraction of the penis; a larger amount caused a marked contraction of the leg and a slight (passive) expansion of the penis.

Submaxillary gland. Acetyl-cholin, injected intravenously, caused a brief increase in the outflow of blood from the submaxillary gland (fig. 15, exp. 504); a more prolonged acceleration of the outflow could be obtained by a slow intravenous infusion or by applying a drop of a rather strong solution (e.g., 1:1000) to a muscle. The vasodilator action of acetyl-cholin upon the vessels of the submaxillary gland was abolished with great ease by atropine. Thus (exp. 461), 0.0002 mgm. acetyl-cholin caused a pronounced but brief increase in the outflow

and a marked fall of blood pressure; after 0.05 mgm. atropine sulphate, 0.001 mgm. acetyl-cholin had no effect on the outflow but caused a slight fall of blood pressure. After 2 mgm. of atropine 0.1 mgm. of acetyl-cholin caused a slight fall of blood pressure but had no effect on the venous outflow. After a further injection of 5 mgm. atropine, 2 mgm. acetyl-cholin caused a very slight fall of blood pressure and a very slight diminution of the outflow. In this and similar experiments stimulation of the chorda tympani continued to cause a marked increase in the venous outflow. No secretion of saliva (as observed in a cannula in the gland duct) resulted from these rapid intravenous injections of acetyl-cholin. In one experiment the venous outflow from the submaxillary gland was reduced by about one-half by stimulation of the cervical sympathetic; during the stimulation acetyl-cholin was

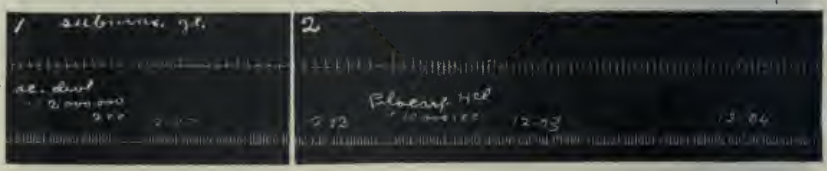

Fig. 15. Experiment 504. Cat, 3.2 K; ethyl carbamate; hirudin. Drops of blood from vein of submaxillary gland. Time in seconds, 10 seconds and minutes. (1) *12*, < 0.001 mgm. (old preparation) of acetyl-cholin into saphenous vein; blood pressure fell from 100 to 59 mm. Hg. (2) *12–02+*, 0.1 mgm. pilocarpine; blood pressure fell from 90 to 52 mm. The fall of blood pressure from the pilocarpine was slightly more prolonged than that from the acetyl-cholin.

injected intravenously and the outflow increased to almost normal for about ten seconds.

Thyroid. The outflow from a vein on the anterior surface of the trachea (cat), formed chiefly by the union of veins from the thyroid, was slightly diminished (passively?) by doses of acetyl-cholin causing a moderate fall of blood pressure. Applied, on filter paper, to the surface of the thyroid there was, according to the dose a slightly increased outflow or a diminished outflow (vasoconstriction). (Both pressor and depressor doses of epinephrin caused a marked diminution of the outflow—confirmatory of Gunning (20); that the diminished outflow from depressor doses was not entirely passive was indicated by the fact that the diminished outflow continued longer than the fall of blood pressure.)

Nasal mucosa. The nasal mucosa, examined by the plethysmograph method Tschalussow (Chalussov) (21), uniformly showed a contraction after the injection of acetyl-cholin although this was often preceded by an expansion (fig. 5, exp. 471; fig. 16, exp. 469). With

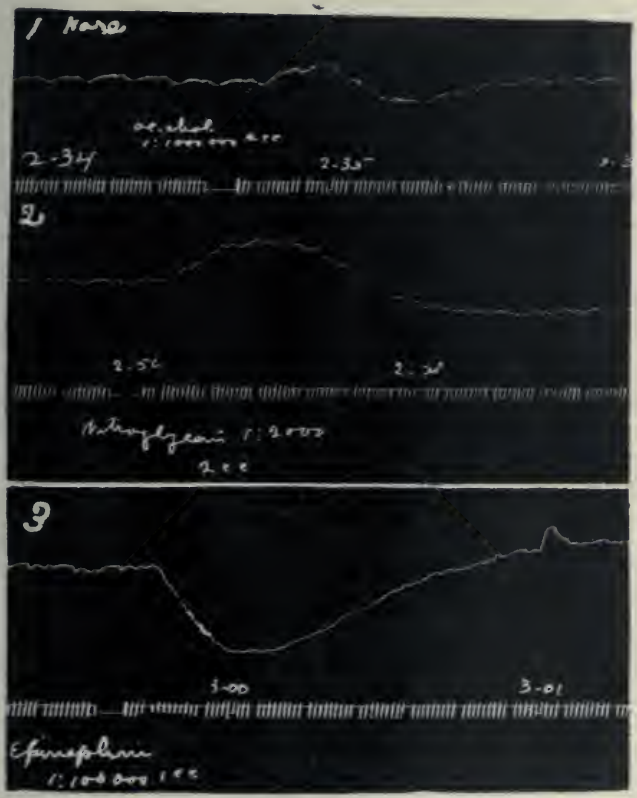

Fig. 16. Experiment 469. Dog, 5.84 K; morphine; chlorbutanol; vagi cut. Plethysmograph records from nose. Up = expansion. (1) *2–34+*. 0.002 mgm. acetyl-cholin into right saphenous vein; blood pressure fell 23 mm. (2) *2–50*, 1 mgm. nitroglycerin; blood pressure fell 26 mm. (3) *3–00–*, 0.01 mgm. epinephrin; blood pressure rose 39 mm. Control injections of normal saline had no effect.

very small doses of acetyl-cholin (but sufficient to cause a pronounced fall of blood pressure) I consider this diminution of the volume of the nasal mucosa to be a passive effect for it is prevented by atropine; atropine does not prevent, but makes more prominent, the vaso-constrictor

(nicotine-like according to Dale) action of acetyl-cholin. The primary expansion which frequently occurs is due to vasodilation; the pulse waves are usually more distinct (fig. 16).

The difficulty of interpreting with certainty the action of a drug like acetyl-cholin (and the same is true of epinephrin) which has both a vasodilator and a vasoconstrictor action (according to dose, the organ studied and other factors) is well illustrated by the experiment (471) in which the tracings shown in figure 5 were obtained. Figure 5 shows that 0.002 mgm. acetyl-cholin caused a distinct diminution of the volume of the nasal mucosa and a slight increase in the volume of the foreleg; the blood pressure fell 38 mm. Hg. (from 96 to 58 mm.). After 0.5 mgm. atropine sulphate this amount of acetyl-cholin did not have the slightest effect upon the blood pressure or the nasal mucosa or the leg. But 0.1 mgm. acetyl-cholin caused a fall of blood pressure of 12 mm. Hg. a greater diminution in the volume of the nasal mucosa and no change in the volume of the leg (or the heart rate). The diminution in this case was probably due in part to the beginning vasoconstrictor ("nicotine-like") action of the acetyl-cholin (for the fall of blood pressure was less and the contraction of the mucosa greater than above; the fall of blood pressure was probably due to a vasodilatation occurring elsewhere than in the leg. After the further injection of 3.5 mgm. atropine 0.1 mgm. acetyl-cholin had no effect on the blood pressure but there was a diminution of the volume of the nasal mucosa and an expansion of the leg. Standing alone it would be impossible to interpret this result (and similar problems arise with epinephrin); thus it might be supposed *a*, that there was an active constriction in the nasal mucosa and an active dilatation in the leg; or *b*, that there was an active contraction in the mucosa and elsewhere, with a passive expansion of the leg; or *c*, that there was an active dilatation in the leg with a passive contraction in the mucosa. Taken in connection with the results preceding and following this injection, however, the second seems to be the more probable explanation. The further injection of atropine had no effect upon the changes caused by the above dose of acetyl-cholin but larger doses of acetyl-cholin caused a rise of blood pressure, a greater contraction of the mucosa and a greater expansion of the leg. The tracing at *1—38+* in figure 5 shows the effect of 5 mgm. of acetyl-cholin; the blood pressure rose 34 mm. Hg. In this case there seemed to be an active constriction in the nasal mucosa, and doubtless in other organs, which led to the rise of blood pressure and a passive expansion of the leg.

Small doses of epinephrin caused in this experiment a fall of blood pressure, a marked contraction of the nasal mucosa (fig. 5) and no change in the volume of the leg. Standing alone the contraction of the mucosa might be interpreted as a passive result of the fall of blood pressure. With doses causing a rise followed by an equal fall of pressure there was a greater contraction of the mucosa and a slight expansion of the leg. With a purely pressor dose of epinephrin curves almost identical with those from pressor doses of acetyl-cholin were obtained, although the rise of pressure was less after epinephrin (fig. 5). The expansion of the leg might easily have been interpreted as due to an active vasodilatation but considered in connection with the other results it was almost certainly passive, the change in the mucosa being due to an active vasoconstriction.

In this experiment a dose of nitroglycerin causing the same fall of blood-pressure as a dose of acetyl-cholin, caused about the same change in the leg volume but had less effect upon the nasal mucosa.

In an experiment upon a dog (fig. 16, exp. 469), acetyl-cholin caused a slight contraction of the mucosa or a slight expansion followed by a contraction or a slight contraction followed by a longer but slight expansion; nitroglycerin in doses causing an equal or less fall of blood pressure uniformly caused a marked expansion of the nasal mucosa followed by a prolonged (probably passive) contraction. A pressor dose of epinephrin caused a marked contraction followed by a long-continued expansion.

The above experiments indicate that depressor doses of acetyl-cholin have relatively little effect upon the nasal mucosa although in the dog there was at times a distinct dilatation, but this was usually obscured by a passive contraction due to the fall of blood pressure resulting from a greater dilatation elsewhere; the marked tendency of the nasal mucosa to respond passively to changes in blood pressure was emphazised by Fofanow and Tschalussow (Fofanov and Chalussov) (22). These experiments also indicate that the nasal mucosa is relatively less sensitive to acetyl-cholin than it is to nitroglycerin or epinephrin.

Intestines. Dale, using a plethysmograph, found an expansion of the intestine (cat) after acetyl-cholin. My experiments were performed upon rabbits and a dog; they were not very satisfactory. I am not certain that the results were not complicated by contractions of the intestine although Dale found that it required a relatively very large dose of acetyl-cholin, given intravenously, to affect the intestinal movements even for a brief period. In the rabbit there was sometimes a slight contraction, sometimes a contraction followed by a more prolonged expansion (fig. 17, exp. 456). The latter type of curve was also obtained in a dog (fig. 18, exp. 469). (The usual effect of epinephrin in pressor doses in these experiments was to cause an expansion of the intestine; whether this was passive or active I do not know.)

In the only experiment in which the outflow from a vein from the small intestine (cat) was determined, acetyl-cholin caused a diminished outflow (probably passive) with a pronounced fall of blood pressure.

A large loop of the isolated small intestine of a rabbit was perfused with Ringer solution; acetyl-cholin caused intestinal movements with a diminution of the outflow After atropine the same (small) doses of acetyl-cholin had no effect either upon the movements or outflow. (Sodium nitrite caused an increased outflow.)

Spleen. The effect of acetyl-cholin upon the volume of the spleen, as determined by the plethysmograph, was somewhat variable and sometimes puzzling on account of the development of rhythmical

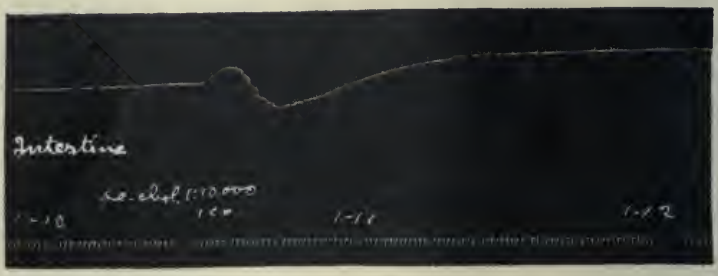

Fig. 17. Experiment 456. Rabbit, 1.74 K; paraldehyde. Plethysmograph record of loop of small intestine. Up=expansion; 0.5 mgm. atropine sulphate had been given; this had greatly diminished the action of acetyl-cholin upon the blood pressure. At *1–10*+, 0.1 mgm. acetyl-cholin into femoral vein; blood pressure rose, briefly, 8 mm. (probably mechanical effect of injection) and then fell 21 mm. The fall of blood pressure was prolonged, but not as prolonged as the expansion of the intestine.

Fig. 18. Experiment 469. Dog, 5.84 K; morphine; chlorbutanol. Plethysmograph record of loop of small intestine. Up = expansion. *12–42*+, 0.02 mgm. acetyl-cholin into saphenous vein; blood pressure fell 23 mm. Hg.

changes in the volume of the spleen. Sometimes there was a diminution of the volume, apparently passive, during the fall of blood pressure, or a diminution followed by an expansion (fig. 19, exp. 468);

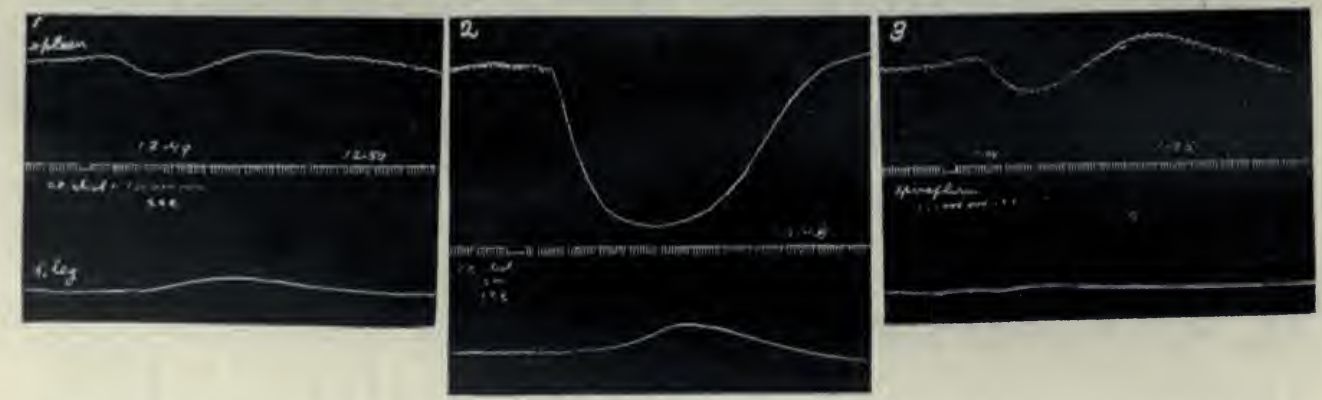

Fig. 19. Experiment 468. Cat, 2.4 K; paraldehyde; vagi cut. Plethysmograph record of spleen (above) and right foreleg (below). Up = expansion. (1) *12–49–*, 0.00002 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell 13 mm. Hg. (2) *2–44+*, 5 mgm. acetyl-cholin; blood pressure fell 6 mm., then rose 13 mm. Before this injection a total of 19.5 atropine had been injected. (3) *1–04–*, 0.001 mgm. epinephrin; blood pressure rose 7 mm.

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expansion was often followed by two or more extensive rhythmical variations in volume during which the volume of the spleen was greater than before the injection. I am inclined to think that the records can be interpreted as showing an active vasodilation in the spleen after acetyl-cholin; during the diminution of the volume the vessels may have relaxed but the organ diminished in size as a result of greater dilatation elsewhere (in the skin of the legs, for example).

The experiment (468) in which the tracing in figure 19 was obtained presented many of the problems as regards both acetyl-cholin and epinephrin which were discussed in connection with the experiments on the volume changes in the nasal mucosa. As figure 19 shows, 0.00005 mgm. of acetyl-cholin caused a contraction, followed by a slight expansion, of the spleen and an expansion of the foreleg; the blood pressure fell 13 mm. All of these effects from small doses of acetyl-cholin were completely prevented by 1 mgm. of atropine; 0.01 mgm. acetyl-cholin, however, caused a slight contraction followed by a distinct expansion (complicated, however, by extensive rhythmical changes) of the spleen and a marked expansion of the leg; the blood pressure fell 15 mm. Hg. After 2 mgm. of atropine the above dose of acetyl-cholin (0.01 mgm.), and also 0.1 mgm., had none of these effects but 1 mgm. of acetyl-cholin caused the blood pressure to fall 23 mm., the spleen to contract to a far greater extent than it had previously, although in some cases the fall of blood pressure had been greater (hence the contraction now was probably in part active) and the leg expanded markedly. After a further injection of atropine, acetyl-cholin had either no effect upon the blood pressure or caused an insignificant fall followed by a rise or only a rise; that is, the dilator action of acetyl-cholin had been paralyzed. Doses, however, which caused no change in the blood pressure continued to cause a contraction of the spleen and a dilatation of the leg. With larger (essentially pressor) doses of acetyl-cholin there was a very great contraction of the spleen (evidently not passive, for the blood pressure rose) and a marked expansion of the leg (perhaps passive) (fig. 19).

Small, but weakly pressor doses of epinephrin caused a marked contraction of the spleen usually followed by an equally marked dilatation and a slight dilatation of the leg (fig. 19); similar changes in the spleen were described by Hoskins and Gunning (23) and by Hartman and McPhedran (24). I was not certain whether this after-dilatation of the spleen was to be considered active or passive; it may be that the spleen, on account of its relative proximity to the heart, responds first with an active constriction and then, as other more distant structures are constricted, passively dilates. As the dose of epinephrin was increased only a marked contraction of the spleen and a correspondingly greater dilatation of the leg was obtained; such a result was obtained with doses of epinephrin causing a rise of blood pressure of only 10 mm. Hg. In this case the dilatation of the leg was probably only passive although the volume curve of the leg considered alone, or with the blood pressure curve, might easily have been interpreted as showing an active dilatation of the leg.

Any doubt as to the ability of acetyl-cholin to cause a dilatation of the vessels of the spleen was removed by perfusing the isolated organ with Ringer solution containing the drug: small amounts caused a greater and more prolonged increase in the outflow than occurred in similar experiments on the rabbit ear. The relation to atropine was similar; the latter of itself in small doses caused a marked increase in the outflow and also rendered the vessels more sensitive to acetyl-cholin. After large doses of atropine, acetyl-cholin had no dilator effect. Large doses of acetyl-cholin caused a marked diminution of the outflow. The records of these experiments are so similar to those obtained with the rabbit ear (see figs. 10 and 11) that it is not necessary to reproduce them; the chief difference was that the changes were of longer duration in the case of the spleen. Epinephrin greatly diminished, sodium nitrite increased, the outflow from the spleen.

Liver. Changes in the volume of the left lobe of the liver, after acetyl-cholin, were determined by the plethysmograph method in one dog and two cats. In all cases there was first a diminution in volume (fig. 3, exp. 488; fig. 2, exp. 481; fig. 20, exp. 486). With large doses the contraction was followed by an expansion in the dog. After atropine large (pressor) doses of acetyl-cholin caused only an expansion. After incomplete atropinization comparatively large doses of acetyl-cholin (e.g., 2 mgm.) caused a slight fall followed by a slight rise of blood pressure and a contraction followed by an expansion of the liver (fig. 20).

The diminution in liver volume from small doses of acetyl-cholin was probably passive due to the lowered blood pressure. It apparently resulted from a diminution of the blood supply to the liver through the hepatic artery for it did not occur when this was clamped. Similarly the expansion of the liver from pressor doses of acetyl-cholin after atropine was probably in part at least passive.

The only indication of an active dilatation of the liver observed was the expansion, following a contraction, often seen in the dog after a comparatively large (but still purely depressor) dose of acetyl-cholin (fig. 2); this result was similar to that usually seen in the volume changes of the intestine of the dog. For reasons given below it scarcely seems probable that these changes in the liver volume result from changes in the portal vessels. Hence they may be taken as an indication that acetyl-cholin has a dilator action upon the terminations of the hepatic artery in the liver but that the first effect upon

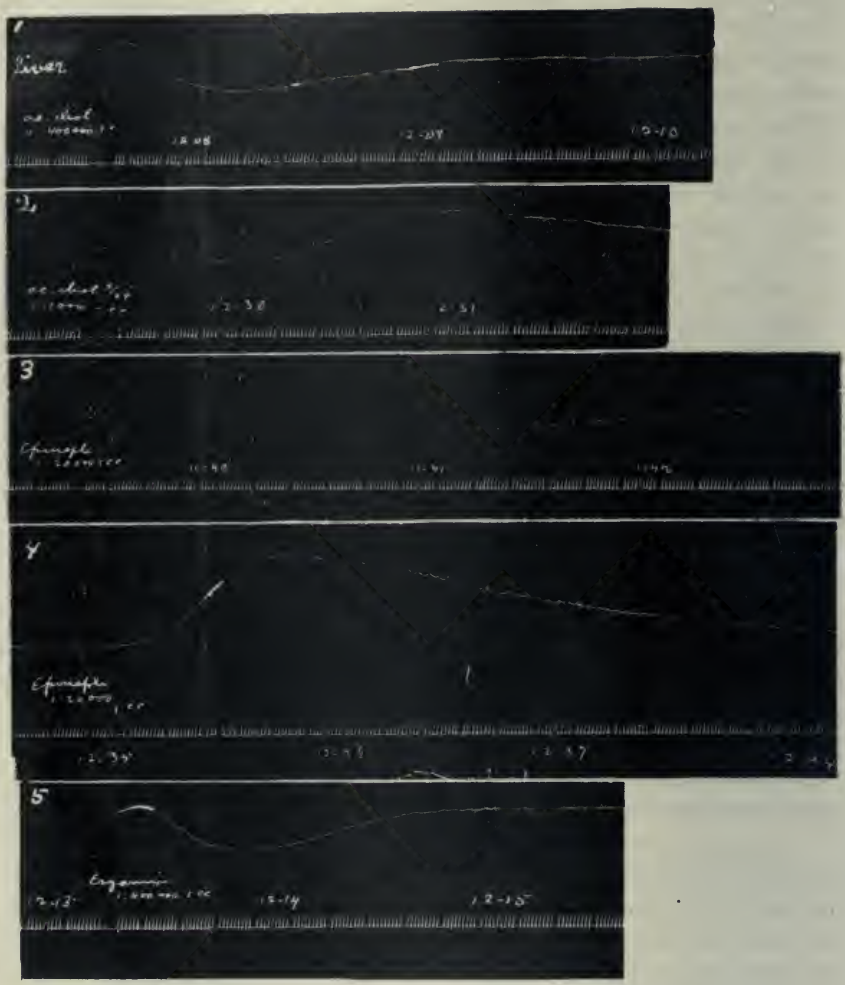

Fig. 20. Experiment 486. Cat, 2.26 K; paraldehyde. Plethysmograph record of left lobe of liver. Up = expansion. (1) *12–07*+, 0.0025 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell 24 mm. Hg. *12–17*, 2 mgm. atropine injected. (2) *12–29*+, 2 mgm. acetyl-cholin; blood pressure fell 9, then rose 9 mm. Hg. (3) *11–39*+, epinephrin 0.05 mgm; blood pressure rose 56 mm. (4) *12–35*, epinephrin 0.05 mgm.; blood pressure rose 68 mm. (5) *12–13*, histamin ("ergamine") 0.0025 mgm., blood pressure fell 22 mm.

the volume of the liver is a diminution due to a greater vasodilation elsewhere.

The results of the injection of epinephrin upon the liver volume in these experiments are of some interest. Edmunds (25) found the usual effect upon the liver of epinephrin (in pressor doses) to be a diminution of the volume in the case of the dog; in rare cases there was an expansion. The latter result, an expansion, was the usual response in cats; the expansion he attributed to an increased efficiency of the heart. Mautner and Pick (26) report an active contraction of the liver from epinephrin in the dog and cat. In my experiment upon a dog and in one of the experiments on cats, epinephrin (in pressor doses) caused at first only a marked contraction; the latter was as pronounced in the cat (fig. 20) as in the dog. Peptone was injected into the dog; this caused a marked expansion of the liver and a fall of blood pressure which, however, later returned to normal. After the peptone injection epinephrin caused only an expansion of the liver. Peptone was also injected into the cat; there was a contraction of the liver and a fall of blood pressure; the latter remained low. Epinephrin continued to cause a contraction of the liver but after 2 mgm. atropine sulphate, which caused a further fall of blood pressure, epinephrin caused only an expansion (fig. 20). In the second experiment upon a cat the blood pressure fell from the beginning, perhaps from an overdose of paraldehyde; epinephrin caused only an expansion of the liver (fig. 3; exp. 488).

Thus in the experiment on a dog and in one of the experiments on cats the primary effect of epinephrin was to cause a contraction of the liver. Later, after various insults, the liver responded with only an expansion; animals at this time may be considered to have been in a condition somewhat analogous to experimental shock, although the blood pressure in the dog was as high as at the beginning (about 100 mm.) when epinephrin caused only a contraction of the liver. The second cat was in a condition analogous to shock from the beginning. The results in these three experiments were so striking as to suggest the thought: May not one of the features of experimental "shock" be a change in the blood vessels of the liver such that they can no longer respond with a contraction to epinephrin or rather, perhaps, that they are no longer able to offer a resistance to a rise of blood pressure? The expansion of the liver after epinephrin seemed to be passive; it was accompanied by a marked contraction of the leg, and was, in the only experiment (cat) in which it was tested, prevented by clamping the hepatic artery. In the latter case there was not a late expansion of the liver described by Edmunds when the hepatic artery was clamped and ascribed by him to a rise of vena cava pressure.

As stated above there seems no reason for believing that the change in the liver after acetyl-cholin are due to an effect upon the terminations of the portal vein. Another reason for doubting such an effect was the failure to obtain more than at most an extremely slight increase in the outflow from the liver (guinea pig) when this was perfused through the portal vein with Ringer solution containing various amounts of acetyl-cholin; higher concentration caused a diminution of the

outflow but the vasoconstrictor action of large doses of acetyl-cholin seemed to be less marked in the liver than in most of the organs studied.

Epinephrin in this experiment caused only a moderate diminution in the outflow: this confirmatory of Mautner and Pick (26); the vasoconstrictor action was far less marked than in the case of most organs examined. Atropine caused a very slight increase; sodium nitrite, in relatively large doses, caused a considerable increase, followed in one case by a diminution.

In the only experiment (cat) in which it was tested the portal pressure fell with doses of acetyl-cholin causing a marked fall of arterial pressure. This was probably a passive effect for although there is evidence that acetyl-cholin has a dilator action upon the vessels of the intestine this does not seem to be sufficiently great (in some cases at least), in comparison with this action in other areas, to lead to an increased outflow from the intestine and so to a rise of portal pressure; as was stated above there was a diminished outflow from a mesenteric vein in the only case in which this was determined. (In the experiment in which the portal pressure fell after acetyl-cholin, epinephrin caused a slow but pronounced rise.)

Injection of acetyl-cholin into a mesenteric vein. I have assumed in the above that even if acetyl-cholin has a pronounced dilator action upon the terminations of the portal vein this would not be in evidence when the drug is injected into a systemic vein. I have assumed that there would be a considerable destruction of the compound during its relatively slow passage through the vessels of the intestine and that it would reach the liver in too great a dilution to affect this organ. I have performed no experiments (Eck fistula, for example) to test this assumption. The following experiments, however, may be of interest in this connection as showing the effect of passing the drug through the liver. Small doses of acetyl-cholin which, when injected into a systemic vein caused a pronounced fall of blood pressure, had not the slightest effect when injected into a mesenteric vein. In an experiment (cat) in which comparisons were made it was found that to cause an equal fall of blood pressure fully one hundred times as much acetyl-cholin had to be injected into a mesenteric as into a saphena vein. Thus 0.01 mgm. injected into a mesenteric vein caused the blood pressure to fall 31 mm.; 0.0001 mgm. into a saphena vein caused a fall of 58 mm. (Similar but less marked differences were observed in connection with nitroglycerin and, in different experiments, with pressor and depressor doses of epinephrin; but in one case in which a depressor dose of epinephrin was injected the fall of pressure

was greater when it was injected into a mesenteric vein and in another case as the dose was gradually increased from a depressor to a pressor dose the change occurred first from the mesenteric injection, i.e., the same dose injected into the mesenteric vein caused a rise of pressure and when injected into the saphena vein caused a fall.)

Experiments upon the absorption, as judged by the effect upon the blood pressure, of acetyl-cholin when applied to the surface of various organs are of interest in this connection. The experiments were made by applying small squares of filter paper moistened with solutions of acetyl-cholin to various organs; it was determined beforehand how much, by weight, the pieces of paper absorbed. I made no effort to determine how much acetyl-cholin actually reached the circulation. In one experiment when squares of paper moistened with approximately 4 mgm. of a 1 per cent solution of acetyl-cholin were applied for thirty to forty seconds to the surface of various organs, the following results were obtained:

Liver: blood pressure fell 62 mm. Hg. (from 144 to 82).
Stomach: no effect.
Adrenal: blood pressure fell 76 mm. Hg. (from 139 to 63).
Small intestine: (4 and 8 mgm.) no effect.
Liver: blood pressure fell 72 mm. Hg. (from 146 to 74).
Kidney: blood pressure fell 60 mm. Hg. (from 143 to 83).
Spleen: no effect.

Thus the acetyl-cholin was either very imperfectly absorbed from the surface of the small intestine, spleen and stomach or, what is more probable, it was absorbed and rendered inert in its passage through the liver. Acetyl-cholin was absorbed with great ease from the surface of the lung; this is a very convenient method of obtaining a prolonged and fairly uniform lowering of the blood pressure. A small amount, e.g., 0.002 mgm., injected into the trachea also caused a prolonged fall of pressure. Absorption occurred, but to a lesser extent as judged from the effect on the blood pressure, when acetyl-cholin was applied to the conjunctiva and the nasal mucosa; it was very slight from the prepuce. It was well absorbed from the surface of a muscle. Acetyl-cholin injected peripherally into the artery of a limb caused a fairly marked fall of blood pressure: thus 0.0005 mgm. injected peripherally into the femoral artery of a cat caused the arterial (carotid) pressure to fall 20 mm.; the same amount injected into a saphena vein caused the pressure to fall 33 mm.

Kidney. Acetyl-cholin caused only a diminution of the kidney volume as determined by the plethysmograph (fig. 12, exp. 465); this was prevented by a dose of atropine which prevented a fall of blood pressure. The contraction seemed to be a passive effect. The only indication of a dilator action I observed in these experiments is shown in figure 12: when sufficient epinephrin was added to the acetyl-cholin to greatly diminish the fall of blood pressure there was no distinct effect upon the kidney although a smaller (pressor) dose of epinephrin caused a marked contraction of the kidney. A contraction of the kidney would have been expected from the acetyl-cholin and epinephrin mixture if the acetyl-cholin did not have a dilator or antagonistic action to the epinephrin; the fact that a contraction did not occur suggests that the acetyl-cholin prevented in some way the constrictor action of the epinephrin. Perfused through the isolated kidney (rabbit) acetyl-cholin caused a slight increase in the outflow; this was very small in comparison with the effects of sodium nitrite. (The usual effect of epinephrin in pressor doses was, in these experiments, to cause a contraction of the kidney volume; with very large doses, causing a great rise of blood pressure, the contraction was frequently followed by an expansion, evidently passive.)

Lung. A lobe of a lung (cat, rabbit or guinea pig) was perfused with Ringer solution containing acetyl-cholin; in no case was there a distinct increase in the outflow. There was, on the contrary, with large doses, in all three cases a great and very prolonged decrease in the outflow; in some cases the outflow did not return to normal until after thirty minutes. Atropine (at least in the comparatively large doses employed) did not prevent this action.

Atropine itself, which so often causes an increased outflow from perfused organs, had no effect. Sodium nitrite caused in the experiments with the rabbit and guinea pig lungs (it was not tried with the cat lung) first a brief increase (50 to 100 per cent) in the outflow followed by a great and very prolonged diminution. It was sometimes an hour before the outflow returned to the previous rate; the result was strikingly like the vasoconstriction often seen in the perfusion of other organs with epinephrin. The rabbit lung showed the same reactions twenty hours after the first injection. Macht (27) has shown that the nitrites cause a contraction of strips of the pulmonary artery and he cites earlier experimental and clinical work which indicated that these bodies constrict the pulmonary vessels *in vivo;* I do not know of any previous perfusion experiments on this subject.

SUMMARY

It has been shown in the above that acetyl-cholin has an intense vasodilator action on the vessels of the skin and of the ear; the action on the skeletal muscles is slight. It dilates the vessels of the penis, of the submaxillary gland and of the spleen; it seems also to dilate the vessels of the intestines and liver. Only slight evidence of a dilator action was found in the case of the kidney and none in that of the lung. The nasal mucosa seemed relatively less sensitive to the vasodilator action of acetyl-cholin than many other vascular areas. The vasodilation in all of these cases was diminished or prevented by atropine.

As little as 0.000,000,002,4 mgm. acetyl-cholin per K caused a pronounced fall of blood pressure.

Acetyl-cholin injected into the trachea or applied to the surface of the lung, kidney, liver, adrenal and various muscles was very active in causing a fall of blood pressure; similar doses applied to the surface of the stomach, spleen and small intestine had no effect on the blood pressure.

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VASODILATOR REACTIONS. II

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Received for publication December 7, 1917

1. THE RELATION OF THE ACETYL-CHOLIN VASODILATOR REACTION TO VASOMOTOR NERVES

It has been shown by the work of Dale (1) and also by that reported in the preceding paper that acetyl-cholin causes vasodilation in many organs and that this action is prevented by atropine. It is usually assumed that a drug, the effect of which is prevented by atropine, acts upon "nerve endings" or upon "receptive substances" in connection with nerve endings; moreover, such an antagonism is often interpreted as showing the presence in an organ of a parasympathetic nerve innervation. Hence it was of interest to see if this vasodilator reaction could be correlated with the action of any of the nerves to which vasodilator actions have been attributed. "Vasodilator nerves" have been described for many organs; but in nearly every case their presence has been questioned. In fact the existence of "vasodilator" nerves (in the usual sense in which the term is employed) often has been the subject of discussion (2), (3), (4). Further, some authors believe that certain "vasodilator nerves" should be classed with one group of nerves, others that they should be placed in a different category. For present purposes we may consider the following groups of alleged vasodilators and see what evidence there is that they are involved in the action of acetyl-cholin: (a) Parasympathetic vasodilators, (b) Posterior root vasodilators, (c) Sympathetic vasodilators.

a. Parasympathetic vasodilators. The chorda tympani, stimulation of which causes a dilatation of the vessels of submaxillary gland, and the pelvic nerve, the stimulation of which causes, among other effects, dilatation of the vessels of the penis, have long been considered typical examples of parasympathetic vasodilators. It has been shown in the preceding paper that acetyl-cholin causes vasodilation in the submaxillary gland and in the penis; does it act upon the "nerve-endings"

of the parasympathetic nerves? That this is not the case (at least in the conventional sense) is shown by the different effects of atropine in the two cases: the action of acetyl-cholin is easily prevented by atropine; that of the stimulation of the nerves is not. Thus it was shown in the preceding paper that a small dose of atropine (2 mgm., for example) prevented the marked dilator effect of acetyl-cholin upon the penis; Langley and Anderson (5), Piotrowski (6) (and others cited by Piotrowski) found that atropine (even up to 60 mgm.) did not prevent the vasodilator action of the pelvic nerve.

As regards the chorda tympani: I found, for example, that 0.05 mgm. atropine prevented the vasodilator action of acetyl-cholin upon the submaxillary gland, whereas stimulation of the chorda continued to cause a marked vasodilation after more than 5 mgm. atropine. It has long been a common physiological demonstration that atropine while "paralyzing the secretory fibers" of the chorda tympani does not paralyze its vasodilator fibers (7). It has however been shown (8) (and I have confirmed the results) that atropine does diminish the "vasodilator" action of the chorda tympani and some, Barcroft for example, are inclined to hold that the vasodilation is the result of the secretory processes caused by the stimulation of the nerve. (See (2), (3), (4)). Of course, an interpretation could be placed upon my results with acetyl-cholin, similar to that given by Henderson and Loewi (8) for pilocarpine, namely, that the vasodilation from this was simply the result of increased secretory activity; but similar relations between atropine and acetyl-cholin hold for organs in which an analogous explanation can scarcely be offered. Since, when comparable degrees of vasodilation are caused on the one hand by acetyl-cholin and on the other by stimulation of the chorda tympani, the action of the latter is not perceptibly impaired by an amount of atropine a hundred times greater than that which suffices to prevent the action of the former it seems simpler to suppose that the vasodilation caused by acetyl-cholin is not the result of a stimulation of the chorda "nerve-endings."

It has also been shown that the vasodilators to the tongue are not paralyzed by atropine (6).

b. Posterior root dilators. The most widely distributed and apparently the most powerful vasodilator nerves described are those in the posterior roots which were discovered by Stricker but which have been investigated with especial care by Bayliss (9). Bayliss considers that the vasodilator supply to the limbs, skin of the trunk and probably

of the ears and face, and of the intestine belong to this system. It is in these regions that acetyl-cholin exerts its most pronounced vasodilator action; is this the result of the stimulation of the "endings" of these nerves? Atropine prevents the action of acetyl-cholin upon the vessels in this area, as in all other cases. So far as I can learn the effect of atropine upon the vasodilator action of the posterior roots has not been directly tested. There is, to be sure, some evidence that atropine does not paralyze these nerves. Thus Ostroumoff (10) reported that the vasodilation resulting from weak or slow stimulation of the peripheral end of the sciatic was not prevented by atropine; Pick (11) found that whereas large doses of atropine diminished the action of the vasoconstrictors of this nerve that of the vasodilators was not affected. Bayliss believes that the only sources of vasodilators to the limbs are the posterior root fibers, but others have described sympathetic vasodilators to the limbs, and Gaskell (4) has recently stated that he thinks it impossible to consider that the posterior root fibers are the same as those found in the sciatic by slow stimulation. It seemed desirable therefore to test the effect of atropine upon the result of the direct stimulation of the posterior root fibers. This seemed especially desirable in view of Gaskell's suggestion that the posterior roots affect the metabolism of the skin as the chorda tympani controls the cells of the submaxillary gland and that the vasodilation from the stimulation of the nerves is, in both cases, the indirect result of changes in metabolism (production of "metabolites" which cause vasodilation.) The acceptance of this suggestion would not necessarily lead to the expectation that atropine would prevent the vasodilation caused by the posterior roots for there is no information as to the character of the metabolites supposed, on the above hypothesis, to be produced. But if atropine does not prevent the vasodilator action of the posterior roots whereas it does prevent the action of acetyl-cholin the conclusion would be justified that the vasodilation in the two cases is different.

As a matter of fact, I found that when comparable degrees of vasodilation were caused in a posterior extremity of a dog by stimulation of a posterior lumbar root on the one hand and by acetyl-cholin on the other, the effect of the latter was completely prevented by a small dose of atropine whereas that of the former was not diminished even by large doses (fig. 1, exp. 476).

According to the current conceptions of the posterior root vasodilators (3), (9), (see however, (12)), the nerve fibers of these divide, one

division supplying receptors of the skin, etc., and the other the blood vessels; the former are stated to be readily paralyzed by cocaine. It

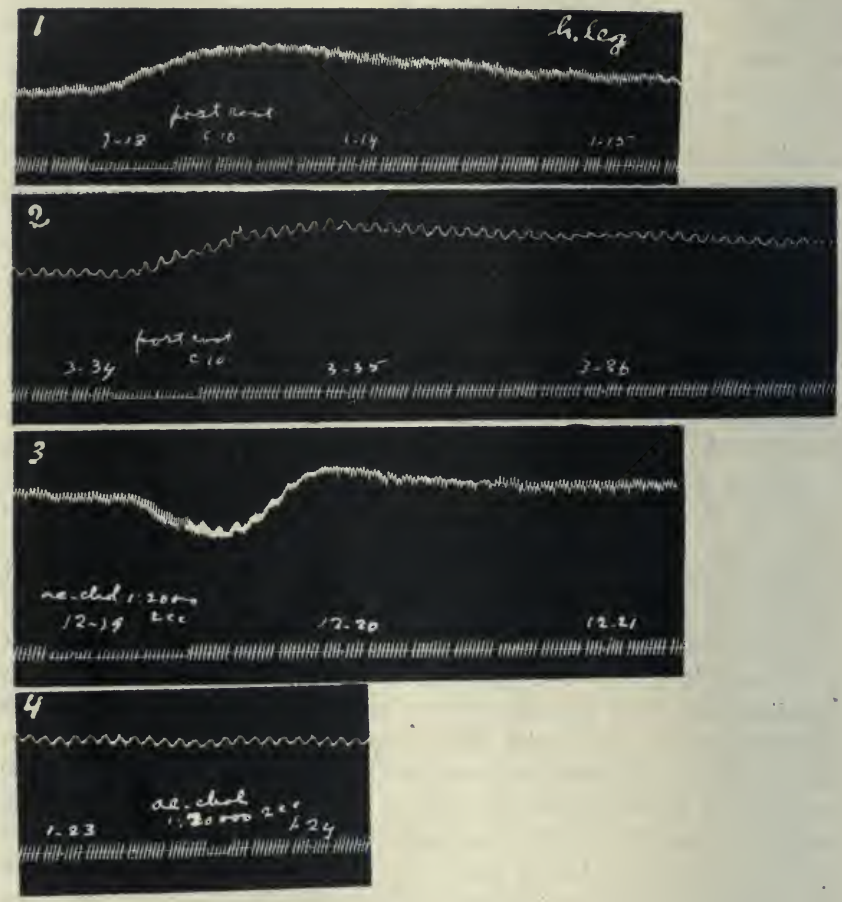

Fig. 1. Experiment 476. Dog, 12.8 K; morphine; ether. Plethysmograph record of hindleg; up = expansion. Time in seconds, 10 sec. and min. (1) *1–13*, stimulation of 7th lumbar posterior root before, and (2) *3–34*, after intravenous injection of 38 mgm. atropine sulphate. (3) *12–19*, 0.1 mgm. acetyl-cholin injected into a paw vein before, and (4), *1–23* after 2 mgm. atropine sulphate. At *12–19* the blood pressure fell from 130 to 71 mm. Hg; at *1–23* there was no change.

apparently has not been determined whether the "vasodilator branches" are paralyzed by cocaine. The acetyl-cholin vasodilator mechanism is not paralyzed by cocaine: thus the dilatation of a cat's leg (plethys-

mograph) caused by injecting acetyl-cholin peripherally into the artery was not in the least diminished by the injection of 30 mgm. of cocaine into the artery; the absolute dilatation was much increased as the acetyl-cholin completely overcame the marked vasoconstrictor action of the cocaine.

c. Sympathetic vasodilators. Several writers have described vasodilator nerves belonging to the sympathetic system; this interpretation has been questioned in nearly every case. Thus Carlson (13) reported the presence in the cervical sympathetic of the cat of vasodilator fibers to the submaxillary gland; he found that these fibers are paralyzed by relatively large amounts of atropine. Some more recent writers (2), (3), have attributed the vasodilation observed by Carlson to the effects of metabolites resulting from the stimulation of the secretory fibers of the sympathetic (a possible explanation considered by Carlson but held by him to be insufficient); the primary action of the atropine would, according to this interpretation, be upon secretory and not upon vasodilator nerves. A similar explanation has been suggested (4) for the vasodilation seen in the bucco-facial region of the dog following stimulation of the cervical sympathetic.[1] Special interest in "sympathetic vasodilators" has been aroused in recent years by the work of Dale, (16), (17), (18). Dale found that after the injection of ergotoxine or of ergot preparations containing ergotoxine into certain animals stimulation of the splanchnic (also after removal of the adrenal), or of the spinal cord, caused a fall of blood pressure; stimulation of the abdominal sympathetic caused a dilatation of the vessels of the foot instead of the usual constriction. Epinephrin (and also nicotine) caused only a fall of blood pressure in these animals and only a dilatation of the intestines and spleen.[2] Dale interpreted these results as probably additional evidence for the existence of sympathetic vasodilator nerves the action of which is usually obscured by that of the sympathetic vasoconstrictor nerves. Similarly the vasodilation, often resulting in a fall of blood pressure, usually obtained (without the previous injection of ergotoxin) in certain animals after the injection of small amounts of epinephrin has often been attributed to a stimula-

[1] This vasodilator action was described by Dastre and Morat (14) and confirmed by Langley and Dickinson (15) and by Dale (16). None of these writers report experiments with atropine.

[2] This effect upon the spleen and also the abolition, by ergot, of the pressor action of epinephrin and of splanchnic stimulation was observed independently by Sollman and Brown (19).

tion of such nerves. The work of Dale and others shows that this vasodilator mechanism is widely distributed in the body;[3] hence it was of interest to see if it was involved in the vasodilator action of acetyl-cholin. There were some reasons for suspecting that the latter would be found not to be the case; for instance, no evidence based upon experiments such as those just described has been found for the presence of sympathetic vasodilators in the rabbit and yet the vasodilator action of acetyl-cholin is very pronounced in this animal.

So far as I am aware Carlson (13) is the only one who has, in recent years, investigated the action of atropine upon so-called sympathetic vasodilators; as was stated above, doubts have been expressed as to the vasodilator functions of the nerve he studied. Ostroumoff argued that the vasodilators in the sciatic which he investigated belonged to the sympathetic system; as was stated above he found that these nerves were not paralyzed by atropine. It has been shown, however, that there are many posterior root vasodilators in the sciatic—perhaps they are the only ones present—these, as was shown above, are not paralyzed by atropine.

If the vasodilation from acetyl-cholin is due, in whole or in part, to an action upon the endings of sympathetic vasodilators or to the same mechanism, whatever it may be, responsible for the fall of blood pressure from epinephrin we should expect to find the action of the latter diminished or prevented by atropine. I have been unable to find any record of experiments in which the effect of atropine upon the vasodilator action of epinephrin after ergotoxine was tested. The only reference which I have found as to the relation of atropine to the depressor action of epinephrin is an incidental remark by Chiari and Fröhlich (24): they found epinephrin to cause a fall of blood pressure in cats poisoned with oxalates and in a normal cat with high blood pressure; they state that the vagi had been cut or atropine administered.

[3] Meltzer and Meltzer (20) reported experiments which suggest that epinephrin may have a central vasodilator action; Pilcher and Sollmann (21) found vascular dilatation to result exceptionally from a central action of epinephrin but believed it to be caused by an increased blood supply relieving an asphyxial stimulation; Hartman and Fraser (22) believe the epinephrin vasodilator reaction to be of central origin. That the epinephrin vasodilation after ergotoxine is not, at least solely, due to a central action is shown by the dilatation observed by Dale in the cat's foot when epinephrin was injected peripherally into the femoral artery after ergotoxine and by the work of Cannon and Lyman (23) who showed that epinephrin may cause a fall of blood pressure, after ergotoxine, when the brain and entire spinal cord have been destroyed.

I have frequently observed, and have no doubt that others have made the same observation, although I do not find it recorded, a fall of blood pressure from epinephrin after atropine had been administered, i.e., in animals which had not received ergotoxine. From a quantitative standpoint such experiments are often unsatisfactory; the results are often obscured by a fact noted incidentally by others (24), (25), and more fully investigated by Cannon and Lyman (23) namely, that the extent of the fall of blood pressure and at times even its occurrence is dependent to a considerable degree upon the level of the blood pressure when the epinephrin is injected. If, as often happens, atropine has caused a fall of blood pressure the depressor action of a given dose of epinephrin may be less or even replaced by a rise. However, a fall of blood pressure from epinephrin after the administration of sufficient atropine to prevent the vasodilator action of acetyl-cholin has been so often observed as to leave no doubt that the fall of blood pressure from epinephrin is of a different character from that after acetyl-cholin; experiments of this kind are shown in tables 3 and 4 (p. 243.)

The same is also shown in the following experiment (which also shows that the depressor action of acetyl-cholin is not altered by ergotoxine).

TABLE 1

Experiment 436. Cat; 1.96 K; pithed from second vertebra upwards; injections into external jugular

TIME	DOSE	BLOOD PRESSURE
11–29	Epinephrin 1: 100,000, 1 cc.	Rose 29 mm. (90 to 119)
36+	Acetyl-cholin 1: 5,000,000, 1 cc.	Fell 16 mm. (80 to 64)
40	Ergotoxine, 5.2 mgm.	Rose 67 mm. (78 to 145)
5	Epinephrin 1: 100,000, 1 cc.	Fell 9 mm. (105 to 96)
9	Acetyl-cholin 1: 5,000,000, 1 cc.	Fell 18 mm. (87 to 69)
51	Epinephrin 1: 10,000, 1 cc.	Fell 15 mm. (83 to 68)
4	Ergotoxine, 3.25 mgm.	0
5	Acetyl-cholin 1: 5,000,000.	Fell 13 mm. (77 to 64)
12–01	Atropine sulphate, 0.5 mgm.	0
5	Acetyl-cholin 1: 100,000, 1 cc.	0
6	Epinephrin 1: 10,000.	Fell 13 mm. (70 to 57)
11	Atropine sulphate, 2.5 mgm.	0
48	Ergotoxine, 3.25 mgm.	Rose 26 mm. (86 to 92)
50	Epinephrin 1: 10,000, 1 cc.	Fell 23 mm. (83 to 60)
12–56 to 1–31	9.6 mgm. atropine sulphate.	
1–34	Epinephrin 1: 10,000, 1 cc.	Fell 28 mm. (92 to 64)
43	Acetyl-cholin 1: 10,000, 1 cc.	Fell 14 mm. (94 to 80)

It will be noted in this experiment that after ergotoxine had been given until the epinephrin effect was reversed and after the injection of 12.6 mgm. of atropine, 0.1 mgm. acetyl-cholin caused a fall of pressure. Ordinarily, i.e., without ergotoxine, such an amount of acetyl-cholin injected after such an amount of atropine caused no fall of blood pressure; frequently it, like larger doses, caused a rise of pressure (nicotine-like action of Dale). Thus it appeared that ergotoxine may have prevented or reversed the pressor action of acetyl-cholin. That ergotoxine does have this action was shown in another experiment: atropine was given until acetyl-cholin (5 mgm.) caused only a rise of pressure (from 78 to 118 mm. Hg.); 6.5 mgm. ergotoxine was injected; the same dose of acetyl-cholin caused the pressure to fall 34 mm. (from 80 to 46). Expressed in the terms of the hypothesis of sympathetic vasodilators and the nicotine-like action of acetyl-cholin this result might be interpreted as showing that acetyl-cholin stimulates ganglion cells of both the sympathetic vasoconstrictors and of the vasodilators; but since the endings of the former had been paralyzed by ergotoxine only the action of the latter appeared, leading to the fall of blood pressure. The injection of nicotine in this experiment was of interest; the first injection (20 mgm.) caused only a pronounced fall of pressure (as was to be expected after ergotoxine: "stimulation of sympathetic vasodilators"): a second injection of nicotine had no effect on the blood pressure ("stage of the paralysis of the sympathetic vasodilators"). But acetyl-cholin caused as great a fall of pressure as before the nicotine, which may be interpreted as indicating that sympathetic vasodilator ganglia are less easily paralyzed by nicotine than are the constrictor ganglion cells. An alternative hypothesis would be that the fall of pressure was due to the acetyl-cholin being able to overcome the peripheral action of the atropine when the possibility of its constrictor action had been eliminated by ergotoxine.

2. THE RELATION OF THE ACETYL-CHOLIN VASODILATOR REACTION TO REFLEX VASOMOTOR CHANGES

It was not possible, as has been shown above, to find a relation between the vasodilator action of acetyl-cholin and any of the groups of nerves to which vasodilator functions have been ascribed: namely, the parasympathetic, the posterior root or the sympathetic nerves. Since, however, the mechanism through which acetyl-cholin exerts its vasodilator action is the most powerful yet found in the body it seemed of interest to determine if there are any indications that the body makes use of it in reflex vasomotor changes and also to compare the areas involved, the degree of activity, etc.

a. Depressor nerve. If the mechanism under discussion is involved in the fall of blood pressure resulting from stimulation of the depressor nerve the effect of the latter should be diminished by atropine. It is, of course, well known that the depressor causes a fall of blood pressure after atropine but no experiments seem to have been reported in

which the possibility was considered that atropine might diminish the effect or possibly prevent one phase of the action. A number of investigators, notably Bayliss (26), have shown that the fall of blood pressure from stimulation of the depressor is due in part to increased activity of vasodilator nerves, in part to an inhibition of vasoconstrictors. Martin and Stiles (27) found two types of reflex fall of blood pressure from stimulation of the central end of the vagus (cat); in one type the threshold is low and the authors believe the fall of blood pressure is due to the excitation of vasodilators; the other type has a high threshold and is believed to represent the inhibition of constrictors. It seemed of interest to determine if atropine might not affect the one type and not the other. My own experiments were performed upon rabbits. The only publication I found bearing upon this subject was by Tschirwinsky (28), who stated that the fall of blood pressure from the depressor was 26.2 per cent before and 29.7 per cent after atropine; he also stated that he sometimes found the depressor to cause a rise of pressure and (if I understand him correctly) that this was facilitated, or increased, by atropine. As regards the latter point: I have occasionally seen the fall of pressure followed by a rise but this seemed to be entirely independent of the administration of atropine. I did not find atropine to have any effect upon the fall of blood pressure resulting from either weak or strong stimulation of the depressor. I do not consider these results to be conclusive evidence that atropine may not modify certain features of the action of the depressor nerves; finer gradations of stimuli might have given different results and plethysmograph experiments might have shown that after atropine the relative extent of dilatation in different organs was changed (see below). But even if such changes were found this would not invalidate the conclusions that the acetyl-cholin dilator mechanism is not involved, to any considerable extent, in the fall of blood pressure from stimulation of the depressor.

Plethysmograph experiments showed that, the blood pressure being high, when equal falls of blood pressure were caused by depressor stimulation and by acetyl-cholin the expansion of the foreleg and the ear was usually greater with the acetyl-cholin. When, as sometimes occurred, the depressor and acetyl-cholin caused a (passive) diminution in the volume of the hindleg that resulting from the former was greater; the fall of blood pressure being the same in both cases. These results suggest that dilatation of the limb and ear vessels are a more important factor in the acetyl-cholin fall of blood pressure than in that

caused by the depressor. Comparisons made upon other organs were not very satisfactory but the following results are of interest: in an experiment in which the blood pressure was very low (39–40 mm.) depressor stimulation and the injection of acetyl-cholin caused about equal falls (9–11 mm.) of blood pressure and a (passive) diminution in the volume of a loop of the intestine; later when the blood pressure was higher (78–82 mm.) acetyl-cholin caused a slight expansion of the intestine and depressor stimulation a slight diminution (the fall of blood pressure being 22 and 24 mm. respectively); a little later, with a blood pressure of about 90 mm. both the depressor and acetyl-cholin cause a marked expansion of the intestine. These results suggest that the dilatation of the intestinal vessels is dependent upon the degree of their tonicity; considered in connection with what follows it seems probable that the depressor dilatation in this area is chiefly due to an inhibition of vasoconstrictors.

There was in one respect, a striking difference between the effects of depressor stimulation and of acetyl-cholin upon the volume of the ear or leg (table 2 and fig. 2). As already stated the acetyl-cholin dilatation was greater than the depressor dilatation although the fall of blood pressure was the same in both cases. Moreover, the dilatation could be obtained repeatedly and without any diminution from acetyl-cholin but the dilatation from depressor stimulation became less and was sometimes replaced by a (passive) diminution of volume, although the effect upon the blood pressure remained the same. A number of factors may be involved (especially in such an experiment as that upon which table 2 is based) in this lessened dilatation of the leg or ear. But the factor apparently most often involved was the repeated stimulation of the depressor itself, and there seems justification for the hypothesis that the dilatation of the limbs and ear from depressor stimulation is chiefly due to a central stimulation of vasodilators and that this mechanism is easily fatigued whereas the acetyl-cholin dilatation is entirely peripheral and is not easily fatigued.

b. Other afferent nerves. Stimulation of afferent nerves other than the depressor will, as is well known, cause a fall of blood pressure, especially under certain conditions. The mechanism of this fall of pressure is not definitely known; that it is different from that involved in the acetyl-cholin reaction is indicated by the fact that it is not prevented by atropine (tables 3 and 4; in both of these experiments the fall was greater after atropine).

TABLE 2

Experiment 457. Rabbit; see figure 2

TIME		BLOOD PRESSURE (FEMORAL)	EAR (PLETHYSMOGRAPH)
12–19	L. depressor stimulated 10 sec., coil 13.5.	Fell 42 mm. (86–44)	Expansion (fig. 2, a)
22	Acetyl-cholin, 0.001 mgm. intravenously..	Fell 36 mm. (86–50)	Expansion (fig. 2, b)
27	L. depressor; 10 sec., coil 13.5.....	Fell 42 mm. (84–42)	Slight expansion (fig. 2, c)
32	L. depressor; 10 sec., coil 13.5.....	Fell 40 mm. (82–42)	0
35½	Acetyl-cholin, 0.001 mgm. (injected more slowly)..	Fell 23 mm. (79–56)	Expansion (fig. 2, d)
37	0.9 per cent NaCl, 1 cc...	0	0
39	Atropine sulphate, 0.5 mgm.....	Fell 8 mm. (83–75)	0
42	L. depressor.....	Fell 40 mm. (81–41)	Slight expansion
48	Acetyl-cholin 0.001 mgm.....	0	0
51	L. depressor.....	Fell 40 mm. (84–44)	Slight expansion
54	Acetyl-cholin, 0.01 mgm.....	Fell 38 mm. (86–48)	Great expansion
58	Chloral hydrate 0.036 gram, Ethyl carbamate, 0.09 gram, (intravenously)		
1–03	L. depressor.....	Fell 35 mm. (91–56)	Great expansion (fig. 2, e)
	Atropine, 2 mgm.....		
1–58	L. depressor.....	Fell 38 mm. (90–52)	Expansion (fig. 2, f)
2–02	Acetyl-cholin, 0.05 mgm.....	Fell 39 mm. (88–49)	Great expansion (fig. 2, g)
07	L. depressor.....	Fell 38 mm. (86–48)	0
	Atropine, 5 mgm.....		
2–39	L. depressor.....	Fell 39 mm. (88–49)	0
42	Acetyl-cholin, 0.05 mgm.....	Fell 20 mm. (88–68)	Great expansion
	Chloral hydrate 0.108 gram, Ethyl carbamate. 0.270 gram, (intravenously)		
3–40	Atropine sulphate 5 mgm.....	Fell 20 mm. (96–76)	Expansion (fig. 2, h)

TABLE 2—Continued

Experiment 457. Rabbit; see figure 2

TIME		BLOOD PRESSURE (FEMORAL)	EAR (PLETHYSMOGRAPH)
3–46	L. depressor.....	Fell 39 mm. (93–54)	0
53	Acetyl-cholin, 0.025 mgm.....	Fell 14 mm. (88–74)	0
4–02	Acetyl-cholin, 0.1 mgm.	Fell 49 mm. (90–41)	Great expansion (like 2–02)
13	Atropine, 10 mgm.....	Fell 42 mm. (93–51)	Great expansion Great contraction
19	Acetyl-cholin, 0.1 mgm.	0	0
30	L. depressor, coil 10....	Fell 22 mm. (72–50)	Contraction

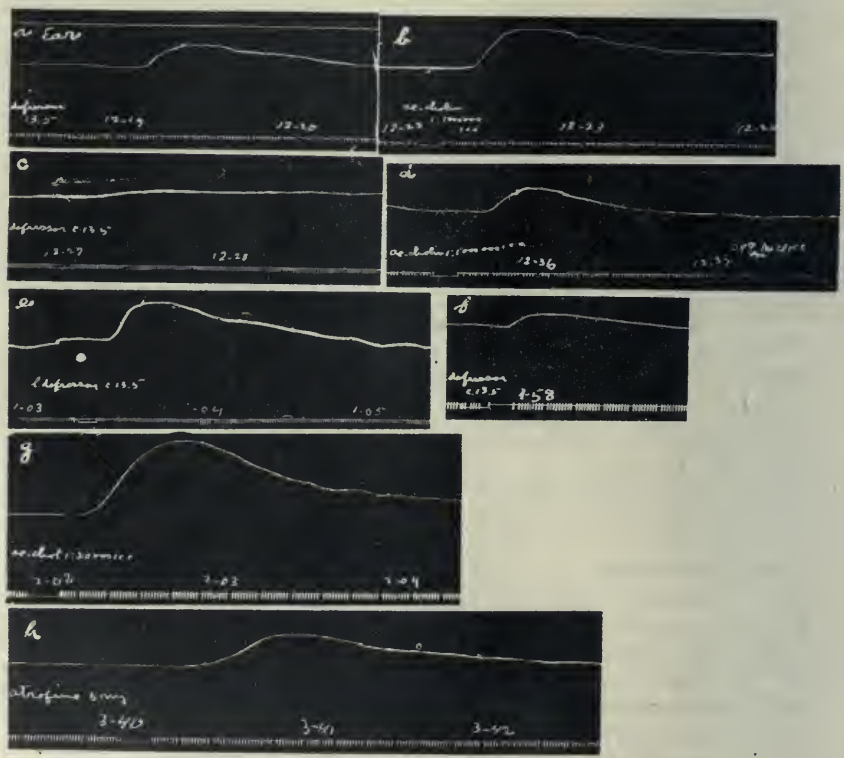

Fig. 2. Experiment 457. Rabbit, 1.84 K; chloral hydrate and ethyl carbamate; vagi cut. Ear in plethysmograph; up = expansion. Time in seconds, 10 sec. and min. See table 2.

TABLE 3

Experiment 473. Cat; 3.78 K; chloral hydrate and ethyl carbamate

TIME	DOSE	BLOOD PRESSURE (FEMORAL)
12–06	Sciatic (Harvard coil; tetanizing current), coil 13	Fell 7 mm. (127–120)*
12–12	Acetyl-cholin, 0.01 mgm. (saphenous vein.)	Fell 50 mm. (114–64)
22	Epinephrin, 0.005 mgm	Fell 36 mm. (122–86)
25	Atropine sulphate, 1 mgm	Fell 17 mm. (122–105)
27	Epinephrin, 0.005 mgm	Fell 29 mm. (11 –88)
31	Acetyl-cholin, 0.01 mgm	0
45	Epinephrin, 0.01 mgm	Fell 49 mm. (124–75)
	3.5 mgm. atropine sulphate	
1–07	Sciatic, coil 13	Fell 12 mm. (118–106)
12	Epinephrin, 0.01 mgm	Fell 45 mm. (120–75)
19	Sciatic, coil 13–45	Fell 15 mm. (127–112)†
22	Epinephrin, 0.005 mgm	Fell 52 mm. (126–74).

*This was the greatest fall of pressure which could be obtained with any strength of stimulus.

† This was the greatest fall of pressure which could be obtained with any strength of stimulus.

TABLE 4

Experiment 460. Cat; chloral hydrate and ethyl carbamate

The sciatic was stimulated a number of times with very weak as well as stronger stimuli; the greatest fall of blood pressure obtainable (coil 13–90) was 4 mm. Cold alcohol was now circulated about the sciatic; the strongest stimuli (which had previously caused only a rise of pressure) now uniformly caused a fall of pressure which was greater than that obtainable with any strength of stimulus before the nerve was cooled; 0.0005 mgm. acetyl-cholin injected intravenously had caused the pressure to fall 27 mm. The nerve was being cooled when the following stimulations were made.

TIME	DOSE	BLOOD PRESSURE (FEMORAL)
12–13	Sciatic, coil 6	Fell 11 mm. (114–103)
14	Epinephrin, 0.002 mgm	Fell 10 mm. (110–100)
15	Atropine sulphate, 0.5 mgm	Fell to 96 mm.
17	Sciatic, coil 6	Fell 14 mm. (96–82)
19	Epinephrin 0.002 mgm	Fell 9 mm. (91–82)
26	Sciatic, coil 6	Fell 10 mm. (98–88)
27	Acetyl-cholin, 0.005 mgm	0
36	Atropine sulphate, 1 mgm	
38	Sciatic, coil 6	Fell 13 mm. (99–86)
40	Epinephrin, 0.005 mgm	Fell 16 mm. (100–84)
43	Acetyl-cholin, 0.025 mgm	Fell 4 mm. (103–99)
44	Atropine sulphate, 5 mgm	
46	Sciatic, coil 6	Fell 19 mm. (96–77)

The circulation of the alcohol was stopped and the temperature of the nerve allowed to return to normal; stimulation now with any strength of current caused no effect or only a rise of pressure; with the coil at 6, for example, the rise was 41 mm. (93–134); no fall could be obtained with any strength of stimulus.

In the second of the above experiments I made use of a method for intensifying the fall of blood pressure, when ordinary "tetanizing" currents are used, originally described by Howell, Budgett and Leonard (29) and more fully investigated by me (30) viz., stimulating the nerve while it was being cooled. So far as I am aware Vincent and Cameron (31) are the only investigators who have repeated these experiments; they state that they obtained the same results (although their interpretation of the results was different from that which I thought could be placed upon them.) Others, without however repeating the experiments, have expressed the opinion that the effect of the cooling is simply equivalent to that of employing a weaker stimulus. Thus Martin and Lacy (32) suggested that the procedures I employed (among them cooling the nerve) resulted in a mere impairment of conductivity:

"Thus strong stimuli were converted into weak ones, and what Hunt observed was the usual effect on blood pressure of threshold stimulation." Ranson and Billingsley (33) stated: "Cooling a nerve would decrease the strength of the impulses passing over it so that the net result would be the same as that of weak stimulation." I thought that I had met objections of this character (which, moreover, at least when expressed in this way, are difficult to reconcile with the current views of the "all or nothing" principle of the nerve impulse) when I stated that I had found that "a much greater fall of pressure can be obtained when the nerve is cooled and stimulated, than can be obtained by the use of a weak stimulus alone"—a fact again illustrated in the experiment cited above. I also found that stimulation of the central end of a regenerating nerve caused a fall of pressure; Vincent and Cameron found the same. These results have been interpreted in the same way as the cooling experiments; it has been said "the conductivity of a regenerating nerve is less than that of a normal one and only weak impulses could be made to reach the cord by way of such a nerve." I had pointed out, however, that a greater fall of pressure could be obtained from a regenerating nerve than could be obtained with any strength of stimulus from the corresponding normal nerve (unless the latter were cooled).

Ranson and Billingsley expressed surprise that Vincent and Cameron should have not always found it possible to satisfy themselves as to the different effects of weak and strong currents; they themselves considered a rise of pressure from weak stimulation atypical. Martin and Lacy also speak of the constancy with which they obtained a fall of pressure with weak stimuli. My experience on the whole has been similar to that of Vincent and Cameron; it has been somewhat exceptional for me to obtain marked falls of blood pressure with weak stimuli. The experiments of Gruber (34) offer a satisfactory explanation of these differences. Gruber found that the rate of stimulation has fully as great an influence upon the result as the strength of the stimulus. I had tried different rates in my original experiments but for some reason failed to find a difference. Martin and Lacy seem also to have obtained negative results in such experiments for Stiles and Martin (35) state: "Martin and Lacy have found that the interruption of the primary current can be made to take place at widely varying rates without affecting the extent of the vasomoter change resulting from the stimulation of a single afferent path." I find it very easy to confirm Gruber's statement as to the difference between the effects of different rates of stimulation; this is a more convenient method of obtaining a depressor reaction than the

method of cooling. Martin and Lacy do not state the rate of stimulation they employed; they simply say that an apparatus was used by which the primary circuit was broken 2 to 8 times a second and that for more rapid rates an apparatus was used by which the circuit could be broken from 4 to 60 times a second. In later papers on this general subject Martin and his coworkers speak of using 8 to 15 (36), 5 to 16 (37) and 8 to 12 (38) interruptions per second. Ranson and Billingsley do not state definitely the rate of stimulation they employed but refer to an earlier paper (39) in which it was stated that the rate "was fairly slow, about 25 per second." Gruber used rates of from 4 to 20 per second. On the other hand Howell, Budgett and Leonard, Vincent and Cameron and I employed the "tetanizing" current; in the coil I used, the rate was about 80 per second. The group of investigators who used slower rates found a fall of pressure to be the usual result of the stimulation of an afferent nerve with weak stimuli; those of us who used more rapid rates often failed to observe more than slight falls of pressure. This difference in the method of experimenting may also explain a difference in the results which have been reported for certain nerves: thus I, using the tetanizing current, found in a considerable number of experiments a fall of pressure from the saphenous nerve in the dog and rabbit, but stated that it "rarely occurred" from stimulation of this nerve in the cat; Langley (40) found the same, although he states that his experiments upon cats were not numerous. Other workers using slower rates of stimulation have easily obtained a fall of pressure from the cat's saphenous. I have recently obtained the same.

I have recently made, incidentally, some observations upon the effects of cooling a nerve (sciatic, cat) and stimulating it with the "tetanizing" current (about 80 interruptions per second) and with a slow rate of stimulation (6 per second). Before cooling only a very slight fall of pressure (4 or 5 mm.) could be obtained whatever was the position of the secondary coil of the inductorium arranged for the tetanizing current. The strength of the latter was increased until a rise of 15 mm. was obtained; a fall of 14 mm. was obtained with the slow stimulation. Cold alcohol was circulated about the nerve in the manner described in my original paper and the nerve stimulated alternately with the slow and with the rapid rate, the secondary coils remaining the same in each case. As the nerve was cooled the rise of pressure from the tetanizing current became less, then there was no change and finally only a fall (15 mm.) was obtained. The fall of pressure from the slow stimulation remained the same. If, as has been suggested, the change from the rise to the fall of pressure in the former case was simply the result of a weakening of the stimulus through cooling it is difficult to see why the depressor reaction from the slow stimulation was not lessened. In this experiment the fall of pressure from the slow rate (before and after cooling), which was the maximum effect which could be obtained, was the same as the maximum fall obtained by cooling the nerve and stimulating it with the tetanizing current.

I thought (30) that the fall of blood pressure which results under certain conditions from the stimulation of afferent nerves (it being assumed that the condition of the center remained unchanged) could, on the whole, be more satisfactorily explained on the hypothesis that there are present in mixed nerve trunks depressor, or reflex vasodilator, nerve fibers rather than on the hypothesis

that stimulation of the same nerve fibers may at times lead to a fall, at other times to a rise of blood pressure. The chief objection to this hypothesis has been based upon the quantitative studies of Martin and Lacy on vasomotor reflexes from threshold stimulation; Ranson and Billingsley (33) found this work to be the "most serious difficulty" in the way of such a hypothesis. As they say: "according to them (Martin and Lacy) the threshold for the depressor reflex is 8.7 units as compared with . . . . 280 units for the pressor reflex." "It is difficult to conceive of two afferent fibers so differently constituted that the stimulation threshold of one should be 30 times greater than that of the other." This particular difficulty seems to have been removed by Gruber's work: Gruber obtained pressor responses with only 5.8 units if the rate of stimulation were increased to but 20 per second; on the other hand, depressor responses were obtained with 383 units (about 70 times stronger) if the rate of stimulation was reduced to 4 per second. With a still slower rate of stimulation a depressor response was obtained with 6643 units, which was 390 times stronger than the current necessary to produce a rise of blood pressure when interrupted 20 times per second. Gruber concludes that the threshold for both pressor and depressor responses may be the same depending upon the rate.

Little positive evidence has been adduced by those who believe that only one set of afferent fibers is involved in both pressor and depressor reactions. Martin and Lacy seemed to think that their studies on the reflex vasomotor thresholds and the assumption that cooling a nerve, etc., was equivalent to merely weakening the stimulus was sufficient to make this hypothesis at least equally possible; in later publications, however, Martin and his coworkers have had occasion to make use of the other conception.

Ranson and Billingsley adopt a hypothesis which is essentially the same as one which I considered in 1895 and thought inadequate; I stated it as follows: "We might suppose one nerve fiber to be connected with both a dilator and a constrictor center; and perhaps we might further imagine that a weak stimulus could reach or excite one center more readily than the other, owing to a difference in irritability." I endeavored, unsuccessfully, to trace the paths of the pressor and depressor impulses in the spinal cord but Ranson and Billingsley have made the very important discovery of tracts in the spinal cord by which "a weak stimulus could reach or excite one center more readily than the other." They found that the "depressor path" is through a tract of long fibers with few relays whereas the pressor path is through a series of short relays. Accepting the views of Martin on the relation between strength of stimulus and the vasomotor response they believed that a weak stimulus could readily reach the centers causing a vasodilation through the former, whereas only stronger impulses could pass through the pressor path to the centers causing a vasoconstriction. This attractive hypothesis, however, has lost its chief support through the work of Gruber although doubtless it could be employed equally well to explain the effects of different rates of stimulation. The possibility that the effect of cooling a nerve is in reality the same as reducing the number of stimuli might also be suggested as a working hypothesis.

On the other hand the fact that pressor and depressor impulses follow different paths in the spinal cord is not in the least inconsistent with the hypothesis that the impulses reach the spinal cord through different nerve fibers. The ana-

tomical relations of the latter are not sufficiently known to make theorizing, beyond the making of working hypotheses, profitable. But there are certain facts and views in this connection which are worthy of consideration. It seems to be generally believed that the posterior root vasodilators are myelinated nerve fibers; the work of Bayliss makes it probable that the stimulation of the central end of these fibers causes at least local reflex vasodilation and my own work indicated that the vasodilation accompanying the fall of pressure from stimulation of afferent nerves occurred in regions supplied by the posterior root dilators. The afferent fibers causing a reflex rise of blood pressure were found by Ranson and Billingsley (41) to be unmyelinated fibers; these authors state that they believe the afferent fibers involved in the depressor reflex to belong to this group although they do not seem to have investigated this point. Should these conceptions prove correct, viz., that the afferent depressor fibers are myelinated and the afferent pressor fibers are unmyelinated they might afford an explanation of the effects of different kinds of stimuli. It is interesting that the methods of stimulation which have proved of value in demonstrating the presence of vasodilator nerves in nerve trunks containing vasoconstrictors, viz., weak stimuli, a slow rate of stimulation, stimulation of a cooled or of a regenerating nerve are the same as those by which a reflex fall of blood pressure may most easily be obtained. To what extent these differences are due to differences in the response of the end or central organs and to what extent they may be due to differences in the nerve fibers themselves has not been satisfactorily determined.

3. ANTAGONISTIC AND OTHER ACTIONS OF DRUGS IN RELATION TO THE VASODILATOR ACTION OF ACETYL-CHOLIN

I have repeatedly called attention to the antagonistic action of atropine to the vasodilator action of acetyl-cholin; in fact this is one of the most characteristic pharmacological reactions of acetyl-cholin yet discovered. Some comparisons were reported in a previous publication (42) between the effects of atropine upon the cardio-inhibitory nerves and its action in diminishing or abolishing the fall of blood pressure from acetyl-cholin. It was found that other members of the "atropine" series (scopolamine, hyoscyamine, homatropine and "eumydrin," or atropinemethyl nitrate) had a similar action but there were marked quantitative differences; in general there was a parallelism between their power to paralyze the cardio-inhibitory nerves and to prevent the vasodilator action of acetyl-cholin.

Recently the action of atropine sulphuric acid ester ("Atrinal") was tested. This was far less active than atropine sulphate; its paralyzing action upon the cardio-inhibitory nerves was also relatively slight, as had already been found by Trendelenburg (43). Thus 1 mgm. of this compound (which when injected intravenously into a

cat caused a fall of blood pressure with a dilatation of the leg and a probably passive contraction of the nasal mucosa) reduced the absolute fall of blood pressure caused by 0.002 mgm. acetyl-cholin by 41 per cent; the percentile fall of pressure was reduced from 35.8 to 30 and the dilating action of acetyl-cholin upon the leg was diminished. Within six minutes, however, the above dose of acetyl-cholin was almost as active as at first; 0.5 mgm. of atropine sulphate completely abolished the effect of 0.002 mgm. acetyl-cholin and greatly reduced that of 0.1 mgm.

Pilocarpine in doses causing no lasting fall of blood pressure, but which usually caused a slowing of the heart, caused a diminution of the acetyl-cholin fall of pressure and less expansion of the rabbit ear; there was a similar diminution in the acetyl-cholin effect in the cat and dog without a slowing of the heart. Unfortunately no injections of acetyl-cholin were made immediately after, or simultaneously with, the pilocarpine injection; it is very probable, from experiments which will be described later, that there would have been a summation of the effects of the two. The depression of the vasodilator action of acetyl-cholin by pilocarpine is doubtless similar to the depression of the vagus effects of "synthetic muscarine" by pilocarpine described by Gaisböck (44); in fact Gaisböck's protocols show effects upon the blood pressure similar to those I obtained with pilocarpine and acetyl-cholin but he attributed the effects to a cardiac action.

Physostigmine which is often classed, pharmacologically, with pilocarpine has an effect the opposite to that of the latter in relation to acetyl-cholin. Physostigmine was shown in a previous communication (45) to have a sensitizing effect on the cardio-inhibitory action of one of the homocholins and also, apparently, on the pupil-constricting action of the latter; physostigmine has been found to have a similar effect on all of the vascular actions of acetyl-cholin: it increases the fall of blood pressure and the slowing of the heart before a large dose of atropine and the rise of blood pressure after atropine. Some of these actions are illustrated in the following experiment: (Table 5).

A large number of other compounds were tested in the hope that some of them might throw more light upon the action of acetyl-cholin. Thus certain substances which have, or which have been supposed to have, some of the actions of atropine were tested; also a number of substances which have been stated to increase, or diminish, the sensitiveness of the parasympathetic nervous system (upon which most of the effects of acetyl-cholin are exerted). It has been claimed that

whereas atropine paralyzes chronotropic and inotropic cardio-inhibitory functions equally, other substances depress especially the inotropic functions. It seemed of especial interest to test some of these, for the negative inotropic action of acetyl-cholin upon the heart

TABLE 5

Experiment 479. Dog; morphine; ether; vagi cut

TIME		BLOOD PRESSURE	HEART RATE IN 10 SECONDS
2–19	0.05 mgm. acetyl-cholin..	Fell 55 mm. (103–48)	Slowed from 33 to 21
25	0.02 mgm. acetyl-cholin..	Fell 49 mm. (104–55)	0
28	2.0 mgm. atropine sulphate		
41	0.02 mgm. acetyl-cholin..	0	0
43	1.0 mgm. physostigmine salicylate.....	Rose 11 mm. (104–115)	0
47	0.02 mgm. acetyl-cholin..	Fell 17 mm. (113–96)	0

Before the injection of atropine acetyl-cholin caused an expansion of a foreleg (plethysmograph); this was prevented by the atropine but occurred again after the physostigmine.

1 mgm. atropine sulphate and curare were now injected.

3–38	0.1 mgm. acetyl-cholin...	0	0
43	0.3 mgm. physostigmine salicylate.....	0	0
44	0.1 mgm. acetyl-cholin...	Rose 14 mm. (67–81)	0
47	0.4 mgm. physostigmine salicylate.....	0	0
48	0.1 mgm. acetyl-cholin...	Rose 28 mm. (73–101)	Increased from 25 to 28
56	1.8 mgm. physostigmine salicylate.....	0	0
57	0.1 mgm. acetyl-cholin...	Rose 40 mm. (86–126)	0
4–08	6 mgm. physostigmine salicylate.....	0	0
13	5 mgm. acetyl-cholin.....	Fell	Stopped for 40 sec.
17	20 mgm. atropine sulphate.....		
22	5 mgm. acetyl-cholin	Rose 59 mm. (41–100)	Increased from 17 to 25

The acetyl-cholin at 3–38 had no effect on the volume of the leg; the subsequent injections (i.e., after the physostigmine) caused an expansion except that at 4–13 there was a contraction (doubtless passive) and at 4–22 a marked contraction (active because the blood pressure rose).

(marked upon the auricle, less distinct upon the ventricle) is more pronounced than the negative chronotropic action, which appears only after large doses; the action of acetyl-cholin upon the blood vessels may be analogous to this negative inotropic action upon the heart.

Since the dilatation of the blood vessels of glands by acetyl-cholin might be interpreted as an indirect effect of the stimulation of secretion (production of "metabolites") a number of compounds supposed to have effects upon certain phases of metabolism were also tested. Some others were tried on the basis of hypotheses which need not be discussed; others were tested incidentally to other studies. It is quite probable that some of the results would not have been reported negative had larger doses been employed or if the experiments had been performed in a different manner; I was looking for striking, undoubted effects like that of atropine.

Curare which has been stated by some to paralyze vasodilator nerves (denied by others (11)), usually diminished the absolute and percentile fall of pressure from acetyl-cholin when it had itself caused a fall of pressure; when curare caused a rise of pressure, as sometimes occurs, especially in rabbits, the action of acetyl-cholin was slightly increased.

Agaric acid the action of which upon the sweat glands has long been supposed to be analogous to that of atropine (although recently an entirely different interpretation has been given (46) and which is also said to have an antagonistic action to the negative inotropic effect of pilocarpine upon the frog heart (47) had, in a dose of 0.1 gram (cat), no effect upon the acetyl-cholin, or pilocarpine, fall of blood pressure. "*Euphthalmin,*" a compound closely related to betaeucaine, which dilates the pupil, presumably by an atropine-like action, seemed to diminish the effect of acetyl-cholin slightly. *Betaeucaine* itself seemed to slightly diminish the fall from acetyl-cholin; it quickly abolished the slowing of the heart from vagus stimulation and for a brief period diminished the slowing from large doses of acetyl-cholin.

"*Novocaine,*" which caused a fall of blood pressure and a slowing of the heart, diminished the percentile fall from acetyl-cholin. "*Alypin*" in a dose causing a considerable fall of blood pressure diminished the effect of acetyl-cholin but the latter returned as the blood pressure rose towards normal. *Cocaine* (cat; 36 mgm.) diminished the absolute but not the percentile fall of pressure from acetyl-cholin.

Ergotoxine in doses which reversed the effect of epinephrin upon the blood pressure did not distinctly reduce the effect of acetyl-cholin (table 1). *Histamin* (B-iminazolylethylamin) increased the fall of blood pressure from acetyl-cholin in the cat; there seemed to be a simple summation of the effects of the two. During the low blood pressure following an injection of *peptone* or *hirudin* both the absolute and percentile fall of pressure from acetyl-cholin were diminished; as the blood pressure returned to normal acetyl-cholin had its full effect. Peptone caused an expansion of the liver; acetyl-cholin usually a diminution at least at

first. The previous injection of extract of *corpus luteum* had no effect upon the acetyl-cholin fall of blood pressure; injected together there was a summation of their depressor effects. The action of corpus luteum extract was diminished but not prevented by atropine.

Nicotine. In my first experiments with acetyl-cholin I found that its depressor action was not prevented by nicotine; the percentile fall of pressure might be the same whether the blood pressure was 160 mm. (under the influence of nicotine) or only 40 mm. Dale showed that after atropine large doses of acetyl-cholin caused a rise of pressure; I had observed the same but had failed to interpret it correctly. Dale attributed this rise of pressure to a nicotine-like action of large doses of acetyl-cholin; it is prevented by nicotine. I have found the same but have also observed that sometimes the effect of nicotine is to convert the pressor action of acetyl-cholin (after a small amount of atropine) into a depressor action; after a further injection of atropine the depressor effect disappeared. The pressor action (after atropine, but before nicotine) was probably due to the preponderance of the stimulation of the ganglion cells over that of the peripheral action which had been reduced but not abolished by incomplete atropinization; the depressor action to the preponderance of the latter after the ganglion cells had been paralyzed by nicotine; this depressor action was in turn prevented by sufficient atropine. But the depressor action of acetyl-cholin (after nicotine) sometimes persisted after much atropine had been injected; this can perhaps be best explained on the hypothesis advanced above: nicotine paralyzes sympathetic constrictors more readily than sympathetic dilators and the vasodilation from acetyl-cholin after nicotine and atropine may be due to a stimulation of sympathetic vasodilators. Nicotine also increased the tendency of acetyl-cholin to cause a slowing of the heart. *Emetine* had no effect on the action of acetyl-cholin. The relation of *epinephrin* to the acetyl-cholin fall of blood pressure was discussed in an earlier publication; fig. 12, (p. 214 of preceding paper) shows the balancing action of the two upon the rabbit ear.

The alkaloids of *Zygadenus*, which I (48) showed in 1902 to belong to the veratrine group (the toxic principle of these plants had previously been supposed by some to be colchicine, by others to be a sapotoxin) had no definite effect upon the action of acetyl-cholin; when they caused an extreme fall of blood pressure the percentile fall from acetyl-cholin was usually lessened but as the blood pressure returned, acetyl-cholin had its full effect. Similar effects were obtained with a commercial specimen of *"veratrine."* The experiments with *Yohimbine* were inconclusive; there may have been a slight diminution of the acetyl-cholin effect. Acetyl-cholin was active after large doses of *brucine* and also after *picrotoxin*.

Sodium oxalate which is stated (24) to increase, through deprivation of calcium, the excitability of the autonomic nervous system to pilocarpine and epinephrin, had, in small doses, no effect upon the acetyl-cholin fall of blood pressure; in one experiment (cat) 190 mgm. of sodium oxalate increased both the absolute and percentile fall of pressure from acetyl-cholin. *Magnesium sulphate* diminished the absolute fall from acetyl-cholin and, when it had caused a pronounced fall of blood pressure, also the percentile fall; calcium chloride, after magnesium sulphate, increased first the absolute fall and then restored the percentile fall. During the rise of blood pressure from *barium chloride*, acetyl-cholin sometimes caused a smaller fall of blood pressure; usually, however, the absolute fall was

the same although the percentile fall was less. If, however, the blood pressure had been very low both the absolute and percentile fall from acetyl-cholin was increased after barium chloride. Both the absolute and percentile fall from acetyl-cholin was increased during the rise from *tetra-hydro-B-naphthylamin*. In one experiment (dog) acetyl-cholin seemed much less active after *sodium nitrite*, although the blood pressure was still moderately high.

The fall of blood pressure from acetyl-cholin injected two or three minutes after the injection of *hydrocyanic acid* was markedly diminished (from 40 to 9 per cent for example); there was also a greater tendency of the acetyl-cholin to cause a slowing of the heart (vagi cut). This effect was of very brief duration. The rise of pressure from epinephrin was similarly reduced. *Hydrochloric acid* in an amount sufficient to cause slowing and irregularity of the heart had no effect upon the action of acetyl-cholin. The fall of blood pressure from acetyl-cholin after the intravenous injection of *chloral hydrate* was sometimes slightly increased, sometimes slightly diminished; a constant effect of the chloral hydrate was to increase the tendency of the acetyl-cholin to cause slowing and often irregularity of the heart, a result similar to that obtained by Loewi (49) with "synthetic muscarine" and pilocarpine. *Chlorbutanol* diminished both the absolute and percentile fall of blood pressure in one experiment. A large dose of *sodium diethyl-barbiturate* diminished the effect of acetyl-cholin; *ethyl carbamate* (0.4 gram intravenously; dog 8, 6 K) had no effect; *morphine* diminished the absolute and percentile fall for a short time.

Camphor injected with acetyl-cholin diminished slightly the fall of blood pressure from the latter; no definite results were obtained with *caffeine*. When acetyl-cholin was injected during the rise of blood pressure from *strophanthin* both the absolute and percentile fall was diminished; later both were increased.

After extensive *hemorrhage*, leading to a low blood pressure, the absolute fall from acetyl-cholin was diminished but the percentile fall was increased; the percentile fall from stimulation of the depressor was increased whereas epinephrin caused little or no rise of blood pressure immediately after the hemorrhage. (These results suggest that at some period after hemorrhage those blood vessels which usually respond to acetyl-cholin and depressor stimulation with dilatation and to epinephrin with constriction are in a condition of strong tonic contraction). *Asphyxia* was very effective in causing a rise of blood pressure when this had been lowered to a considerable extent by the application of acetyl-cholin to a lung; stimulation of the *splanchnic* caused a greater rise (both absolute and percentile) under these circumstances but the level reached was not so high. On the other hand the fall of blood pressure from acetyl-cholin was increased when injected during stimulation of the splanchnic or of the spinal cord, but the level reached was not so low, i.e., there was an algebraic summation of the two effects; this also occurred when a sensory nerve was stimulated (for a pressor response) and acetyl-cholin was injected.

Removal of the *adrenal glands* had no immediate effect on the fall of blood pressure from acetyl-cholin. In one experiment (cat; paraldehyde) the adrenals were removed and the same amount of acetyl-cholin injected every fifteen minutes for four and a half hours; during this time the blood pressure fell from 123 to 32 mm.; the absolute fall from the acetyl-cholin steadily declined but the percentile fall remained about the same. It has been supposed by some (with very

little basis; cf. (50)) that after the removal of the adrenals there is an accumulation of cholin in the blood. The *thyroids* were removed in another experiment (cat; paraldehyde) and the reaction to acetyl-cholin tested every twenty minutes for seven and a half hours; the blood pressure fell from 137 to 59 mm.; the absolute fall from acetyl-cholin diminished but the percentile fall remained about the same until near the end when it increased slightly. In another experiment a standard solution of acetyl-cholin was injected every fifteen minutes; between the injections both cervical sympathetics were stimulated, the pupils being kept in a condition of maximal dilatation; the percentile fall of pressure from acetyl-cholin was the same after more than two hours. Twenty-six cubic centimeters of 1: 200,000 solution of epinephrin were now slowly (in sixteen minutes) injected intravenously; when the blood pressure had returned to normal acetyl-cholin was again injected; both the absolute and percentile fall of pressure were very slightly less than before the epinephrin. It has been reported (51) that stimulation of the cervical sympathetic and the injection of epinephrin increases the secretion of the thyroid. It would seem from these experiments that there is no special relation between the condition of the thyroid and the acetyl-cholin fall of blood pressure. The cat in which the cervical sympathetics were stimulated had been given, per os, sodium iodide for several days preceding the experiment; Seidell and I (52) had found in 1908 that inorganic iodides given per os increased within twenty-four hours the amount of physiologically active iodine in the thyroid. (Marine and Rogoff (53) have found, when the iodide was injected into the circulation, a definite increase in the pharmacological activity of the thyroid after the eighth hour; this was well marked by the twentieth hour).[4]

4. DO OTHER VASODILATOR DRUGS ACT UPON THE SAME MECHANISM AS DOES ACETYL-CHOLIN?

The chief characteristic of the acetyl-cholin reaction is that it is readily prevented by atropine; the best available criterion as to whether other drugs act through the same mechanism is to test their activity before and after the administration of atropine. A large number of drugs have been tested in this manner; only a few need mention here.

[4] I may add in this connection that I found in certain experiments on cats in which the thyroids had been removed and the brain and spinal cord pithed to the mid-thorax a progressive increase in the rise of blood pressure from the injection of a small dose of epinephrin; after two hours the increase reached in one case 400 per cent. Levy (51) had found a similar increase in certain experiments; he attributed it to a discharge of thyroid secretion due to a discharge of epinephrin as a result of struggling under ether. But this explanation would hardly hold in the case of the experiment cited above for the thyroids had been removed twenty-four hours previously. I thought the increased response to epinephrin might be due to a gradual loss of irritability of vasodilator mechanisms; if this is the case the latter are not analogous to the acetyl-cholin reaction for this does not seem to diminish during an experiment.

Vasodilator action of pilocarpine. Few studies seem to have been reported on the action of pilocarpine upon the blood vessels. Kobert (54) stated that it constricts the vessels of the perfused leg and kidney. Brodie and Dixon (55) found it to constrict the blood vessels of the perfused limb and intestine; they stated that its action is identical with that of epinephrin except that larger amounts are necessary; after apocodeine or curare they found pilocarpine to cause no constriction but it may now cause a dilatation. Dixon (56) states that the constriction is prevented by atropine. Brodie and Dixon, and Baehr and Pick (57), found pilocarpine to dilate the vessels of the lung; Berezin (58) reported a constriction. Dixon and Halliburton (59) found a slight dilatation of the cerebral vessels. Fröhlich and Pick (60) found pilocarpine to dilate the blood vessels in frogs. Henderson and Loewi (8) carefully investigated the vasodilation in the submaxillary gland following pilocarpine and concluded that it is probably the result of the vasodilator action of products of secretion; Sollmann (61) suggests that the hyperemia of the skin often seen after pilocarpine may possibly be due to the increased activity of the sweat glands.

Thus no one seems to have reported a direct vasodilator action of pilocarpine upon the systemic blood vessels of mammals; my own experiments, on the contrary, point to such an action and also show that this, like the acetyl-cholin vasodilation, is abolished by atropine. Whether the difference between my results and those of others was due to differences in dose was not determined; it is not improbable that large doses of pilocarpine, like very large doses of acetyl-cholin, have a vasoconstrictor action. Under some conditions pilocarpine causes a marked rise of blood pressure. The vasodilator action of pilocarpine is shown in a number of figures in the preceding paper: figure 4 (p. 205) shows the expansion of the leg of a dog; figure 3 (p. 204) that of the leg of a cat after pilocarpine; similar results were obtained with the rabbit. Figure 13 (p. 214) shows the expansion of a rabbit's ear after pilocarpine; this figure also shows the contraction of the ear after epinephrin (which has been said to have the same effect upon peripheral blood vessels as pilocarpine). The effect of pilocarpine upon the outflow of the perfused rabbit ear is shown in figure 10 (p. 212); the effect on the outflow from the submaxillary gland is shown in figure 15 (p. 216). Pilocarpine caused a slight contraction (passive?) followed by an expansion of the dog's penis (one experiment). In all of the above experiments pilocarpine caused a fall of blood pressure with little or no slowing of the heart; both the fall of pressure and the

effects upon the blood vessels (with the exception of the perfused rabbit ear) were readily prevented by atropine; in none of the experiments had curare (which has been stated to prevent the "vasoconstrictor" action of pilocarpine) been given.

In a number of cases comparisons were made between the effects upon the peripheral blood vessels of pilocarpine and acetyl-cholin in doses causing approximately the same fall of blood pressure; the expansion of the rabbit ear (fig. 13, p. 214) and usually that of the leg (fig. 3, p. 204) was slightly less after pilocarpine than after acetyl-cholin; that of the dog's penis (one experiment) was greater; pilocarpine caused a greater increase in the outflow of the submaxillary gland than did acetyl-cholin; it had practically no effect on the outflow from a muscle when acetyl-cholin caused a slight increase; it was less active in causing an increased outflow from a cutaneous vein of the upper part of the leg of a cat (paw removed) than was acetyl-cholin. The last experiment was performed to determine whether the expansion of the intact limb could be attributed entirely to the effect of pilocarpine upon sweat glands—production of "metabolites;" the fact that pilocarpine dilated the skin vessels independently of those of the paw as well as the dilatation of the vessels of the rabbit ear and of the dog's penis indicate that the dilator action is not due entirely to its stimulation of recognized secretory processes. On the other hand the greater effect of pilocarpine as compared with that of acetyl-cholin upon the outflow from the submaxillary gland (fig. 15, p. 216) suggests that here stimulation of secretion may be an important factor in the vasodilation; pilocarpine is a much more powerful stimulant of secretion than is acetyl-cholin.

As to other effects of pilocarpine upon the blood pressure; in dogs, before atropine, the fall of pressure was frequently followed by a rise; after atropine there was often a rise of pressure in cats, dogs and rabbits but with the doses employed (which seldom exceeded 10 mgm.) the rise was not very great except in one case (dog) in which 10 mgm. caused the pressure to rise 57 mm. (from 75 to 132). In one experiment (cat) the rise of pressure was less after removal of the adrenals. In most of these experiments doses of pilocarpine having little or no effect upon the heart rate were employed. How little effect, however, great changes in the heart rate may have upon the fall of blood pressure from the same dose of pilocarpine was strikingly shown in an experiment on a dog (519): the vagi were in a condition of strong activity from which the heart could be freed by cooling the vagi; the

slow rate could be restored by stimulating the vagi peripherally to the cooled part: the fall of pressure from a given dose of pilocarpine was practically the same, 60 to 67 mm. (from 130 or 134 to 63 or 70) whether the heart rate remained the same (18 in 10 seconds) or whether it, following the pilocarpine injection, increased (from 21 to 39) or whether it was slowed slightly (30 to 28). The percentile fall was the same before and after clamping the auriculo-ventricular bundle in one experiment (dog); the same was true for acetyl-cholin; in both cases the fall was prevented by atropine.

Colchicine. Dixon and Malden (62) stated that after colchicine "the blood pressure falls as the result of a very slight cardiac inhibition, and the effect is not obtained on the atropinized animal." They publish a tracing from a dog showing a fall of blood pressure and a contraction followed by a less marked expansion of the hind leg of a dog. This curve is very like the curve I often obtained with acetyl-cholin when the hindleg of an animal was placed in a plethysmograph (see fig. 1, p. 234). I believe the diminution of volume to be passive and due chiefly to a vasodilator reaction occurring in other vascular areas. For when the foreleg of a cat was placed in a plethysmograph and colchicine injected intravenously there was, with the fall of blood pressure, an expansion of the leg; the curves obtained were practically identical with those obtained from acetyl-cholin and pilocarpine (see figs. 2 to 7 and 19 of preceding paper) and need not be reproduced here. Comparatively large doses (5 to 10 mgm.) were necessary to cause a marked expansion; smaller doses (1 mgm., for example) caused some expansion and increased the size of the pulse waves, another indication of vasodilation. A small dose (1 mgm.) injected peripherally into the femoral artery caused a marked expansion of the hindleg with no change in the blood pressure. The fall of blood pressure and the expansion of the leg were prevented by a small dose of atropine. Thus it seems that colchicine has a vasodilator action similar to that of acetyl-cholin and pilocarpine.

The first effect of a small dose of *physostigmine* was a slight fall of blood pressure and an expansion of the foreleg; this was followed by a rise of blood pressure and a contraction of the leg. These effects were prevented by atropine. Physostigmine thus seems to have a vasodilator action of the acetyl-cholin type but this is generally obscured by other more important actions.

Histamin. Histamin (B-imazolylethylamin) causes a fall of blood pressure and vasodilation in some animals the exact cause of which is

in some doubt (63), (64), (65). I do not find experiments recorded in which this drug was given after atropine and it seemed possible that the vasodilation was similar to that caused by acetyl-cholin and pilocarpine. I found, however, in experiments on cats, that the fall of pressure from histamin was not distinctly affected by atropine; frequently the fall of pressure was the same after doses of atropine which abolished the fall of pressure from acetyl-cholin; sometimes the absolute fall was less but the percentile fall remained the same. With a lowered blood pressure, after atropine, the fall might be less but the level reached remain about the same; if the blood pressure was raised by barium chloride the histamin fall of pressure was increased. As already noted I found, as had Barger and Dale (64) a dilatation of the cat's leg after histamin; this was not as great as that caused by an amount of acetyl-cholin causing an equal fall of pressure (fig. 8, p. 208 of preceding paper) and was frequently followed by a slow contraction. Histamin caused a pronounced diminution of the volume of the liver (fig. 20 of preceding paper, p. 224); it was not determined whether this was active or passive, due to the fall of blood pressure.

Mautner and Pick (65) state that there is in the liver of the dog and cat, either in the end capillaries of the portal or of the hepatic vein or in the area between these, a nervous mechanism which responds with a cramplike contraction to histamin; this, according to these authors, interferes with the return of the venous blood to the heart and leads to the fall of blood pressure. I found, however, that histamin causes a greater fall of pressure (cat) when injected into a saphena vein than when injected into a branch of the portal vein; also that large doses of sodium nitrite or acetyl-cholin injected into a branch of the portal vein were without effect upon the histamin fall of blood pressure.

Yohimbine caused a fall of blood pressure after an amount of atropine which had abolished a greater fall of pressure from acetyl-cholin; whether the depressor action was diminished by the atropine was not clear, for both the atropine and yohimbine caused a long continued lowered pressure and whereas yohimbine continued to cause the pressure to fall to the same level, or lower, both the absolute and percentile fall were less. In any case atropine has no action upon the yohimbine fall of pressure at all comparable to its action upon the effects of acetyl-cholin or pilocarpine. The latter was also true of *curare:* curare caused a fall of pressure after atropine but atropine diminished the fall somewhat. This antagonistic action of atropine towards curare had already been more carefully studied by Sollmann and Pilcher (66); they offer no explanation of the action.

Saline injection. Probably most experimenters are familiar with the fall of pressure which the intravenous injection of a small amount (1 or 2 cc.) of normal saline sometimes causes in certain animals but I have seen no discussion of it. I have observed this effect a number of times in cats but have made no systematic attempt to analyze it; the latter may prove difficult for, in my experience, the effect usually rapidly diminishes after a few injections and then can no longer be obtained. I have never observed this fall in the large number of experiments in which atropine had been injected; if it should be shown that atropine really does abolish the effect this would be of interest as suggesting that perhaps the same mechanism is involved as in the acetyl-cholin reaction. My own observations on this point are of practically no value for, with a single exception, the atropine was not injected until somewhat late in the experiment when the action, even if it had been present at all, would probably have spontaneously ceased. In the exceptional experiment to which reference was made the fall of pressure had been obtained ten times in succession in forty-five minutes and there was almost no indication that it was diminishing; about a minute after the last injection (1 cc. of a 0.9 per cent sodium chloride solution, which caused the pressure to fall from 107 to 80 mm.) 0.05 mgm. atropine sulphate was injected; the next injection of saline, made two and a half minutes later, caused only a very slight rise of pressure. A number of injections of saline were made during the next forty-five minutes but in no case was there a fall of pressure.

This saline fall of blood pressure was most frequently obtained in curarized cats or in cats anaesthetized with paraldehyde (1.7 cc. per K.); it occurred in animals in which the vagi, also in some in which the accelerators had been cut; it occurred not only with 0.9 per cent sodium chloride solutions but with Ringer's solutions of different formulas and with saline solutions freshly made with double glass distilled water and the purest (Kahlbaum) salts. It occurred in one animal in which a myocardiograph was attached to the right auricle; there was no weakening of the latter. The reaction seemed to be greater in animals especially susceptible to acetyl-cholin; and also in those in which the depressor action of epinephrin was pronounced. In one case the fall, which, as always, was of brief duration, was 51.1 per cent; usually it was between 20 and 35 per cent. Nearly all of the injections were made into a saphena vein; a few into a jugular. There was no change in the heart rate.

It is not improbable that this saline fall of pressure is analogous to

the flushings sometimes seen in sensitive individuals after intravenous injections of various kinds ("salvarsan," for example); possibly small doses of atropine would be found to have a prophylactic action.

Compounds related to cholin. The vasodilator action of cholin itself has been the subject of a number of investigations; among the most important of these were those of Mott and Halliburton (67), who found that the fall of pressure caused by cholin was replaced by a rise when atropine was injected, and of Müller (68) who made a further analysis of the action. The results of the studies with cholin have been somewhat contradictory (see (69), (70)) owing to the number of, often opposing, factors involved; no clear conception of the latter was possible until Dale's (1) work on the nicotine-like action of cholin on sympathetic ganglion cells but this does not clear up all points (e.g., the peripheral vasoconstriction after atropine found by Müller and evidently similar to that I have found with acetyl-cholin, and the apparent central action—sympathetic ganglion cell action?—described by Pilcher and Sollmann (71). As regards the vasodilator action of cholin: this has been less thoroughly investigated than that of acetyl-cholin but the action of the two seems to be qualitatively the same; as regards the quantitative results: Taveau and I in 1906 (72) found, in comparisons on the same animal, the acetyl compound to be about one hundred thousand times more active than cholin itself; in other experiments the difference has been even greater, much depending upon the anaesthesia and other factors.

I investigated, in collaboration with Taveau, seventy-nine compounds derived from cholin or analogous compounds (42), (72), (73); later, in collaboration with Menge (45), (74), (75), (76), I made a similar study of about twenty-five derivatives of the homocholins. Dale (1) has recently studied about a dozen other compounds related to this series. There is, therefore, a very large collection of data available for a consideration of the relation between the chemical composition and physiological action in this series.

While a detailed consideration of this subject belongs in a publication devoted to pharmacology and was discussed several years ago there are certain features which have a physiological interest. One of my general conclusions was that "the greatest effects upon the blood pressure are produced by those compounds which depart least from the choline type—that is, by those compounds containing the nucleus $HON\begin{matrix}(CH_3)_3 \\ CH_2CH_2O—\end{matrix}$; all changes in the group $(C_nH_{2n+1})_3$

or in the side chain (except the substitution of the terminal hydrogen atom) tended to diminish the effect upon the blood pressure but increased as a rule, the toxicity"—(there are exceptions to this as regards changes in the side chain); and further: "the greater the variation of the compounds from the cholin type, the less effect does atropine have in preventing a fall of blood pressure." These general principles were illustrated by the study of compounds containing ethyl, propyl, etc., groups in place of the methyl groups of cholin; in such compounds and their acetyl derivatives the acetyl-cholin type of action upon the blood pressure was absent or only slightly developed. An interesting illustration of the influence of methyl groups was seen in a comparison of the effects of the compounds $ClN\begin{matrix}(C_5H_{11})_3(iso)\\CH_2CH_2OH\end{matrix}$ and $ClN\begin{matrix}(CH_3)_2\\-C_5H_{11}(iso)\\CH_2CH_2OH\end{matrix}$; the former apparently had none of the blood pressure lowering property of acetyl-cholin whereas this was present, but to a less extent than with cholin itself, in the latter.

It is interesting to note in this connection that Launoy (77) found that compounds of the cholin type containing three methyl groups were far more active in stimulating pancreatic secretion than were analogous compounds containing ethyl, etc., groups.

I suggested that there may be a connection between the low toxicity of the cholin derivatives, as compared with the triethyl, etc., compounds, and their great physiological activity (when the H of the hydroxyl is substituted by appropriate groups, such as the acetyl group) and "the fact that cholin is a constituent of probably all plant and animal cells; these may have, to use the rather crude comparison of a mosaic (a figure of speech used by Ehrlich, one of the pioneer investigators in this field) places into which the compounds can fit and the cells themselves containing such groups are not injured by them as they would be by new and unusual groups;"[5] and further, "the rôle of this group is probably to carry the compounds to definite cell structures or, to use the comparison of Ehrlich, to make them fit in a certain mosaic; then the groups which have substituted the hydrogen atom enable the entire compound to exert a definite action (groups of the lower fatty series to depress the blood pressure, etc.)."

I also stated that "when this configuration is maintained it is pos-

[5] Joseph and Meltzer (78), found that the toxicity of magnesium, calcium, potassium, and sodium ions varies inversely to the proportion in which they occur in the serum of the animal.

sible to vary the intensity and character of action upon the circulation within extraordinarily wide limits by substituting groups in the place of the hydrogen atom" (of the hydroxyl). I illustrated this by substituting not only the acetyl but a large number of other acyl groups and found that the homologues of acetyl-cholin (propionyl-cholin, etc.) had the acetyl-cholin type of action upon the blood pressure, but that "as we pass up the series from the acetyl to the valeryl compounds we find a rapid diminution in the activity of these compounds in causing a fall of blood pressure" and an increase in their activity in other directions. The introduction of residues of the aromatic acids caused a still further departure from the acetyl-cholin type. Dale found that some of the simple esters and ethers of cholin had the acetyl-cholin type of action, but that none equalled this in intensity.

Another point of perhaps some physiological interest is the method employed to obtain a compound having the effect of acetyl-cholin upon the blood pressure but one with a more prolonged action and which would be sufficiently stable to be effective in small doses when given by mouth or injected subcutaneously. Taveau and I had noticed that acetyl-cholin is not very stable, undergoing hydrolysis somewhat readily, an observation since made by Ewins (79) and Fourneau and Page (80) and the physiological importance of which has been emphasized by Dale. It seemed possible that the compound might be rendered more stable by the substitution of certain groups in the side chain without destroying its physiological activity; it was these considerations which led Menge and me to take up the study of the homocholins with the result that Menge prepared the previously unknown α-methyl-cholin. The acetyl derivative of this compound has the physiological properties desired; it was so stable that a single subcutaneous injection of a few tenths of a milligram would keep the blood pressure uniformly lowered to one-half for hours. This low pressure could be almost instantly released, and to any desired extent, by the injection of atropine. This acetyl-α-methyl-cholin underwent hydrolysis less readily than did acetyl-cholin which probably accounts for its greater activity when injected subcutaneously or given per os. On the other hand certain other esters the chemical constitution of which led to the expectation that they would be very active were found not to be so, and Menge found these to be still more resistant to hydrolysis; thus the great activity of acetyl-cholin when injected intravenously seems to be connected with its instability. (Many interesting relations between the resistance of acetyl-cholin and some other esters and

ethers of cholin to the hydrolytic action of the blood, and, possibly, to the action of an esterase, and the action of these compounds upon various organs were discussed by Dale).

The introduction of the methyl group into acetyl-cholin caused a similar change in its action on the pupil: whereas acetyl-cholin applied to the cornea had no effect upon the pupil and even when injected intravenously caused but a brief constriction, acetyl-α-methyl-cholin applied to the cornea caused such a pronounced constriction as to suggest the possibility of its practical use as a miotic.

5. SUMMARY AND DISCUSSION

The above experiments, and those of the preceding paper, show that there is widely distributed in at least certain animals a vasodilator mechanism which has not hitherto been clearly recognized in physiology, pharmacology or pathology; the outstanding features of it are (1) its ability to respond, with great energy, to a limited group of compounds of the cholin type and to pilocarpine and perhaps a few others; (2) that this action is prevented by atropine; and (3) that it apparently is not connected with any of the known vasodilator nerves. It seems appropriate to speak of a "mechanism," for different blood vessels react unequally, i.e., something other than the contractile elements of muscular tissue is involved, and because the action is prevented by atropine; the latter reaction is usually interpreted as indicating the presence of a "nerve-ending" or of a "receptive substance" i.e., some kind of a "mechanism."

The compound to which this vasodilator mechanism responds most typically, or energetically, acetyl-cholin, has been taken as the typical parasympathetic nerve stimulant; Gaskell (4) speaks of the "acetyl-cholin group" of nerves (although, since most of these actions had been previously discovered in connection with muscarine the term "muscarine action" is more appropriate). As has been shown, especially by Dale, the action of this group "may be summarized, with but small qualification, as a reproduction of the effects of stimulating nerves belonging to the cranial and sacral divisions of the involuntary (autonomic) system." As pointed out by Dale, and investigated in more detail in my experiments, these compounds cause vasodilation not only in areas supplied by cranio-sacral vasodilators but in areas in which the existence of such nerves is anatomically not probable. An equally important qualification to the identification of the acetyl-

cholin vasodilator effect with that of parasympathetic nerve is the difference in the effects, in the two cases, of atropine: atropine readily abolishes the vasodilator action of acetyl-cholin in the regions of pelvic and chorda tympani nerve innervation whereas it has no effect upon the vasodilation caused by stimulating the former nerve and affects that of the latter only in very large doses. For the same reason it is impossible to identify other vasodilator actions of acetyl-cholin with that of the action of other parasympathetic or of any other vasodilator nerves: the effect of the drug is everywhere prevented by atropine, whereas that of nerve stimulation is nowhere so affected.

On the other hand the analogy between the action of acetyl-cholin and that of parasympathetic nerve stimulation upon so many organs is so striking that one is tempted to think of the vasodilator mechanism upon which acetyl-cholin acts as being constituted like the other mechanisms upon which it and the parasympathetic nerves act. It might be supposed that this mechanism has developed but has not become connected with nerves just as Dale and Laidlaw (81) suggested might be supposed to be the case with the uterus (which responds, when isolated, with contraction to pilocarpine and also, though feebly, to acetyl-cholin which actions are inhibited by atropine although there is no evidence that this organ receives a parasympathetic innervation). There may be no "need" for an extension of the parasympathetic nerves to the blood vessels just as there may be no "need" for their extension to the ventricle of the mammalian heart although this occurs in the frog. Especially interesting in this connection is the purely negative inotropic action upon the mammalian auricle which can be obtained with acetyl-cholin; the extent of the beat may be reduced almost to the vanishing point without there being any slowing. In the case of the auricle the substance upon which the drug acts is connected with nerves, whereas the similarly constituted substance in the blood vessels seems not to be connected with nerves. Possibly the substance in the blood vessels responds to the stimulus of some unknown hormone whereas that in the auricle is utilized for the more rapid adjustments under the nervous system. Of course the existence of similar mechanisms not intimately connected with nerves is not without analogy; one instance is the case of the uterus cited above, others are the intestines and urinary bladder: these are stimulated by pilocarpine and other "parasympathetic nerve stimulants" and the action of these is prevented by atropin whereas the effects of the vagus and pelvic nerves upon these organs, although the same as that of the

drugs, are not greatly modified by atropine. There are, however, very obvious differences between the two cases; the known vasodilator nerves can not for the most part be considered analogous to the vagus or pelvic nerves.

Of course it may be argued, as has been done in connection with the dilator action of pilocarpine, and of epinephrin on the salivary glands, that the vasodilator action of acetyl-cholin is due primarily to a stimulation of metabolism and that the dilatation is really due to certain products of metabolism. Such an argument may be applied with some plausibility to the dilator effect of acetyl-cholin upon the salivary glands which it also excites to increased activity. In fact a compound analogous to acetyl-cholin might be suggested as one of the "metabolites" found by stimulation of the chorda tympani to which the "vasodilator" action of this nerve has been attributed; if the cholin in the blood (50) which passes through the salivary glands, or possibly the minute amount which occurs in saliva, were present in the gland in the form of some of its esters or ethers these compounds might account for at least a part of the vasodilation following chorda tympani stimulation. But there is no warrant for suggesting that most of the vasodilator actions of acetyl-cholin are the result of increased metabolic activities.

Gaskell has suggested two "reasons" for the existence of vasodilator mechanisms: one to bring more blood to an active organ, another to regulate the blood pressure for the benefit of the heart. It has not been possible to show that the acetyl-cholin mechanism serves either of these functions; no evidence of any kind could be found that it is involved in the action of the depressor or of other nerves In fact, so far as has been determined, the mechanism seems as useless to the body as the host of receptors for various toxins, etc., postulated by Ehrlich; it may, however, be that it was evolved in connection with the metabolism of one of the constant constituents of the body, cholin.

The fact, however, that as yet no "function" can be ascribed to this vasodilator mechanism does not preclude the possibility that it may be utilized for therapeutic purposes on the one hand and, on the other, that it may be involved in pathological processes. It is generally believed that the body possesses, in the depressor nerve and its connections, a remarkable arrangement for the protection of the heart although it has never been satisfactorily shown how or when this may be called into activity; no suggestion has been made as to how any use of it for therapeutic purposes could be made. By the injection of

some of the cholin, and especially of the homocholin derivatives it is possible to control the blood pressure of an animal as effectively as by stimulation of the depressor nerve; possibly they may be found of therapeutic value not only in relation to general blood pressure but also in connection with local spasmodic contraction of blood vessels.

Whether this mechanism is involved in any pathological conditions is also unknown. It is conceivable that it is maintained in a condition of continued activity by products of metabolism and that its failure may be involved in some of the unexplained cases of hypertension. The fact that atropine, which prevents the action of cholin compounds upon the blood vessels does not ordinarily, unless perhaps, when the vagi are in a condition of great activity, cause a rise of blood pressure does not necessarily show that this mechanism is not in a condition of continued activity; atropine itself has a dilator action upon blood vessels (fig. 2, p. 242; also fig. 10, p. 212 of preceding paper) and moreover there are many compensating factors tending to maintain a normal blood pressure. On the other hand it may be that in some of the obscure pathological conditions in which atropine has been reported to be of value there is an abnormal activity of this mechanism.

CONCLUSIONS

The mechanism involved in the vasodilator action of acetyl-cholin and related bodies is different from that involved in the action of any of the nerves (posterior root, parasympathetic and sympathetic) to which vasodilator functions have been attributed. It is also different from that involved in the depressor action of epinephrin.

This mechanism, although capable of more energetic response than any hitherto described, is not involved in the action of the depressor or of other afferent nerves causing a fall of blood pressure.

The only substances found having the same type of vasodilator action as acetyl-cholin were a limited number of compounds derived from, or closely related to cholin and pilocarpine and colchicine.

Atropine and closely related substances were the only compounds found having a pronounced antagonistic action to the vasodilator action of acetyl-cholin. Pilocarpine diminished the action slightly. Physostigmine intensified all of the actions of acetyl-cholin.

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THE INFLUENCE ON THE THYROID OF ANASTOMOSIS OF THE PHRENIC AND CERVICAL SYMPATHETIC NERVES

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Received for publication December 8, 1917

These experiments represent an attempt to repeat the observations of Cannon, Binger and Fitz (1) on the effects of fusion of the anterior root of the phrenic nerve with the cervical sympathetic upon the thyroid function in cats. They state that symptoms resembling those of Graves' disease in man developed. There was marked tachycardia, loose movements of the bowels and falling of the hair. The animals were unusually excitable. The basal metabolism was greatly increased. The pupil was larger on the operated side. In one of the animals exophthalmos and respiratory hippus developed on that side. In one animal in which the symptoms were well developed (2), removal of the thyroid gland on the operated side stopped the progress of the disease. Whereas other animals had died within three months of the first appearance of the symptoms, this cat lived for seven months after the operation, when it was purposely killed. The conclusion is drawn that the symptoms were due to increased thyroid secretion, owing to the bombardment of the gland by impulses discharged along the phrenic synchronously with the respiratory discharge.

Troell (3), working mainly with cats and dogs, stated that he did not find any change in the pupil corresponding to the respiration. But he mentions that in the animals in which he looked for this, the pupil was still contracted and the nictitating membrane still forward on the operated side, indicating that the innervation had not yet been restored at the time the animals were killed. Accordingly it could not be expected that evidence of rhythmical discharges along the phrenic should be obtained on these animals.

Quite recently Burget (4) has reëxamined the question, with a negative result. The animals (cats and rabbits) after recovery from the operation were apparently normal in every respect.

Our experiments were made on ten cats. Early in 1916, the central end of the anterior root of the phrenic was sutured by a single stitch end to end to the cephalic end of the cervical sympathetic. One of the animals died one week after the operation from pneumonia, another one month after the operation from the common epidemic disease characterized by "snuffles," cough, sneezing, poor appetite, progressive emaciation and falling hair, which was prevalent at the time among our stock cats. Two of the cats were killed eight months after the operation on account of the same disease. One was lost after five months. The remaining five cats were in excellent health at the time they were killed (eight, eight and one-half, nine, eleven and one-half and twenty-one and one-half months, respectively, after the operation). Before the animals were sacrificed, the condition of the nerves above and below the site of the anastomosis was carefully tested by electrical stimulation. In all the ten animals systematic and frequent observations were made from the time of operation. In the eight which were kept for several months, the pupil on the operated side had regained equality with its fellow and the nictitating membrane was retracted to the same extent. One of the protocols is reproduced as a sample in condensed form.

Condensed protocol, cat 5, adult, female

January 29, 1916. Phrenic-sympathetic anastomosis made on left side. Between January 30, 1916 and November 17, 1917, when the animal was sacrificed, observations were made frequently during the first year and at less frequent intervals during the rest of the period. Within the first two or three months the contracted pupil on the operated side gradually became more like the pupil on the right (unoperated) side, equality being finally established and maintained until the animal was killed. Reactions to light and accommodation were equal in both eyes. At no time was respiratory hippus observed. Both eyes responded simultaneously and to the same extent to such stimuli as psychical disturbances. No evidence of any abnormal condition was at any time present. The cat became somewhat of a laboratory pet, feeding well and maintaining an excellent nutritional state. On March 12, 1916, she gave birth to five normal kittens. They were reared in and remained a long time about the laboratory. Towards the end of June, 1916, the animal again became pregnant; normal parturition.

November 17, 1917. Pupils equal, react equally to light, no change with respiration. Changes in the size of the left pupil are always accompanied by similar changes in the right. Anesthetized with ether—the pupils remain equal; the left nictitating appears slightly more forward than the right. The pupils react equally to light, as before the anesthetic. No respiratory hippus. Dissected down to the site of the anastomosis; found neuroma uniting the anterior root of

the phrenic with the cephalic end of the cervical sympathetic. The identity of the nerves was verified at autopsy. The various nerves above and below the neuroma were now stimulated repeatedly with a weak interrupted current, which could be just distinctly felt by the tongue. In all, about twenty stimulations were made. Stimulation of the sympathetic cephalad to the neuroma gave marked dilatation of the pupil and retraction of the nictitating. The same result was obtained on stimulating the anterior phrenic root central to the neuroma. In the meantime the phrenic root was not cut. Stimulation of the sympathetic caudad to the neuroma gave no effect on the eye unless the electrodes were very near the neuroma, when a slight dilatation of the pupil, but no retraction of the nictitating, was elicited. Stimulation of the neuroma itself always gave good dilatation of the pupil and retraction of the nictitating. These results were verified several times. Then the anterior root of the phrenic was ligated as high up as possible and cut central to the ligature. It was now again stimulated with the same result as before, namely, good pupil and nictitating reactions. Stimulation of the sympathetic caudad to the neuroma and cephalad to it, respectively, and stimulation of the neuroma gave the same results as before. On ligation and section of the anterior phrenic root it was observed that the left pupil became smaller than the right and remained smaller for the rest of the experiment. The thyroids were removed for histological examination: they were approximately equal in size and color, and appeared normal. The adrenals were normal in appearance and size; the left weighed, 0.212 gram, the right, 0.219 gram. Sections from the two thyroids, hardened and stained in the same manner, showed an identical histological picture, that of normal thyroid.

RÉSUMÉ OF RESULTS

In none of the animals were any symptoms resembling those of Graves' disease observed. In several it was proved by electrical stimulation that functional union had occurred between the phrenic and the cells of the superior cervical ganglion innervating the iris and the nictitating membrane. It was shown in several of the cats that a tonic dilator effect must have been exerted through the phrenic on the pupil of the operated side. This follows from the fact that in animals in which it was demonstrated by electrical stimulation that the phrenic caused dilatation of the pupil, while the sympathetic below the neuroma did not, the pupils on the two sides were equal. In the cat which was allowed to live longest, it was proved directly by nerve section that the phrenic was exerting a tonic dilator effect, for when the anterior phrenic root central to the neuroma was divided the pupil on that side became smaller than the other and remained smaller. In several of the cats the effect of stimulation of sensory nerves (central end of cut sciatic) on the pupil was noted. Dilatation occurred on the operated side at the same time as on the normal side and in the same

degree. In none of the animals was respiratory hippus seen although carefully and repeatedly looked for. If the dilator innervation is due to impulses from the respiratory center, there does not seem to be any obvious reason why a rhythmical change synchronous with the respiration should not be present. We can only state that it was not seen in any of our cats. Exophthalmos did not develop in any of the animals. The thyroids were always approximately equal on the two sides and of the same color on gross examination. Histologically no difference was observed between the thyroid on the operated and that on the normal side. Variations in the weight of the adrenals were within the normal range.

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OBSERVATIONS ON THE POSTURAL ACTIVITY OF THE STOMACH

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Received for publication December 11, 1917

In a paper recently published Sherrington (1) summarizes our knowledge concerning tonus, both of smooth and of striated muscle. He points out that the definitions of tonus heretofore advanced are unsatisfactory inasmuch as some are ambiguous while others serve only to account for certain phases of muscular activity.

The muscle fiber, he believes, is not to be regarded as an elastic string for it has the property of exhibiting different lengths while exhibiting one and the same degree of tension.

> Unstriated muscle, like skeletal muscle, evidently functions for two main purposes which in some ways it is possible and desirable to consider apart. Of these one is the performance of movements which overcome resistance by the development of tension; the other is the adjustment of contractile length without necessarily any alteration of mechanical tension.

Visceral tonus, in this sense, connotes "postural activity."

The investigations reported in this paper were undertaken with the idea of studying, in more or less detail, the postural activity of the gastric musculature.

Mosso and Pellacain (2) were among the first to observe that the internal pressure of the bladder in man and in the dog might be practically zero when the viscus was empty and when it was full. On the other hand, a considerable degree of pressure might develop in the presence of only a small amount of fluid.

Somewhat similar observations were subsequently made on the stomach by Müller (3), Moritz (4), Kelling (5) and Sick and Tedesko (6). Müller found that in rabbits the intragastric pressure remained constant during the period in which the chyme passed through the pylorus into the small intestine. And in man Moritz noted no appreciable changes in pressure during the period of gastric digestion.

In the course of some investigations concerning the state of tension

of the abdominal, the gastric and the intestinal walls Kelling found that within certain limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus. The administration of morphine, chloral, chloroform or large quantities of ether, however, partially or completely inhibited the activity of the mechanism responsible for this compensation. Light narcotization with ether, on the other hand, did not appear to interfere with the reaction. Kelling concluded that the tonus of the stomach is an expression of the activity of the intrinsic nervous mechanism and that it is more or less independent of the central nervous system.

Sick and Tedesko observed that the gradual filling of a cat's stomach was not accompanied by any rise in internal pressure. The stomach, they thought, might be compared to the heart, the fundus resembling the auricles and the pyloric part the ventricles. The active relaxation of the musculature of the fundus which accompanies the entrance of food into the stomach Sick (7) regarded as the active diastole of the viscus.

While a number of investigators have thus noted the ability of the stomach to accommodate its capacity to a given load without exhibiting any appreciable rise in intragastric pressure, none of them appear to have regarded this form of receptive relaxation as an actual expression of reflex tonus, i.e., postural activity (Sherrington). The prevailing view seems to have been rather that this response on the part of the stomach is brought about by a lowering of the level of gastric tonus, meaning by tonus a state of tension in the gastric musculature. Sick, for example, adopting the hypothesis of v. Uexküll held that the distension of smooth muscle rests upon a compromise between the condition of excitation of the muscle and the load. A persisting condition of excitation (Erregungszustand) in the muscle v. Uexküll (8) termed tonus.

METHODS

Both rabbits and cats were used in the course of the experiments. In order to follow the changes in the postural configuration of the stomach the empty viscus was slowly filled with warm physiological saline solution. The fluctuations in the intragastric pressure were taken as an index of the adjustment of the smooth muscle to its new conditions.

When the observations were conducted on living animals a tube was introduced into the pyloric end of the stomach. This was connected

by means of a two-way stopcock with a chloroform manometer and a reservoir of salt solution. The latter was so arranged that it could readily be elevated or lowered to any desired level. After closing the oesophagus with a ligature the body of the animal was submerged in a bath of physiological saline solution the temperature of which was maintained at 38°C. by means of electric light bulbs. Interposed between the tube entering the stomach and the stopcock mentioned above was a glass coil which was also submerged in the bath. This served to warm the fluid entering the stomach.

In certain experiments the pylorus was ligated and the inlet tube made to enter the oesophagus. This reversal of the direction of the fluid entering the gastric cavity did not appear to affect, materially, the behavior of the stomach. Ultimately it was found that for the purposes of a comparative study (in the cat) additions of 5 cc. of fluid made every minute afforded the most satisfactory rate of increase of gastric contents. While a number of experiments were prolonged until the stomach ruptured, in order to determine the limits of muscular relaxation, in most instances the observations were discontinued some time previous to this.

Manometric readings were made with each addition of fluid. The values were then plotted. In the accompanying charts representative curves are presented which illustrate graphically the postural activity of the gastric wall under the conditions imposed by the experiments. Figure 1 is plotted on a different scale from that used in the remaining figures since in this experiment the additions of fluid were continued until the viscus ruptured.

The animals were all anaesthetized with ether. After being placed in the bath, they were kept in the primary stage throughout the periods of observation. Fairly deep anaesthesia, however, did not appear to interfere with the postural activity of the stomach.

When the vagi were used a cannula was introduced into the trachea. The nerves were then transected in the neck. The splanchnics were reached externally through a lumbar incision on either side.

Where observations were made on the excised stomach the viscus was connected with the manometer and reservoir, and then either immersed in the warm saline bath or placed in a moist chamber.

No attempt was made to ascertain the exact pressure in the normal starving stomach. Such determinations involve certain difficulties which it did not seem advisable to meet, since the objects of this study had nothing to do with the empty viscus.

During the preparation of the animal care was exercised to prevent the entrance of air into the stomach. A few centimeters of fluid were permitted to flow into the viscus. By adjusting the manometer the intragastric pressure was brought to zero. The experiment was then started.

The part played by the abdominal wall in these experiments is naturally not a negligible one. Even though the gastric musculature exhibits a compensatory relaxation with each addition of fluid the intragastric pressure must rise unless there is a compensatory increase in the capacity of the abdominal cavity. Such changes in the abdominal musculature have recently been demonstrated by Pike and Coombs (9). They believe that their experiments show that the regulation of the length of the abdominal muscles corresponding to the increase in volume of the contents of the abdominal cavity is a reflex process which falls into line with the other known instances of postural activity of muscle and nerve.

Care was exercised in the experiments conducted with the abdomen closed not to interfere with this reflex. In each instance, moreover, the experiment was repeated on another animal in which the abdominal wall had been widely opened in the saline bath.

RESULTS AND DISCUSSION

Normal postural activity. Figure 1 gives a graphic record of the postural adaptation of the stomach of the rabbit to slow increments in the volume of its contents. In the experiments represented by curves *A* and *B*, 2.5 cc. of saline solution were allowed to enter each stomach every two and one-half minutes until the viscus ruptured. Curve *A* shows a rather marked fluctuation in the degree of intragastric pressure up to near the conclusion of the experiment. This was probably due, in part at least, to peristaltic contractions—the animals had been starved for two days.

The striking feature of curve *A*, however, is the low average pressure which was maintained throughout the greater part of the experiment. The intragastric pressure only rose abruptly shortly before the organ ruptured. In contrast to this, curve *B* is shown. It represents the changes in pressure which occurred in an excised stomach.

The changes observed in the course of approximately similar experiments conducted on cats are pictured in curves *A* and *B*, figure 2 and curve *A*, figure 3.

In certain experiments fluid was withdrawn and re-introduced several times in succession. As compared with the level of intragastric pressure noted during the course of the primary filling, the levels during the second and third fillings were somewhat higher in some instances and a little lower in others. In general the same nice adjustment of the configuration of the viscus to its load was manifest.

Kelling (5) and Sick and Tedesko (6) found that within certain

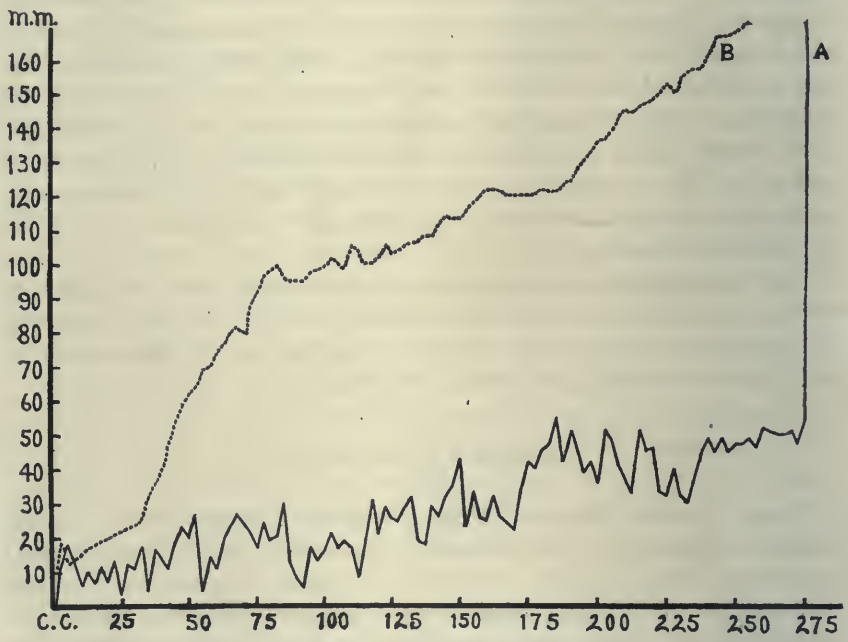

Fig. 1. *A*. Curve of intragastric pressure in a living rabbit; 2.5 cc. added every two and one-half minutes. Abdomen closed. *B*. Curve of intragastric pressure in an excised rabbit stomach immersed in physiological saline solution at 38°C., 2.5 cc. added every two and one-half minutes.

limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus. By this it might be inferred that the relaxation of the walls becomes complete after each addition to the contents. It is probable, however, that in their work small increments of pressure were disregarded. In the fifty odd animals used throughout the course of this work no exception was found to this rule that any considerable increase in volume of contents was accompanied by a measurable rise in pressure. Often this was masked temporarily by the

effects of peristalsis; at other times a temporary drop took place, the exact explanation of which remained obscure though it suggested certain experiments of Rogers and Hardt (10) in which they observed that both in man and in the dog the fundic end of the stomach, during normal digestion, exhibited a slow tonus rhythm.

These results are in no way conflicting with our knowledge of the physiology of the gastric musculature. Cannon (11) has shown that "gastric peristalsis is augmented or weakened, is caused to appear or disappear as the tension on the contents is increased or decreased." And Alvarez (12) who places the seat of origin of the waves at a higher level in the stomach (cardia) believes that they may deepen into appreciable peristalsis at the place where the conditions defined by Cannon are right. It seems that a certain low level of intragastric pressure is essential to the motility of the stomach.

These conditions, then, serve to explain why the postural relaxation of the stomach seldom appears to be complete. A certain low degree of tension—dependent, in part at least, upon the volume of the contents—prevails so long as the viscus functions in the process of digestion.

Kelling (5) noted that time is an essential factor in what he termed the regulation of the intragastric tension. When the viscus was rapidly filled there was comparatively little compensatory relaxation of the walls. Curve *D*, figure 2, pictures the range of intragastric pressure observed subsequent to the sudden introduction of 150 cc. of saline solution into the empty stomach of a cat. During the course of the first five minutes there was a progressive rise of pressure up to 42 mm. of chloroform, a level which is almost half again as high as that observed after the gradual introduction (one-half hour) of the same quantity of fluid (curve *A*, fig. 2). Following this point the pressure very slowly sank to 28 mm.

Splanchnics and vagi. Carlson (13) has shown that section of the splanchnic nerves increases gastric tonus and augments hunger contractions. Section of both the splanchnics and the vagi, on the other hand, leads to a permanent hypotonus, except under conditions of prolonged starvation. Gastric hunger contractions, nevertheless, do persist after isolation of the stomach from the central nervous system.

In cats Cannon (11) found that cutting the vagi caused a temporary loss of tonus and an absence or marked weakness of peristalsis. He concludes that,

their function is solely to set the gastric muscles in a tonic state, to make them exert a tension, so that they are as if stretched by the contents, and the result of this condition is peristalsis.

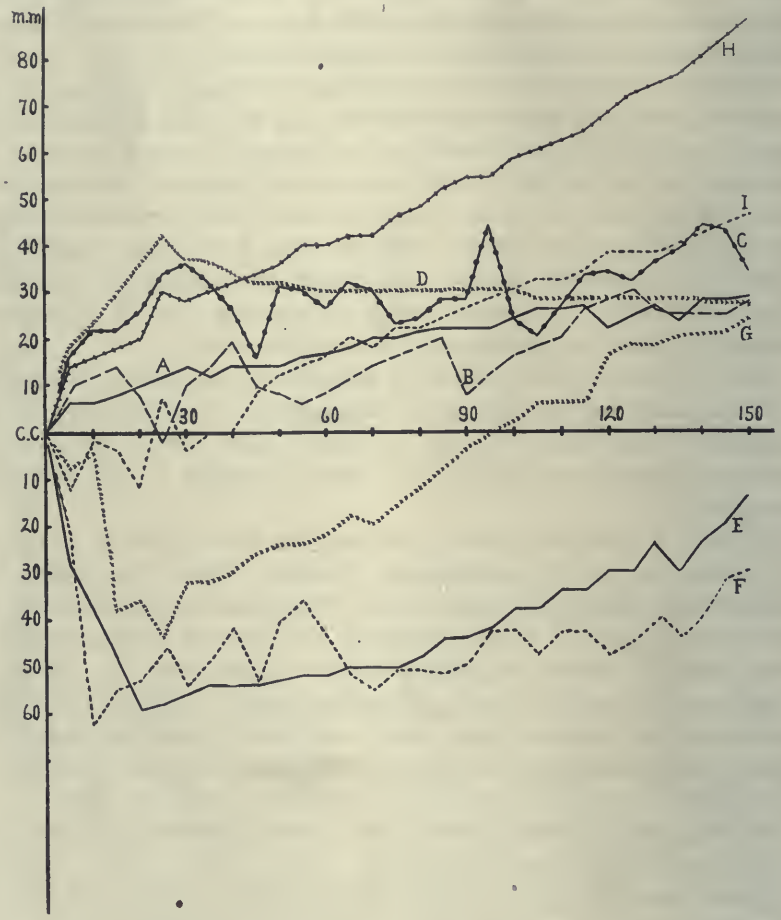

Fig. 2. *A*. Curve of intragastric pressure in a living cat; 5 cc. added every minute. Abdomen closed. *B*. Cat. Living. Abdomen open in physiological saline solution at 38°C., 5 cc. added every minute. *C*. Cat. Living. Abdomen closed. Splanchnic nerves divided; 5 cc. added every minute. *D*. Cat. Living. Abdomen open in salt solution; 150 cc. added at once. *E*. Cat. Living. Abdomen closed. Splanchnics and vagi divided; 5 cc. added every minute. *F*. Cat. Living. Abdomen open in salt solution. Splanchnics and vagi divided. *H*. Cat. Living. Abdomen closed. Splanchnics and vagi divided. 1 cc. 0.1 per cent solution of nicotine into jugular vein. *I*. Cat. Living. Abdomen open. Vagi divided.

Kelling (5) likewise concludes from his experiments that the vagi exert a very decided influence upon the tension of the gastric musculature. His results, however, were somewhat variable. He found that bilateral section might show no effect on the total tension of the viscus; again it might cause some decrease of tone, or it might raise it. Unilateral section (left) caused an increase of tonus which disappeared when the other vagus was cut. But these findings were not always the same.

Curve *C*, figure 2, affords a picture of the effects of bilateral section of the splanchnics on the postural activity of the stomach. The absence of the high tension which characterized the behavior of the excised stomach (curve *A*, fig. 3) pointed to the adaptation of the musculature to its burden. On comparing it with curve *A*, figure 2, it becomes evident that subsequent to the division of the nerves the pressure continued slightly more elevated throughout the entire period of observation. The fluctuations in pressure were due, in greater part at least, to exaggerated peristalsis.

The effects observed after section of one and of both vagi varied greatly from animal to animal. In the experiments represented by curves *I*, figure 2, and *F*, figure 4, the two vagi alone were divided; in those represented by curves *E*, *F* and *G*, figure 2, the splanchnics were also transected. From these curves it is apparent that the loss of the vagi results in no consistent change in the behavior of the musculature, so far as the postural activity of the organ is concerned. While the tension of the wall is observed to fall in certain instances (curves *E*, *F*, *G*, fig. 2), in others it rises to a level somewhat higher than that observed in the intact stomach (curve *I*, fig. 2). The fall in intragastric pressure, when it occurred, did not take place immediately subsequent to the section of the nerves. It appeared only as fluid was introduced into the stomach, during the process of accommodation in the musculature of the wall. The behavior of the wall here was very different from that of an inert, elastic body.

The development of a negative pressure in these experiments is explicable on mechanical grounds. In the course of the readjustment accompanying each increase in load, the absence of the pressor nerve fibers led to an exaggerated relaxation of the musculature, and this sufficed to lower the level of atmospheric pressure established at the outset of the observations. This of course does not imply that a negative pressure may develop in the stomach of an otherwise intact animal when the extrinsic nerves are sectioned, since normally there

exists a positive intragastric pressure. It simply illustrates how the degree of tension of the gastric wall, which characterizes the activity of the intrinsic nervous mechanism, may be influenced by means of its extrinsic nerve supply. Pressure changes of this character may be studied by means of a simple apparatus constructed from glassware and rubber dam. These changes in intragastric pressure, of course, are something distinct from the postural activity of the musculature.

The explanation of the differences in reaction among the animals is not clear. The findings, however, which are more or less consistent with those obtained by Kelling, serve to indicate that just as the essential characteristics of the hunger contractions of the empty stomach are determined by the local gastric motor mechanism rather than by the character of the central innervation (Carlson (13)), so the essential characteristics of postural activity are determined by the local neuro-muscular mechanism.

Cannon and Lieb (14) have shown that the inhibition which follows deglutition and leads to the receptive relaxation of the stomach is produced by way of the vagus nerves. From the considerations given above it is clear that a receptive relaxation may also take place in the absence of any extrinsic nerve supply.

Intrinsic nervous mechanism. In the course of their work on the law of the intestine Bayliss and Starling (15) found that both the local use of cocain and the intravenous administration of nicotine caused the true peristaltic movements to cease. The pendulum movements however persisted. This led them to believe that by the use of these drugs one might bring about a paralysis of the local nerve ganglia.

This conclusion has since been disputed, particularly by Magnus (16). The fact, however, as Gunn and Underhill (17) have pointed out, that the isolated intestine may continue to beat rhythmically in a solution of nicotine 1 to 1000 would suggest either that those movements are independent of nerve ganglia or that these ganglia are unusually resistant to nicotine.

Curve *C*, figure 3, furnishes a graphic record of the changes in intragastric pressure which were observed in an experiment in which the external surface of the stomach was painted with a 2.5 per cent solution of cocain. On comparing it with curve *A*, figure 2, (representing the reaction of a normal stomach) it became evident that the application of the drug led to a moderate increase in the tension of the gastric wall. This may have resulted from the local stimulating effects of the drug.

Curves *H*, figure 2, and *D*, figure 3, illustrate the changes in intragastric pressure observed following the intravenous injection of a large dose of nicotine (1 cc. of a 0.1 per cent solution into the jugular vein.) In one experiment, subsequent to the division of the vagi and splanchnics, the stomach was slowly filled with saline solution. Curve *E*, figure 2, furnishes a record of the changes in pressure noted during this period of the observations. The stomach was then emptied. After an interval of rest nicotine was injected intravenously and the

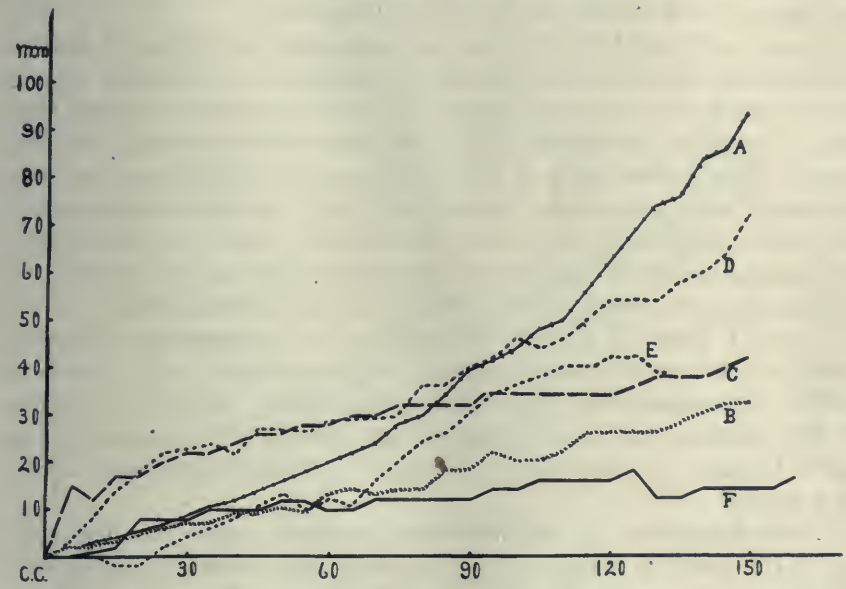

Fig. 3. *A*. Curve of intragastric pressure in an excised stomach (cat) in salt solution; 5 cc. added every minute. *B*. Cat. Living. Abdomen open. Pilocarpin $\frac{1}{120}$ grain. *C*. Cat. Living. Abdomen closed. Stomach rapidly painted with 2.5 per cent cocain solution. *D*. Cat. Living. Abdomen open. Splanchnics and vagi divided; 1 cc. 0.1 per cent solution of nicotine into jugular vein. *F*. Cat. Living. Abdomen open. Atropin $\frac{1}{300}$ grain in two doses.

filling process repeated. Curve *H*, figure 2, depicts the changes occurring in this half of the experiment.

The striking difference in the behavior of the muscle fibers in the two instances would appear to indicate that the drug had affected either the intrinsic nervous mechanism or the muscle cells directly. The fact, however, that the isolated intestine may continue to beat rhythmically in a solution of nicotine suggests that the latter possibility is the less likely.

The results from another experiment (not illustrated by a curve) also point toward the importance of Auerbach's plexus in the postural activity of the stomach. In this case the viscus was excised while the animal was in the primary stage of ether anaesthesia and placed immediately in oxygenated Locke's solution at 38°C. where it was kept throughout the period of filling. A curve plotted from the series of pressures observed corresponded closely to curve *A*, figure 3; that is, it showed an absence of the delicate adjustment of the musculature to its load which was noted in the normal stomach.

Gunn and Underhill (17) have demonstrated that strips of gastric musculature may continue to beat rhythmically for hours at a time under conditions of temperature and nutrition similar to these; and this occurs when the ganglia have been completely removed from the strips. The muscle cells of the gastric wall, in the presence of the necessary salts and oxygen, accordingly, may function independently of nervous influences. It seems fair to assume, then, that the imperfect relaxation of the walls in the experiment cited above was due, in part at least, to changes in the intrinsic nervous mechanism.

Curve *A*, figure 3, represents the effects on intragastric pressure of excising the stomach and keeping it in physiological saline solution at 38°C. throughout the period of the experiment. In curve *D*, figure 4, approximately the same changes are figured. The organ in the latter instance was kept in the ice chest for twenty-four hours before it was used.

Curve *B*, figure 4, on the other hand, shows a higher range of pressures, the result probably of a still greater interference with the postural activity of the muscle cells. The stomach, in this case, was kept for twenty-four hours at room temperature. This observation is in line with the experiences of Gunn and Underhill (17) who found that while the maximal time of survival of the intestine is somewhat over twenty-four hours when it is kept at 15°C., it is only about a third of that time when kept at 37°C. Alvarez (18) had much the same experience with specimens from the stomach.

A still poorer exhibition of postural adjustment in the freshly excised organ was observed when the stomach was kept in a moist chamber instead of in the usual saline bath (curve *C*, fig. 4.) This may have been due, in part, to vascular conditions since Hooker (19) has shown that the predominating effect of CO_2 upon the alimentary tract is to cause contraction of the muscle when in an arhythmic state. Curve *A*, figure 4, shows the effects observed from freely painting such a

viscus with cocain. In this concentration the drug probably acted as a general protoplasm poison.

Pilocarpin, atropin, adrenalin. Curve *B*, figure 3, pictures the results obtained with pilocarpin. The drug was injected immediately before the observations started in sufficient amounts to produce saliva-

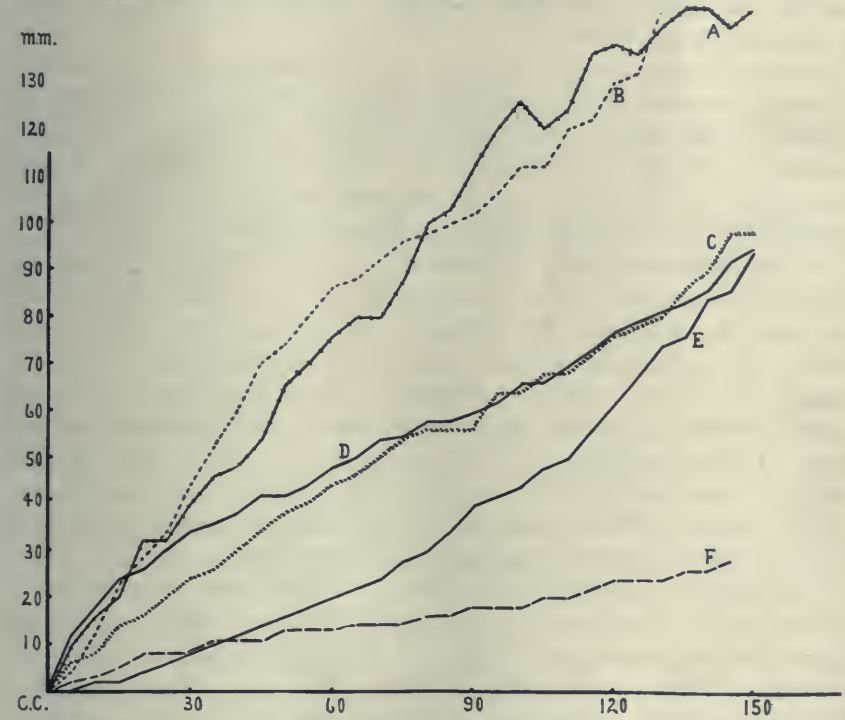

Fig. 4. *A*. Curve of intragastric pressure in an excised stomach (cat) kept in a moist chamber. Freely painted with 2.5 per cent solution of cocain. *B*. Excised stomach of cat in salt bath. Kept for twenty-four hours at room temperature. *C*. Excised stomach of a cat (fresh) in moist chamber. *D*. Excised stomach of a cat in salt bath. Kept for twenty-four hours in ice box. *E*. Excised stomach of a cat (fresh) in salt bath. *F*. Cat. Living. Abdomen open. Vagi divided.

tion. In general the reaction of the stomach was very similar to that noted when no drug was used (curve *B*, fig. 2). Larger doses, of course, in bringing about exaggerated peristalsis raise the intragastric tension considerably. Pilocarpin, it would appear then, exercises no direct influence on the mechanism responsible for postural activity; and affects it indirectly only when it is used in large amounts.

To study the effects of atropin the drug was injected both at the outset of the observations and also after 75 cc. of fluid had been run into the stomach (curve *F*, fig. 3). On the whole the musculature adapted itself to the increments of load with very slight changes in tension. The pressure at the conclusion of the work was approximately one-half that noted at a similar point in the experiment with a normal stomach (curve *B*, fig. 2).

Observations similar to this have led to the statement that atropin in proper dosage decreases gastric tonus. Atropin does lessen the tension of the gastric musculature through its action on the vagus terminals. Tonus or postural activity however, is something different from this. In eliminating certain extrinsic influences the drug nevertheless acts in a way which favors postural changes in the wall.

In the experiments with adrenalin 1 cc. of a 1: 10,000 solution was injected intravenously immediately before the first 5 cc. of saline were run into the stomach. Subsequent to the introduction of 50 cc. of fluid, 2 cc. additional of the drug were administered. Curve *E*, figure 3, illustrates such an experiment. For a few minutes following each injection of the drug there was a slight fall in intragastric pressure. This is suggestive of the experience of Cannon (11), namely, that the administration of a small dose of adrenalin will abolish intragastric pressure. Subsequent to each of these temporary depressions, however, the intragastric pressure rose rather rapidly.

The fall after each injection may be accounted for through the action (stimulation) of the drug on the depressor fibers of the sympathetic system in the gastric wall. The rises in pressure are less readily explained. There are several possibilities, however. The drug might lead eventually to a depression of the depressor fibers, or a heightened vagus action might follow after the drug had ceased to act.

CONCLUSIONS

The experiments outlined above were carried out in order to study, in some detail, the postural activity (Sherrington) of the gastric musculature. From the results of the work the following points may be emphasized.

The normal stomach possesses a striking capacity for adapting its size to the volume of its contents with only minimal changes in intragastric pressure. This capacity disappears only shortly before the viscus ruptures.

Time is an essential factor in the expression of this form of muscular activity—as the rate of change in volume of contents increases, the extent of the postural activity decreases.

The extrinsic nerves have nothing directly to do with the postural configuration of the viscus. The mechanism responsible for these changes concerns solely the musculature itself together with the intrinsic nervous mechanism.

Pilocarpin, atropin and adrenalin likewise have only an indirect influence upon postural activity. They neither increase nor decrease gastric tonus, but, like the extrinsic nerves, serve to regulate the tension of the stomach walls.

Excision of the stomach leads to a marked decrease in its postural activity, due in greater part probably to changes in the intrinsic nervous mechanism.

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THE RÔLE OF CATALASE IN "SHOCK"

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Received for publication December 12, 1917

Henderson (1) observed that the oxygen intake in "surgical shock" is decreased by about 45 per cent and that it is accompanied by a condition of acidosis. Presumably, therefore, oxidation must be decreased in the condition of "shock." We have shown that when oxidation is increased as for example in the excitement stage of anaesthesia, or in combat or by increasing the amount of work, there is a corresponding increase in catalase, an enzyme in the tissues which can liberate oxygen from hydrogen peroxide, and that when oxidation is decreased as for example by decreasing the amount of work, or in deep narcosis, or rendered defective as in pancreatic diabetes, there is a corresponding decrease in catalase. The object of the present investigation was to determine whether or not there is a decrease in the catalase of the tissues in "shock" corresponding with the decrease in oxidation. In view of the fact that catalase is decreased by the administration of anaesthetics and that the decrease is proportional to the depth of anaesthesia, precautions were taken in all the experiments reported in this paper to see that just sufficient ether was administered to keep the animal quiet and unconscious and that this amount was kept as uniform as possible. Determinations of the catalase of the blood were made in all the experiments during the administration of ether and previous to the production of "shock" to see that the catalase of the blood had become practically constant. When this was found to be the case "shock" was produced in the ways described and the catalase determined. Dogs and cats were used in the experiments. "Shock" was produced in the cats by exposure and manipulation of the intestines, and in the dogs by bleeding.

The cats were etherized and determinations of the catalase of the blood made and when these determinations were found to be constant, they were taken for comparison as the normal catalase content of the blood of the animal. The abdominal wall of the cat was then opened

by making a mid-ventral incision and the intestines were drawn out and handled. The catalase of the blood was determined at intervals of twenty minutes after the opening of the abdomen, as had been done previous to the opening of the abdomen. The blood pressure was taken in the carotid artery by means of a mercury manometer at the time of each determination of catalase. The catalase was determined by adding 0.5 cc. of blood to 250 cc. of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted graduated cylinder previously filled with water. After the volume of gas thus collected in ten minutes had been reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The bottles were shaken in a shaking machine during the determinations at a fixed rate of about one hundred and eighty double shakes per minute.

The curve in figure 1 was constructed from data obtained from a cat previous to the production and during the development of "shock." The lower figures (0 to 360) along the abscissa indicate time in minutes while the upper figures indicate blood pressure in millimeters of mercury. The figures along the ordinate (0 to 540) indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood taken from the external jugular vein. It may be seen that during the two 20-minute periods or 40 minutes, during the administration of ether but previous to the opening of the abdomen, 0.5 cc. of blood liberated 450 and 450 cc. of oxygen in ten minutes from hydrogen peroxide, and that the blood pressure was 105 and 103 mm. of mercury respectively; that during the succeeding five periods of 20 minutes each after the abdominal wall was opened and the intestines exposed, the blood liberated 440, 420, 420, 420 and 425 cc. of oxygen respectively, and that the blood pressure had fallen from 103 to 60 mm. of mercury. Hence during the five periods of 20 minutes each, or 100 minutes of exposure and handling of the intestines and administration of ether, the blood pressure had not fallen to a very low level, and there was a very small decrease in the catalase of the blood as is indicated by the small decrease in the amount of oxygen liberated from the hydrogen peroxide. During the succeeding ten periods of 20 minutes each, however, it will be noted that the blood pressure fell from 60 to 26 mm. of mercury and that during this time the catalase of the blood decreased by about 37 per cent as is indicated by a decrease in the amount of oxygen

liberated from 425 to 270 cc. It should be mentioned in this connection that the condition of the animal during the last two hours of the experiment was such that it was unnecessary to administer any ether,

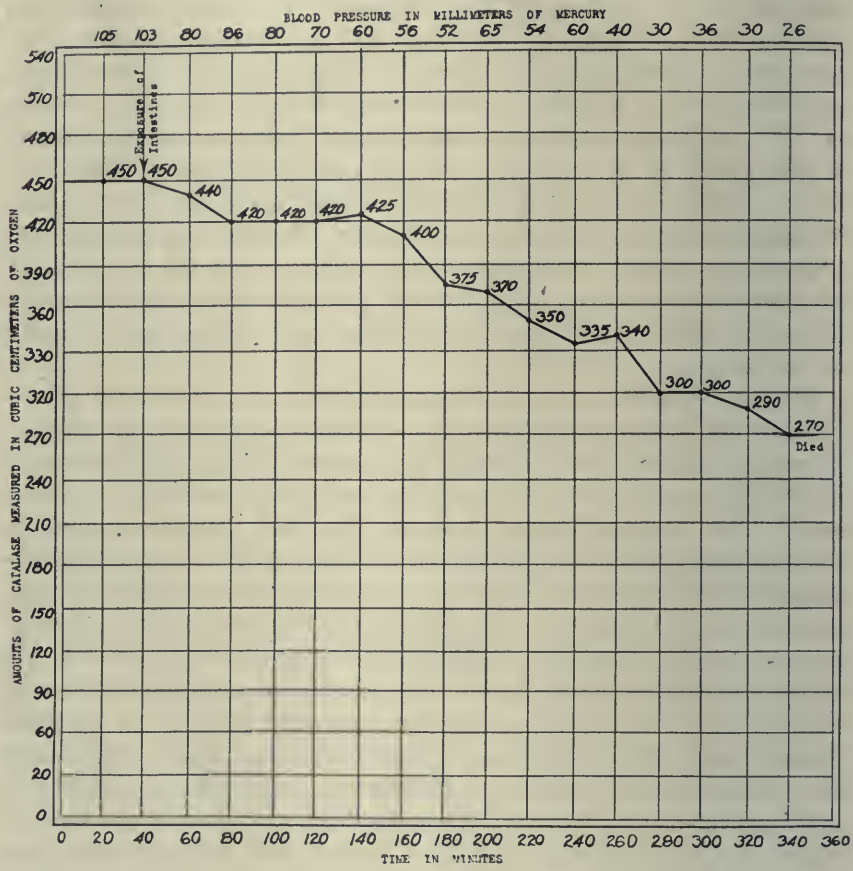

Fig. 1. Curve showing the effect of "shock" on the catalase content of the blood. The lower figures (0 to 360) along the abscissa indicate time in minutes, the upper figures along the abscissa indicate blood pressure in millimeters of mercury. The figures (0 to 540) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

hence the ether could not have played any part in this decrease in catalase. A discussion of the cause of the decrease in the catalase of the blood of the cats in the condition of "shock" will be given in the discussion of the results from dogs in this same condition.

Several dogs, weighing about 10 kgm. each, were bled approximately 5 per cent of the body weight. The blood was taken from the external jugular by means of a hypodermic needle attached to a flask in which a partial vacuum was created. Previous to being bled the dogs were slightly etherized, and during this period of slight anaesthesia determinations of the catalase of the blood were made. When these determinations were found to be constant, they were taken for comparison as the normal catalase content of the blood of the animal. The blood was then drawn from the external jugular at the rate of approximately 15 cc. per minute until 500 cc. of blood had been taken. After losing this amount of blood, the dogs were too weak to walk and would lie more or less quietly in apparently a semi-conscious condition. It was assumed that these animals were in the condition known as "shock." At the intervals given along the abscissa in chart 2, samples of blood were taken from the external jugular and the catalase content determined. The determinations were made in the same manner as those with the cat's blood except that 50 cc. of hydrogen peroxide were used instead of 250 cc. because of the low catalase content of the dog's blood.

Curves *a*, *b* and *c* in figure 2 were constructed from data obtained from dogs in the condition of "shock" produced as described. It will be seen that 0.5 cc. of the samples of blood taken from the different dogs during the administration of ether, but previous to the production of "shock," liberated 52, 48 and 42 cc. of oxygen respectively, and that two hours after the hemorrhage, when the animals were in the condition of "shock," the catalase had decreased by approximately 40 per cent as is indicated by the decrease in the amount of oxygen liberated to 32, 28, and 20 cc. respectively.

The explanation that suggests itself for the decrease in the catalase of the blood of the dogs and of the cats in the condition of "shock" is the decreased output of catalase from the liver and possibly the dilution of the blood by the diffusion of liquid, poor in catalase, from the tissues into the blood stream. The following observations are offered in support of the part played by the liver in this respect. The portal vein and the hepatic artery of a dog were tied off at 12.45 a.m. At 1.45 a.m. the blood pressure had fallen from 140 mm. of mercury to 22 mm. and the catalase of the blood had decreased by 50 per cent. Only the portal vein of another dog was tied off at 2.20 a.m. At 3.20 a.m. the same great fall in blood pressure was observed but the decrease in the catalase of the blood was not so extensive being only 30

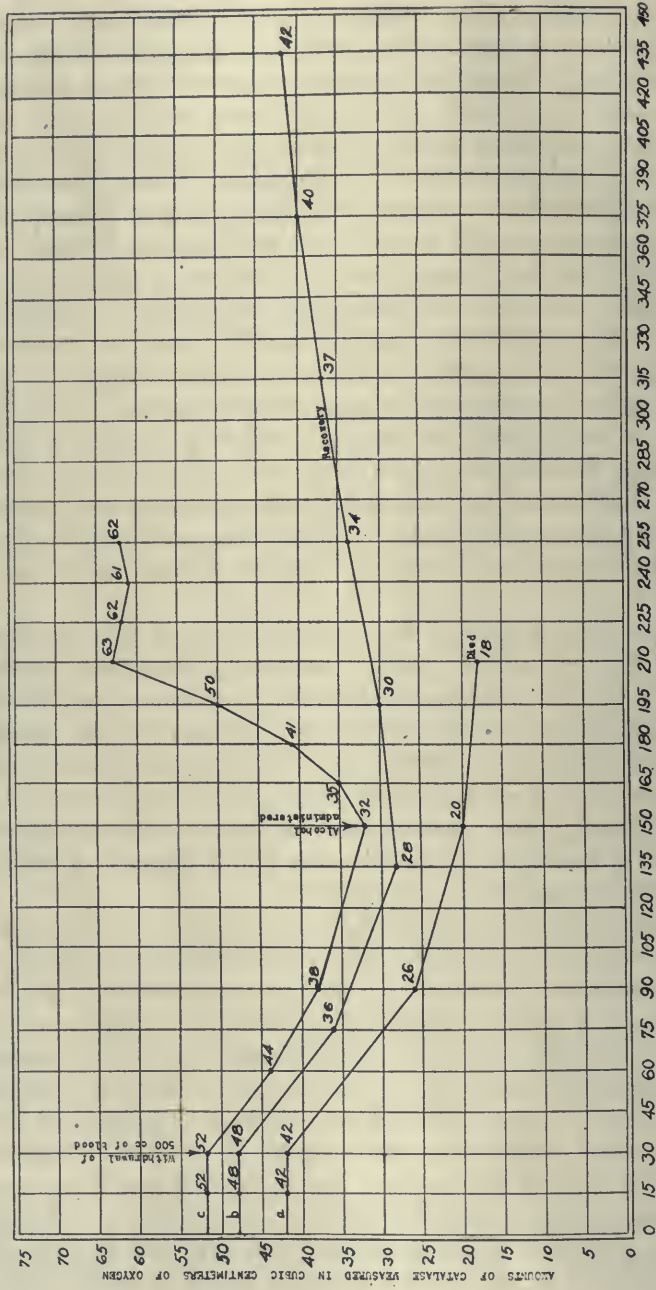

Fig. 2. Curves showing the effect of "shock" on the catalase content of the blood and the effect of the administration of alcohol. The figures (0 to 450) along the abscissa indicate time in minutes. The figures (0 to 75) along the ordinate indicate amount of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

per cent as opposed to the 50 per cent decrease when the entire blood supply was cut off from the liver. In the first case all the blood supplied to the liver being cut off, the output of the catalase from the liver was reduced to zero with the corresponding great decrease in the catalase of the blood. In the second case the blood supply being partially cut off, the liver continued to pour catalase into the blood with the resulting smaller decrease in catalase. We had found that when the liver was cut out of the circulation by an Eck fistula and by tying off the hepatic artery, the decrease in the catalase of the blood was not so great as when the portal vein as well as the hepatic artery was tied off with resulting decrease in general arterial pressure. This led us to the adoption of the second part of the explanation for the decrease in the catalase of the blood in the condition of "shock" namely, the dilution of the blood by the diffusion of liquid from the tissues. I know there is evidence that in "shock" there may be a diffusion of liquid from the blood into the tissues instead of from the tissues into the blood stream; however, as I have seen the evidence, the question seems to be an open one still. If there is a diffusion of liquid and the contained catalase from the tissues into the blood in "shock" this should produce a decrease in the catalase of the tissues. The catalase content of the different tissues of all the animals used in this investigation was determined and the results were found to be very irregular, although on the whole there was probably a decrease.

The dog from which data for curve *a* in figure 2 were obtained died about three hours after the production of "shock," when the catalase of the blood had been decreased by approximately 60 per cent as is indicated by a decrease in the amount of oxygen liberated from 42 to 18 cc. The condition of the dog from which data for curve *b* were obtained, began to improve about four hours after the production of "shock," and along with this improved condition, as may be seen in the chart, there was a gradual increase in the catalase content of the blood. Reasons will be given shortly for believing that the increase in catalase during the period of recovery from "shock," was due to the increased output of catalase from the liver. One hundred cubic centimeters of 40 per cent ethyl alcohol were introduced into the stomach of the dog from which data for curve *c* were obtained about three hours after the production of "shock." At the time of the introduction of alcohol it will be seen that the catalase had decreased by approximately 40 per cent from the normal as is indicated by a decrease in the amount of oxygen liberated from 52 to 32 cc. It may be seen

that the catalase of the blood increased very rapidly after the introduction of the alcohol, and that after one hour it had increased more than 90 per cent, as is indicated by the increase in the amount of oxygen liberated from 32 to 63 cc. In some unpublished results, we found that the introduction of alcohol into the stomach of an animal produced a very rapid increase in the catalase of the blood provided the liver was not excluded from the circulation, but that when this organ was excluded by an Eck fistula and by ligating the hepatic artery, alcohol produced very little or no increase. This observation was interpreted to mean that the great increase produced in the catalase of the blood by alcohol was due to the stimulation of the liver to an increased output of the enzyme.

In some unpublished results we found that the catalase of the liver was decreased in pancreatic diabetes by 72 per cent, and that of the heart by 48 per cent. In view of the fact that catalase content is so inseparably connected with oxidation in the body, the assumption was made that the defective oxidation in pancreatic diabetes was due to the decreased catalase in the tissues, this being brought about by the decreased output from the liver. Benedict and Török (2) found that the use of alcohol as a food in diabetes reduced the output of acetone, nitrogen and glucose. Neubauer (3) showed that red wine reduced the sugar output and the acidosis in diabetes. Allen and Dubois found that the administration of whiskey increased the oxidation of glucose in diabetes. From the results of these observers, it would appear that alcohol aids oxidation in diabetes. It may be that the helpful effect of moderate amounts of alcohol in diabetes is due to the stimulating effect of alcohol on the liver which causes an increased output of catalase from this organ and hence an increase of catalase in the blood and tissues thus facilitating the oxidative processes. If alcohol is helpful in diabetes, a disease in which there is a decrease in the catalase content of the tissues with resulting defective oxidation and acidosis, it would seem that it should be helpful in "shock" where these same conditions prevail.

SUMMARY

In the condition of "shock" the catalase of the blood and probably of the tissues is decreased. The decrease in the catalase of the blood is brought about by a diminished output of catalase from the liver owing to the lowered blood pressure and probably by a dilution of the blood due to a diffusion of liquid, poor in catalase, from the tissues.

The administration of alcohol in the condition of "shock" greatly increases the catalase of the blood and hence of the tissues by stimulating the liver to an increased output of this enzyme.

Since catalase content is so inseparably connected with oxidation in the body, the assumption is made that the decrease in catalase is principally responsible for the decreased oxidation with rsesulting acidosis in the condition of "shock," and that the beneficial effect of alcohol as a stimulant in "shock" and in conditions of general depression is due to the increase it causes in the catalase of the blood and tissues with resulting increase in oxidation and decrease in acidosis.

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SECRETIN

II. Its Influence on the Number of White Corpuscles in the Circulating Blood

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Received for publication December 18, 1917

In a previous report (1) we have shown that the subcutaneous injection of even a small dose of secretin is able to produce a marked increase in the number of erythrocytes in the circulating blood. In the present report we wish to show that such injections are likewise capable of increasing the number of white corpuscles in the blood stream.

The secretin which we used was in all cases prepared from the intestine of the dog. The mucous membrane was scraped off with a dull knife from the upper half of the small intestine, triturated with 50 cc. of 0.4 per cent hydrochloric acid and after standing for two hours was boiled actively. The preparation was neutralized while boiling and filtered. To it was then added sufficient glacial acetic acid to make 2 per cent by volume and the acid extract evaporated to dryness. We have found that such a preparation retains its activity for at least six months. We obtained about 10 mgm. of this dried acid extract per cubic centimeter of original solution. In all of the present series of experiments such an acid extract was used, a sufficient quantity of the dried preparation being dissolved in normal saline solution as needed to make a solution of the same strength as the original filtrate.

As in our previous experiments rabbits were used exclusively because it has been shown by Lamson (2) that they do not respond to fright, pain, etc., by an increase in the number of erythrocytes in the circulating blood, as do the cat and dog, and we wished to avoid the use of an anaesthetic. Also to exclude the factor of digestion leucocytosis food and water were withheld from the animals during the experiments.

The blood was obtained from the ear of the rabbit with as little manipulation as possible. Specimens were taken simultaneously for counting both white and red corpuscles, 0.5 per cent acetic acid being

used as the diluting fluid for the former and normal saline solution for the latter. The counts were made in the usual manner with the Thoma-Zeiss apparatus.

TABLE I

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
1	W. B. C.	4,800	7,800	3,000	62.5	30	65
	R. B. C.	(*)					
2	W. B. C.	10,400	13,600	3,200	30.76	30	60
	R. B. C.	4,960,000	5,608,000	648,000	13.06	30	30
3	W. B. C.	9,600	11,250	1,650	17.18	90	90
	R. B. C.	5,331,000	6,240,000	909,000	17.05	90	90
4	W. B. C.	6,200	14,200	8,000	129.03	30	90
	R. B. C.	7,349,000	7,840,000	491,000	6.68	30	30
5	W. B. C.	11,600	16,600	5,000	43.10	60	90
	R. B. C.	6,054,000	7,184,000	1,130,000	18.66	60	60
6	W. B. C.	20,000	34,800	14,800	74.00	60	90
	R. B. C.	5,234,000	7,200,000	1,966,000	37.56	60	90
7	W. B. C.	15,600	11,786†	3,814	24.44	30	60
	R. B. C.	6,427,000	6,631,000	204,000	3.17	90	90
8	W. B. C.	12,654	15,800	3,146	24.86	60	90
	R. B. C.	6,615,000	7,760,000	1,145,000	17.30	60	60
9	W. B. C.	10,900	13,100	2,200	20.18	60	60
	R. B. C.	5,605,000	6,912,000	1,307,000	23.31	60	60
10	W. B. C.	7,400	12,200	4,800	64.86	60	90
	R. B. C.	5,343,000	6,246,000	903,000	16.90	60	60
Averages	W. B. C.	10,915	15,113	4,198	44.2	51	78.5
	R. B. C.	5,879,777	6,846,777	967,000	17.07	60	63.33

* Red corpuscle counts were not made in experiment 1.

† Experiment 7 shows a decrease in the white corpuscle count

Experiment 5, November 9, 1917

9.50 a.m. White blood corpuscles 11,600 per cubic millimeter. Red blood corpuscles 6,054,000 per cubic millimeter.
9.55 a.m. 1 cc. secretin solution (representing 10 mgm. of dried extract) per kilogram of body weight given hypodermatically.
10.25 a.m. White blood corpuscles 14,400 per cubic millimeter. Red blood corpuscles 6,496,000 per cubic millimeter.
10.55 a.m. White blood corpuscles 16,600 per cubic millimeter. Red blood corpuscles 7,184,000 per cubic millimeter.
11.25 a.m. White blood corpuscles 14,400 per cubic millimeter. Red blood corpuscles 5,984,000 per cubic millimeter.
11.55 a.m. White blood corpuscles 12,400 per cubic millimeter. Red blood corpuscles 5,216,000 per cubic millimeter.
12.25 p.m. White blood corpuscles 8,800 per cubic millimeter. Red blood corpuscles 5,574,000 per cubic millimeter.

We had determined in our previous experiments that 1 cc. of the secretin solution, equivalent to approximately 10 mgm. of the dried extract, per kilogram of body weight was the most efficient dose to produce an increase in the number of erythrocytes per unit volume of blood and that the preparation was effective when injected subcutaneously. Therefore we selected this dose as our starting point and administered it subcutaneously in all cases. Table 1 summarizes the results of ten such experiments, the effect upon both red and white corpuscles being recorded. Following the table is the protocol of a typical experiment of this group.

These experiments show conclusively that not only is secretin solution, when injected subcutaneously, able to produce an increase in the number of erythrocytes in the circulating blood but that it is capable of producing an even greater effect on the number of white blood corpuscles. In addition, however, it shows that the duration of the effect on the number of the corpuscles and the time of appearance of the maximum count are very nearly the same in the two cases—duration of effect on the red blood corpuscles 63.33 minutes, on the white corpuscles 78.5 minutes; maximum count of red blood corpuscles per cubic millimeter in 60 minutes, of white blood corpuscles per cubic millimeter in 51 minutes—the effect being produced quicker in the case of the white corpuscles and persisting longer.

We next sought to determine if the dose of 1 cc. of secretin solution per kilogram of body weight was the most efficient dose in the case of the white blood corpuscles as it had been shown to be with regard

to the erythrocytes. To do this we performed four experiments using in each a dose of $\frac{1}{2}$ cc. of secretin solution per kilogram of body weight and four experiments using in each 2 cc. of secretion solution per kilogram of body weight. The results of these experiments are shown in tables 2 and 3 respectively. An experiment typical of each group is also given in detail.

TABLE 2

Dose: 0.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
11	W. B. C.	16,800	20,600	3,800	22.61	30	60
	R. B. C.	6,961,000	7,671,000	710,000	10.19	30	30
12	W. B. C.	9,200	10,600	1,400	15.21	60	90
	R. B. C.	5,440,000	6,560,000	1,120,000	20.58	60	90
13	W. B. C.	9,200	14,200	5,000	54.34	90	60
	R. B. C.	4,290,000	4,650,000	360,000	8.39	90	60
14	W. B. C.	16,800	18,400	1,600	9.54	60	60
	R. B. C.	6,640,000	6,928,000	288,000	4.33	60	60
Averages	W. B. C.	13,000	15,950	2,950	25.42	60	67.5
	R. B. C.	5,832,750	6,452,250	619,500	10.87	60	60.0

Experiment 11, November 20, 1917

11.05 a.m. White blood corpuscles 16,800 per cubic millimeter. Red blood corpuscles 6,961,000 per cubic millimeter.

11.15 a.m. 0.5 cc. secretin solution (representing 5 mgm. of dried extract) per kilogram of body weight given hypodermatically.

11.45 a.m. White blood corpuscles 20,600 per cubic millimeter. Red blood corpuscles 7,671,000 per cubic millimeter.

12.15 p.m. White blood corpuscles 18,400 per cubic millimeter. Red blood corpuscles 6,624,000 per cubic millimeter.

12.45 p.m. White blood corpuscles 16,000 per cubic millimeter. Red blood corpuscles 6,168,000 per cubic millimeter.

1.15 p.m. White blood corpuscles 14,500 per cubic millimeter. Red blood corpuscles 5,860,000 per cubic millimeter.

TABLE 3

Dose: 2 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
17	W. B. C.	11,600	13,000	1,400	12.06	30	90
	R. B. C.	6,576,000	7,263,000	687,000	10.44	30	60
18	W. B. C.	11,700	19,400	7,700	65.81	60	90
	R. B. C.	4,960,000	5,900,000	940,000	18.95	30	90
21	W. B. C.	7,600	7,300*	300	3.94	40	80
	R. B. C.	6,001,000	7,313,000	1,312,000	21.86	30	30
22	W. B. C.	5,400	10,200	4,800	88.88	30	60
	R. B. C.	6,880,000	7,376,000	496,000	7.20	30	60
Averages	W. B. C.	9,075	12,475	3,400	38.21	40	80
	R. B. C.	6,104,250	6,963,000	858,750	14.61	30	60

* Experiment 21 shows a decrease in the white corpuscle count.

Experiment 17, November 27, 1917

11.00 a.m. White blood corpuscles 11,600 per cubic millimeter. Red blood corpuscles 6,576,000 per cubic millimeter.

11.05 a.m. 2 cc. secretin solution (representing 20 mgm. of the dried extract) per kilogram of body weight given hypodermatically.

11.35 a.m. White blood corpuscles 13,000 per cubic millimeter. Red blood corpuscles 7,263,000 per cubic millimeter.

12.05 p.m. White blood corpuscles 12,000 per cubic millimeter. Red blood corpuscles 6,956,000 per cubic millimeter.

12.35 p.m. White blood corpuscles 12,600 per cubic millimeter. Red blood corpuscles 6,118,000 per cubic millimeter.

1.05 p.m. White blood corpuscles 11,500 per cubic millimeter. Red blood corpuscles 6,288,000 per cubic millimeter.

3.05 p.m. White blood corpuscles 8,500 per cubic millimeter. Red blood corpuscles 6,052,000 per cubic millimeter.

A dose of 1 cc. of secretin solution per kilogram of body weight produces an average increase of 44.2 per cent in the number of white corpuscles in 51 minutes, while 0.5 cc. of secretin solution per kilogram produces an increase of only 25.42 per cent in 60 minutes and 2 cc. of secretin solution per kilogram an increase of 38.21 per cent in 40 min-

utes. Therefore the conclusion is justified that 1 cc. of secretin solution per kilogram of body weight is the most efficient dose to increase the number of both white and red corpuscles.

In our previous work on the red blood corpuscles we also found that by repeating the dose of secretin solution at short intervals the increase in the erythrocyte count could be kept up for several hours but dropped promptly after the administration of the last dose. In table 4 the results of four experiments are recorded in each of which 1 cc. of secretin solution per kilogram of body weight was injected subcutaneously at hourly intervals for three doses.

It will be seen that the increase in the white corpuscle count produced by the first dose is partly maintained by the succeeding doses but rises after the administration of the last dose so that at the end of five hours the number of white corpuscles in the blood stream is very decidedly greater than at the beginning of the experiment. In the same table are given the erythrocyte counts in the same experiments which confirm the results that we obtained previously. A comparison of the effects produced when repeated doses of secretin are given at short intervals shows that the total effect on the white corpuscles is more marked and more persistent than is the effect on the erythrocytes.

A similar comparison of the effect of a single dose of secretin solution on the red and white blood corpuscles shows the same relation. In practically all cases the effect on the white corpuscles appears as quickly, or more quickly, than the effect on the erythrocytes and persists for a longer time. Also the average percentage increase in the white corpuscle count per unit volume of blood is in all cases nearly or quite double the percentage increase in the erythrocyte count.

In our previous paper we suggested as the most probable explanation of the increase in the number of erythrocytes in the circulating blood produced by secretin that it is due to a direct stimulating action of the secretin on the red marrow of the bones. We are still inclined to believe that this is the true explanation and further work is being done in an endeavor to determine this. It is conceded that there are two sources for the white blood corpuscles, the bone marrow and the lymphatic tissues in general. It would seem probable because of the much greater effect of secretin on the number of the white corpuscles that it stimulates their production by both the bone marrow and the lymphatic tissues. Further work is also being done along this line.

TABLE 4

Dose: 1 cc. secretin solution per kilogram of body weight at hourly intervals for three doses

EXPERIMENT NUMBER		INITIAL COUNT	FIRST HOUR	SECOND HOUR	THIRD HOUR	FIFTH HOUR	SIXTH HOUR	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN
											hours
15	W. B. C.	11,000	13,300	11,600	11,400	14,600	11,800	14,600	3,600	32.72	5
	R. B. C.	4,677,000	4,896,000	5,867,000	5,696,000	5,343,000	5,100,000	5,867,000	1,190,000	25.44	2
16	W. B. C.	9,200	15,300	13,200	12,600	16,200	12,200	16,200	7,000	76.08	5
	R. B. C.	5,280,000	6,608,000	6,768,000	7,160,000	6,076,000	4,708,000	7,160,000	1,880,000	35.60	3
19	W. B. C.	6,800	7,400	7,000	6,800	8,100	7,000	8,100	1,300	19.11	5
	R. B. C.	7,584,000	7,968,000	6,408,000	8,176,000	8,128,000	7,026,000	8,176,000	592,000	7.80	3
20	W. B. C.	6,400	8,800	8,000	6,400	7,800	5,800	8,800	2,400	37.5	1
	R. B. C.	6,288,000	6,672,000	6,608,000	7,488,000	6,886,000	5,876,000	7,488,000	1,200,000	19.08	3
Averages	W. B. C.	8,350	11,200	9,950	9,300	11,675	9,200	11,925	3,575	42.81	4
	R. B. C.	5,957,250	6,536,000	6,412,750	7,130,000	6,603,250	5,677,500	7,172,750	1,215,500	20.40	$2\frac{3}{4}$

CONCLUSIONS

1. It is possible to produce an increase in the number of white corpuscles per cubic millimeter o blood by the administration of secretin, even in small doses and by subcutaneous injection.

2. The most efficient dose is 1 cc. of secretin solution per ki'ogram of body weight.

3. The increase in the count appears quickly and is very transient, but is greater and more persistent than the increase in the erythrocyte count produced by the same means.

4. By repeating the dose of secretin solution at short intervals the increase in the number of both the erythrocytes and the white corpuscles can be kept up for several hours but is more marked and persists somewhat longer after the last dose in the case of the white corpuscles than in the case of the red corpuscles.

5. It is suggested that the effects described are due to a direct stimulating action of secretin on both the bone marrow and the lymphatic tissues in general.

6. The results of previous experiments on the number of erythrocytes in the circulating blood are confirmed.

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VI. FURTHER STUDIES ON THE EFFECT OF ADRENALIN UPON THE BLOOD FLOW IN MUSCLES

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Received for publication December 20, 1917

In 1895 Oliver and Schäfer (1) demonstrated that adrenal extract has a pressor action upon the vasomotor system in both frogs and dogs. In the dogs they plethysmographed several organs simultaneously. These did not of necessity respond in the same manner, at times one contracted and the others expanded and vice versa, and sometimes all the organs under observation expanded. This increase they attributed to a passive expansion due to swelling of the large blood vessels in the limbs.

It has been shown that adrenalin injected into animals (cats and dogs) in small doses (0.1 to 0.5 cc. of a 1: 100,000 solution) produces a fall in blood pressure. This fall Cannon and Lyman (2) say results not only from a lessened peripheral resistance due to vasodilation in the cutaneous system but also to dilation in the splanchnic vessels. Plethysmographic records taken simultaneously with blood pressure records showed that the limb volume increases as the blood pressure falls. Dale (3) came to the same conclusion. He advanced the theory that small amounts of adrenalin stimulate vasodilator endings while large quantities bring the vasoconstrictors into play.

Hartman (4) does not share absolutely in this belief, but believes that adrenalin in small doses is selective in its action. He found that if the limb vessels were clamped adrenalin produced a rise in blood pressure, and if the vessels of the splanchnic area were clamped and the limb vessels left intact adrenalin produced a fall in pressure. He says that toneless relaxed muscles probably do not relax further by injection of adrenalin. Recently Hartmen and Fraser (5) advanced the

[1] A number of the earlier experiments were performed in the Laboratory of Physiology in the Albany Medical College.

theory that the vasodilator action of adrenalin in muscle vessels does not depend upon the tonicity of the vessel wall but upon a center in the central nervous system elsewhere than in the cerebral hemispheres. They obtained dilation in the perfused limbs of dogs upon injecting adrenalin into the circulation when the nervous connections were intact.

Hoskins, Gunning and Berry (6) pointed out that adrenalin injected intravenously in small doses dilates the muscle vessels and constricts the cutaneous. Gruber (7) was unable to obtain vasodilation in muscles the nerves of which had just been cut. His results were confirmed by Hartman and Fraser (5). Nor was he able to obtain dilation in active muscles whose nerves were cut and stimulated at a rate favorable to dilation.

The present research was carried out to determine whether or not adrenalin acted centrally or peripherally in producing vasodilation in muscles.

METHOD

Cats, anaesthetized with ether, were used. The blood pressure was registered by a mercury manometer from a cannula in the carotid artery. A time marker was placed at the atmospheric pressure line of the manometer.

Usually adrenalin chloride, but in some cases crystalline adrenalin (epinephrin) (0.5 to 2 cc. of a 1: 100,000 solution) and in some cases (0.5 to 1 cc. of a 1: 10,000 solution), was injected into a cannula placed in the external jugular vein. In many experiments hirudin (10 mgm. per kilo) was injected intravenously as an anticoagulant.

In all cases the cutaneous vessels were ligated and cut and either the skin removed or the limb mass ligated above the hock. Both methods satisfactorily separate the skin circulation from the muscle circulation. A paraffined glass cannula of small bore (3 mm. diameter) was placed in the femoral vein, all the branches to which were tied off except the deep anterior tibial vein which comes from the tibialis anticus muscle and other muscles of that region. The drops of blood flowing through it were recorded on the smoked drum surface by a signal magnet operated by an automatic circuit breaker (7).

Experiments were performed on several animals in which either the sciatic nerve or the peroneal nerves in the left limb had been cut 2 to 10 days earlier. As many animals were used in experiments on the normal limb, on the limb in which the nerve was cut just previous to

the experiment and on perfused limbs. In these last cases the fluid was forced through a cannula placed in the femoral artery.

RESULTS

Adrenalin injected into a cat in small doses (0.5 to 2 cc. 1: 100,000 solution) brings about a fall in arterial pressure and produces an active

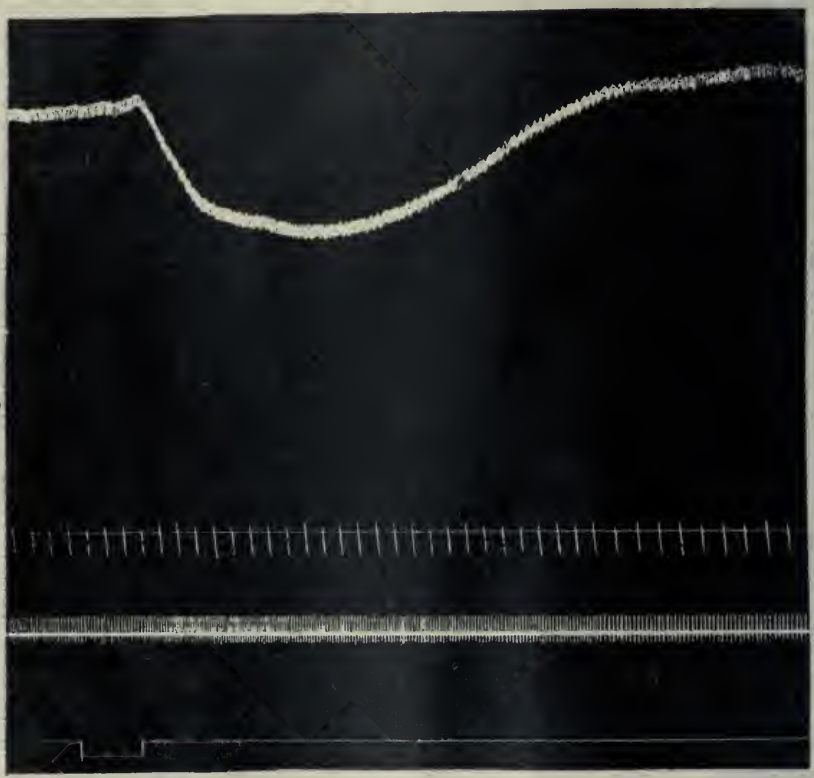

Fig. 1. In this and the following records the upper curve records blood pressure with mercury manometer, below it the time marker and zero blood pressure. Except in Fig. 2 the third line is that of blood flow through the muscle in drops. Unless otherwise stated the time is in 5 second intervals. Hirudin used as an anticoagulant. Nerve intact, time in 15 seconds.

vasodilation of the muscle vessels (see fig. 1). The same dose of adrenalin was always found to produce vasoconstriction of the skin vessels. These results corroborate those obtained by Hoskins, Gunning and Berry on dogs and Hartman and Fraser on cats with the

plethysmograph. The animal from which this figure was taken weighed 2 kilos and received 20 mgm. of hirudin intravenously. At the point indicated in the record 0.5 cc. of adrenalin (1: 100,000 solution) was injected. There resulted a fall in blood pressure from 110 to 80 mm. of mercury or 32 per cent, and an increase in blood flow through the muscle vessels from 32 to 48 drops per 30 seconds.

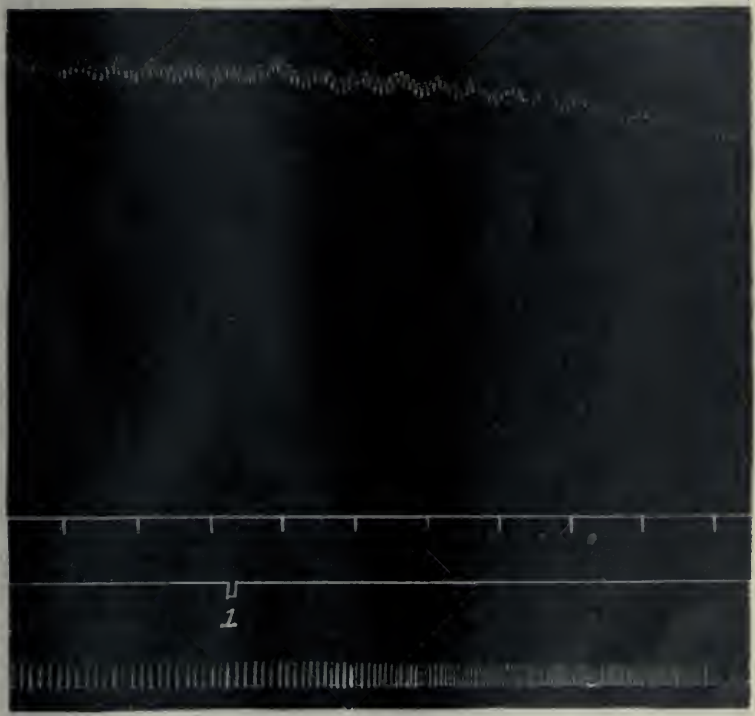

Fig. 2. Curve showing the effects of cutting the nerve upon the muscle circulation. Nerve cut at *1*.

In many instances adrenalin in larger doses produced a rise in arterial pressure with the muscle vascular system still showing a dilation. In order to compare the blood pressure in the limb with that of the general circulation two mercury manometers were employed, one in the carotid and the other in the femoral artery of the limb not under investigation. By looping a ligature around the aorta just above the iliac axis it was possible to control the blood pressure in the vessels

of the limb under investigation. Even though the blood pressure did not increase in this limb, there was a marked increase in blood flow through the muscle.

Figure 2 is given to show the effect of cutting the nerve to the muscle. For the 10 seconds preceding the cutting of the nerve at *1* the rate of

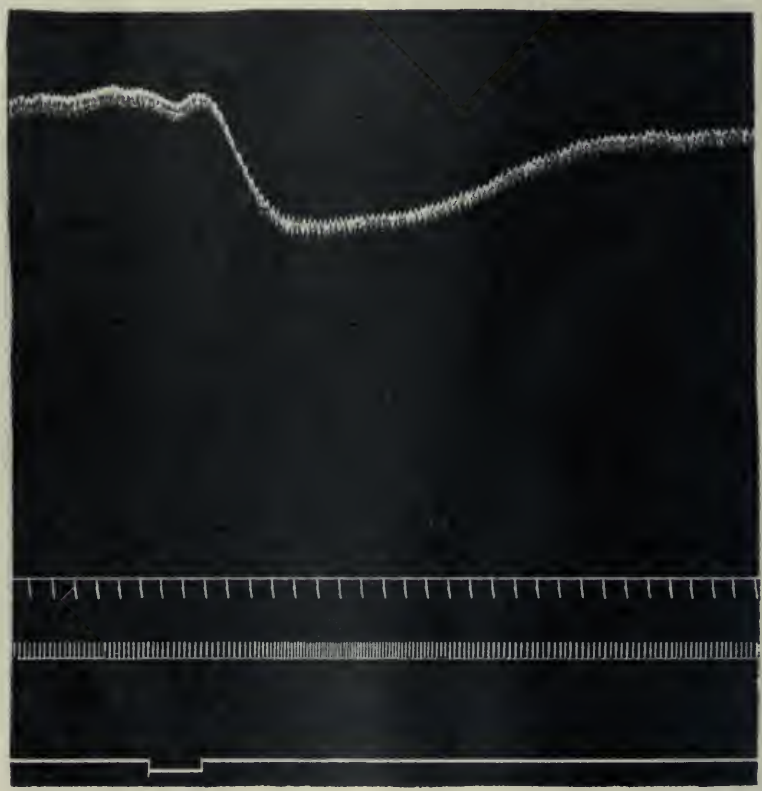

Fig. 3. Peroneal nerve cut 4 days and the sciatic cut previous to this record; 0.5 cc. of 1: 100,000 adrenalin.

blood flow through the vessel was 16 drops. After cutting, the rate of flow was increased to 47. In all cases it was noted that within 15 to 30 seconds after the nerve was cut the rate of blood flow through the muscle was from 2 to 3 times faster than before. This is of course due to loss of tonicity in the vessel wall.

There is in muscles under such experimental conditions no increase

in flow upon injection of adrenalin unless there is considerable rise in blood pressure during which a slight passive dilation may be recorded. This confirms Gunning's (8) work with large doses of adrenalin. If the pressure falls there is either a passive constriction due to the fall in the blood pressure, especially when the pressure is low, or an active vasoconstriction due to the action of the adrenalin itself. Hartman suggests that, since "a depressant substance obtained from ox pituitaries" caused further dilation in the denervated limbs, the inability of adrenalin to bring about further dilation under similar conditions is not due to the absence of muscular tonicity but to the loss of connection with a vasodilator center. It might be questioned in this connection whether or not the points of action of adrenalin and the depressant substance from ox pituitaries are the same.

It is my belief that this lack of vasodilator action of small doses of adrenalin in denervated limbs is due to the loss of tonicity; that when the tonicity of the vessel wall is normal adrenalin causes vasodilation and when the tonicity is lost it causes constriction. In both cases it acts upon the peripheral vasomotor mechanism. To substantiate this theory figures 3 and 4 are presented.

The effect of nerve degeneration (2 to 10 days). It is a well known fact that if time is allowed for recovery after cutting the nerves to vessels a certain amount of tonicity of the vessel wall is regained (9). Bowditch and Warren (10) demonstrated that the vasoconstrictor nerve fibers are the first to degenerate after section of a mixed nerve. In this present research some experiments were performed upon animals in which either the peroneal or the sciatic nerves were cut from 2 to 10 days previous to the experiments. Somewhat variable results were obtained but this variation was due to the degrees of inflammation in the sectioned limbs. A dilation was never observed in limbs in which there existed marked inflammation and edema. In eleven animals in which healing took place without infection, the vessels responded with dilation to adrenalin as well as did the vessels of normal uninjured muscles.

Figures 3 and 4 are records obtained from animals in which the nerves had been cut 4 and 6 days, respectively, previous to the experiments. In the former the peroneal, in the latter the sciatic nerve was sectioned. In order to make sure that the vasomotor center was inoperative on the area drained by the venous cannula the sciatic nerve was cut just before figure 3 was made. As a result no change in blood flow was noted. At the point indicated in the record 0.5 cc.

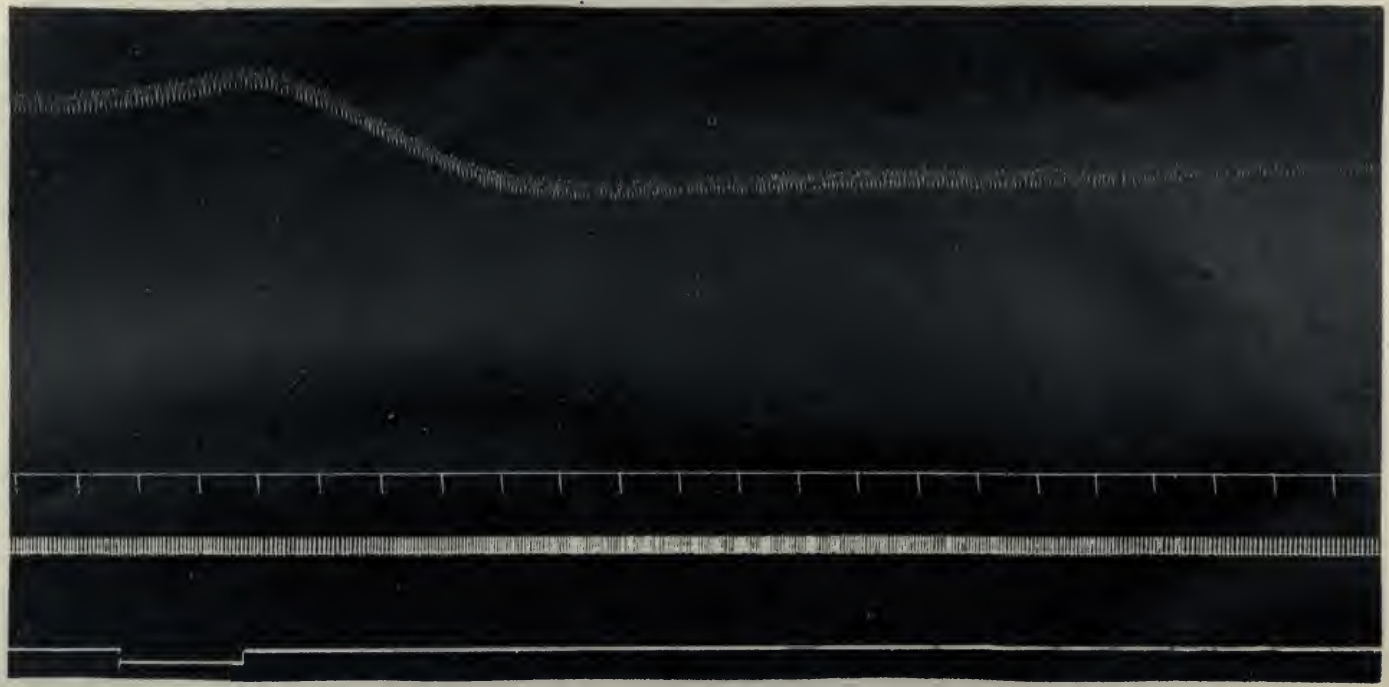

Fig. 4. Sciatic nerve cut 6 days; 0.5 cc. of 1: 100,000 adrenalin.

adrenalin (1: 100,000) was injected intravenously. There resulted a fall in blood pressure from 124 to 90 mm. of mercury with a resultant increase in flow of blood through the muscle from 25 to 43 drops per 30 seconds. In figure 4, 0.5 cc. of adrenalin (1: 100,000) slowly injected intravenously caused a fall of blood pressure from 98 to 74 mm. of mercury and an increase in the rate of blood flow from 90 to 150 drops per 30 seconds.

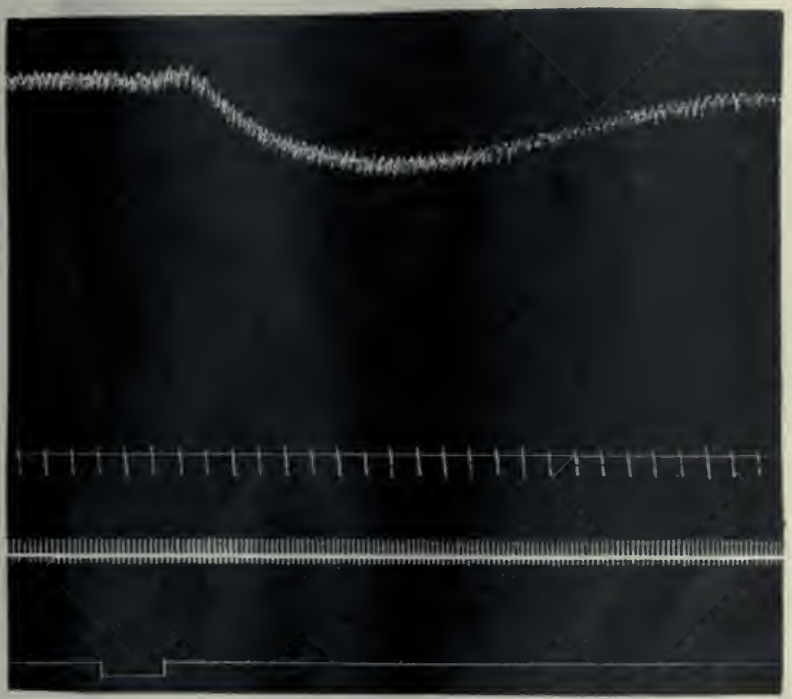

Fig. 5. Perfused limb with nerve intact; 0.5 cc. of 1: 100,000 adrenalin.

In two animals with denervated limbs all the muscles were severed at the thigh leaving only the artery intact and the femur to support the anterior tibial muscle and other muscles of the lower leg. In both instances when there could be no possible connection with a vasodilator center there resulted from injections of 0.5 cc. of adrenalin (1:100,000) the usual vasodilation.

In the animals in which the nerves were cut some time previous to

the experiment an increase in the blood flow was obtained even with a blood pressure as low as 30 mm. of mercury. It was observed that a larger dose of adrenalin was necessary to produce vasoconstriction.

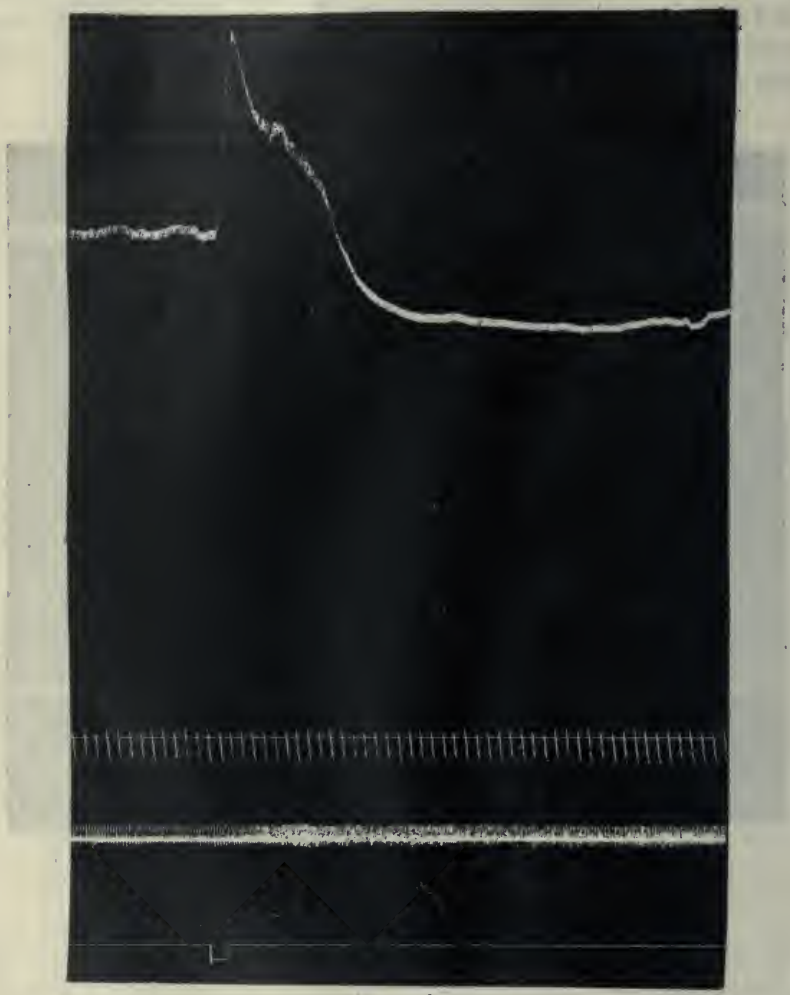

Fig. 6. Perfused limb with nerve intact; 0.5 cc. of 1: 10,000 adrenalin.

In one animal with nerve cut 6 days, 3 cc. adrenalin (1: 100,000) rapidly injected was necessary to produce vasoconstriction, whereas 0.1 to 0.5 cc. (1: 100,000) produced the same result in the limb in which the nerve had just been severed.

Perfusion experiments. Eleven experiments were performed upon animals in which the limb was perfused with oxygenated Ringer's solution or oxygenated defibrinated blood at a constant temperature. This varied in the different animals from 37° to 38.5°C. but was constant for each experiment. The pressure for the perfusion fluid was 90 cm. of water. The flask containing the perfusion fluid was placed within a container filled with water at a constant temperature of 38.5°C. The fluid then flowed into a 100 cc. flask placed within another container through which a stream of water at 38.5°C. ran to maintain a constant temperature within the perfusion flask. In order to make sure that the temperature was constant a thermometer was placed in the perfusion fluid just before it entered the cannula.

The limb was first ligated above the hock. The femoral vein and artery were isolated without injuring the nerves, as in my previous experiments, and ligatures were looped loosely around them to make ready to insert the cannulae.

The abdominal aorta was isolated and a ligature placed loosely around it. The venous cannula was then inserted and the limb tested for normal vasodilation. The artery was quickly clamped, a cannula inserted and the perfusion started, after which the loop around the aorta was tightened. This reduced the manipulation of the tissues between the normal test and the perfusion test to a minimum. In a few experiments one limb was perfused and the other limb was bled from the venous cannula. In all but two cases a fall in blood pressure was obtained by injecting 0.5 cc. 1: 100,000 solution of adrenalin intravenously. In two animals this amount gave a rise in blood pressure followed by a fall. In every instance it produced a dilation of the normal intact limb (fig. 1) but no change in the perfused limb with the nerve intact (fig. 5). That this difference in reaction cannot be due to injury through manipulation of the vessels is shown by the fact that the normal limb still acts by vasodilation to small doses of adrenalin and that a fall in blood pressure is still obtained. Moreover the only injury caused after the operations on the normal limb is that of placing a cannula in the previously isolated femoral artery and the tying of the ligature already looped around the aorta. If adrenalin exerted its influence entirely through a vasodilator center it should produce the same results in these two cases where the only difference in the conditions of the limbs is that one has and one has not the circulation intact.

Dilation of the perfused limb was recorded in cases where much

stronger solutions (0.5 to 1 cc. 1: 10,000 solution) were used. In every case the blood pressure was markedly increased (fig. 6). These results corroborate Hartman and Fraser's results for they present a similar rise in blood pressure and active vasodilation.

My results with small doses of adrenalin (0.5 cc. 1: 100,000 solution) support Cannon and Lyman's view and Dale's theory based upon experiments with ergotoxin and adrenalin showing that adrenalin acts peripherally rather than centrally. With large doses of adrenalin (0.5 to 2 cc. 1: 10,000 solution) active vasodilation is observed in perfused limbs, which fact supports Hartman and Fraser's theory that adrenalin excites a vasodilator center. It may be, however, that this dilation is due to depression of the vasoconstrictor center through increased blood pressure (9). Pilcher and Sollman's (11) experiments render this latter view untenable.

SUMMARY

Adrenalin in small doses (0.5 to 2 cc. 1: 100,000 solution) produces active dilation of the vessels in cats' muscles when the nerves are intact. These results coincide with those obtained by Hoskins, Gunning and Berry experimenting on dogs and Hartman and Fraser on cats.

In my experiments, adrenalin, in any strength, produced no active vasodilation in muscles in which the nerves were recently cut.

Small doses of adrenalin (1: 100,000) slowly injected intravenously produce active vasodilation in muscles in which the nerves were previously cut and allowed to degenerate from 2 to 10 days. This renewal of the action of adrenalin may be due to a partial recovery of the tonicity of the vessel walls.

Small amounts of adrenalin (0.5 cc. 1: 100,000) injected intravenously did not produce vasodilation in the eleven experiments in the perfused limb intact with the central nervous system. A fall in blood pressure was obtained in every case showing dilation somewhere in the vascular system.

In perfused limbs large amounts of adrenalin (0.5 cc. 1: 10,000) produced active vasodilation accompanied by a rapid increase in blood pressure followed by a fall. Hartman and Fraser attribute this dilation to excitation of a vasodilator center. Further work is necessary to prove conclusively whether or not this vasodilation is due to depression of the vasoconstrictor center by the increased blood pressure or to excitation of a vasodilator center by the adrenalin.

The vasodilation in muscles caused by small amounts of adrenalin is dependent upon the tonicity of the vessel wall.

Small doses of adrenalin (0.5 cc. 1: 100,000) bring about vasodilation by their action on the peripheral vasodilator mechanism.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 45 MARCH 1, 1918 No. 4

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. NO. 81

ON SENSORY ACTIVATION BY ALKALIES

W. J. CROZIER

Received for publication December 14, 1917

I. Weak acids and weak alkalies have in general a more powerful physiological action than would be predicted from their ionization. The explanation of this condition depends in part upon the fact that weak acids and weak alkalies are known to penetrate cells with relative ease (9). It is possible to utilize the observed relative speeds of cell penetration by different acids and alkalies in attempting to account for the manner in which these substances act upon receptor organs of the chemical sense. If an increase in the permeability of the cell surface is a frequent or an invariable concomitant of the process of excitation, it is important to discover in particular instances the rôle played by this change in permeability, as well as the manner in which the change is produced. It should be possible to obtain some light upon this matter through the study of receptor organs which may be normally activated by direct chemical means.

The present experiments were made in order to compare the stimulating powers of NaOH and NH_4OH, the former representing the strong alkalies which penetrate cells with difficulty, the latter a weak alkali entering cells with ease (6), (5). The animals used were earthworms, Allolobophora sp., obtained from a "fertilizer" pit containing a large amount of earth and vegetable material together with a small proportion of manure. The method of stimulation has been described previously (3); the worms were placed, one at a time, upon a low

ridge separating two shallow tanks, one of these containing an activating solution, the other holding boiled rain water. At the moment of adjustment, the worm was situated with one-half in the stimulating solution, the other end being in water. The stimulated part was, as a result, caused to be pulled into the water. The time occupied by this movement, that is, from the instant of adjustment in the activatting solution until the part concerned had been retracted from this solution, was measured with a stopwatch.

The interval so timed is regarded as an indication of the intensity of the activation of the worm under the particular conditions. When precautions are taken to standardize the procedure, using worms of uniform size and previous history, it is possible to obtain in these experiments "retraction-time" figures which are, to a fairly high degree, reproducible in successive experiments.

In these tests the *posterior* half of the worms was stimulated, since it was desired to eliminate consideration of the special sensitivity of the prostomium, and, in addition, to apply to the gross "retraction-time" figures as measured a correction increasing their significance. This "correction" consisted in taking account of the fact that stimulation of the posterior end of the worm (when not too intense) merely increases its normal tendency to move in an anterior direction; while there is an appreciable minimum time required for the fastest possible retraction of the worms; this correction seems legitimate because only one principal type of motor response is being considered (which is not the case with anterior stimulation according to this method). For most purposes it proved sufficient to subtract from each average "retraction-time" the average shortest interval required for retraction, since this factor would be appreciable only under conditions of rapid movement following strong stimulation of the worm. The percentage increase in rapidity of movement of the worm, induced by each stimulating solution, may also be calculated upon the basis of an ascertained average rate of progression when a special stimulant is absent; this procedure is less accurate than the former, and leads qualitatively to the same conclusions as the simpler method first outlined.

The "reduced retraction-time" figures are regarded as inversely proportional to the intensity of the activation experienced by the worms.

II. Average measurements of the "retraction-times" of the earthworm from solutions of NaOH and of NH_4OH are given in tables 1 and 2 (see also fig. 1). These figures are each the average of twenty

determinations. A single earthworm was used but once, thus avoiding "after effects." The averages were reduced, as previously described, by subtracting from each figure the minimum time required by these worms to effect the creeping movement of retraction. This amounted

TABLE 1

Retraction-time of earthworms from NaOH *solutions; concentration* = $N. \times 10^3$; *R.T. = retraction-time. 27°.0.*

CONCENTRATION $N \times 10^3$	*R. T.*	$\frac{1000}{R.T.}$
	seconds	
62.5	5.00	200.0
50.0	5.40	185.0
37.5	7.25	138.0
25.0	11.6	86.2
12.5	18.9	52.9
6.2	44.3	22.6
5.0	50.0	20.0

TABLE 2

Retraction-time of earthworms from NH_4OH *solutions; concentration* = $N. \times 10^3$; *R.T. = retraction-time. 27°.0.*

CONCENTRATION $N \times 10^3$	*R. T.*	$\frac{1000}{R.T.}$
	seconds	
125.0	4.11	244.0
119.0	4.80	208.0
95.3	6.62	151.0
81.7	8.50	118.0
61.2	11.2	89.2
59.5	11.3	88.5
50.0	13.8	72.5
40.9	19.8	50.5
31.2	17.1	58.5
30.6	15.6	64.5
25.0	26.7	37.5
20.4	31.9	31.4
15.3	40.6	24.6

to 1.3 seconds, and was obtained from tests made with worms stimulated several times in quick succession; the correction figure cannot be obtained from experiments with concentrated solutions because of their toxic effect.

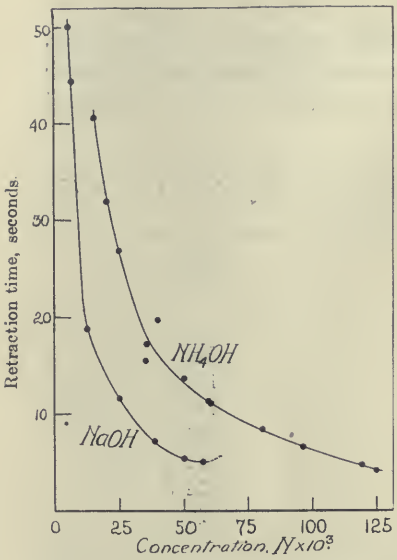

Fig. 1. Retraction-time of earthworms from alkaline solutions. See tables 1, 2.

The solutions employed were prepared by finding a maximum concentration of each alkali which would permit normal escape of the worms without producing obvious toxic consequences. Dilutions were then made from such a solution, analyzed by titration, and tried in succession.

Within the limit of error imposed by the nature of these experiments, the results agree satisfactorily with the requirements of the principle of mass action. For each alkali, over a considerable range of concentrations, the product of the concentration of alkali by the (corrected) "retraction-time" is sensibly constant. The individual observations deviate from the rule to the extent shown graphically in figure 2, where the logarithm of the concentration is plotted against logarithm $\frac{1000}{R.\ T.}$. The average "mass action constants" were used in drawing the straight lines shown in figure 2; the slope of these lines is 45°.

If the degree to which the earthworm is stimulated by alkali depends upon the amount or concentration of a substance, S, formed in its receptors through the action of alkali upon some receptor constituent, the rate of formation of S should be proportional to the concentration of alkali, according to the law of reactions of the first order. In the present instance, the intensity of stimulation is

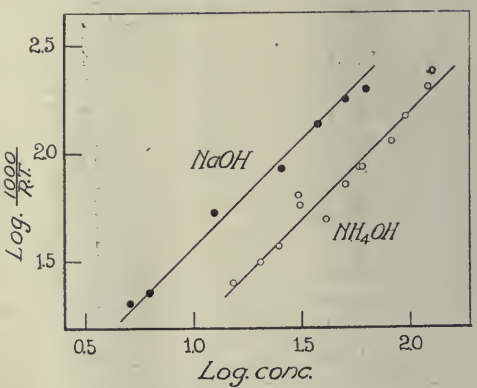

Fig. 2. The stimulating efficiency of alkaline solutions (measured by the reciprocal of the retraction-time of the earthworm), plotted against concentration of alkali.

assumed to be directly measurable by the reciprocal of the (corrected) "retraction-time," hence the product (retraction-time) × (concentration of alkali) should be constant for each alkali, as is found to be the case.

It should especially be noted that the "time" here considered does *not* have the significance of t in the reaction velocity equation. This "time" is, it is true, roughly equivalent to the period during which the worm has remained in the alkaline solution; and from this standpoint a parallel might be suggested between the outcome of these experiments and such a condition as that described by Lillie (8) with regard to the activation of Asterias eggs by butyric acid. In each of these instances a definite physical result appears: the starfish eggs form a normal fertilization membrane, the earthworms move out of the stimulating solution; and in both cases the time required to effect this physical result is inversely proportional to the concentration of the activating agent (within physiological limits). But this analogy is readily seen to be inadequate. When the worm is placed in alkali it begins, after an interval which varies with the concentration of alkali, to creep forward, and the "retraction-time" as measured with the stopwatch includes this period as well as a following one during which (at a rate depending upon the activity of the worm) a gradually, decreasing length of the animal is being exposed to the action of the alkali; the sensitivity of the skin of the earthworm is different at different axial levels. For these reasons the "retraction-time" may not be regarded as a measure of the time of action of the alkali. There is some reason to believe that the actual period of stimulation may be very brief indeed, and amount only to a fraction of the total "retraction-time" (except for the highest concentrations used).

On the other hand, the constancy of the relation illustrated in figure 2, which is significantly displayed also in the effects of acid solutions (cf. the following paper), fully justifies the contention that the intensity of activation of the earthworm is directly proportional to the acting concentration of alkali.

From this fact alone it cannot be concluded that a "monomolecular" reaction is at the basis of stimulation in this case. The same equation applies in other heterogeneous reactions,[1] such as the solution of a

[1] The stimulation of the skin of an earthworm by immersing the animal, or a part of it, in a solution brings into play several sources of "heterogeneity." The earthworm is covered with a resistant cuticle pierced by nephropores, gland cell openings, and numerous apertures through which access is had to the spe-

metal plate by acid; in that case hydrodiffusion of acid (governed by the concentration of acid in the body of the solution) is the slowest, or limiting, process the speed of which is measured in estimating the course of the reaction (13). Such a process has, however, a low temperature coefficient, that of hydrodiffusion. Some figures given by Shohl (21) point to a temperature coefficient of $Q_{10} = 2 +$ for the stimulation of the anterior end of the earthworm by alkali (NaOH); this finding I can confirm, for the posterior end, from experiments with NaOH and NH_4OH in which the observations were corrected for the effect of temperature upon the rate of locomotion of the worms, which were at the same temperature as the activating solutions (20).

It may therefore be suggested that sensory activation in this case depends upon a reaction between the alkali and some constituent of the receptor cells. It is to be supposed that this reaction is "reversible," and that it conforms to the law of reactions of the first order.

"Monomolecular" effects of this type are encountered in the measurement of toxicity (19), (16), and in the action of salts on protoplasmic permeability (14); apparently the action of NaOH in producing an increase in the permeability of protoplasm also follows this law, according to Osterhout's measurements (15). A similar interpretation has been put upon the course of erythrocyte haemolysis by bases (Arrhenius, cited by Lillie, (8)).

The fact that the response which is here regarded as a measure of the intensity of stimulation does not follow the Weber-Fechner rule,

cialized distal ends of sensory cells. These sensory cells are presumed to be concerned in chemoreception, since when *small* areas of the worm's surface are stimulated the reaction time of the worm varies inversely with the number of sense organs in that area (1). Whether or not all the sense organs in the posterior region of the worm are activated by immersion in alkali, cannot be decided, but it seems probable that a sufficient number of them is always stimulated to overcome the objection that the number of sense organs activated determines the speed of locomotion of the worm (as might be the case if the "all or none" principle were applied). This objection is likewise combatted by experiments in which varying lengths of the worms were immersed in alkali. These tests gave no indication that the degree of stimulation effected is proportional to the total area of the worm acted upon. The "specific excitability" of the worms varies for different regions of the animal's surface, as previously stated, but no evidence has been obtainable, from a great number of tests with various substances, that there exist any specializations into different kinds of chemoreceptors (such as the salt-, acid- or bitter-sensitive organs on the human tongue), but it is possible that in some cases free nerve terminals of a 'common chemical' sense are concerned in stimulation.

need cause no inconvenience in this connection, since we know of other instances where the motor result is directly proportional to the physical intensity of the activating agent—such as those photic reactions which obey the Roscoe-Bunsen law, (11), (18). Weber's rule appears to hold particularly when it is a question of the balance or discrimination between two stimulating intensities acting either simultaneously or in quick succession, upon the same receptive area; for example, in certain kinds of salt antagonism (10), (17), or, in the case of the eye, when the retina has become "adapted" to one light intensity before being acted upon by another.

III. It remains to compare the stimulating powers of NaOH and NH_4OH; the latter is much more effective than if the C_{OH} external to the worm were the determiner of activation. This difficulty is similar to that arising in connection with other physiological actions of ammonia, concerning which some complicated explanations have been advanced (12). It is well known that ammonia easily penetrates to the interior of cells. From Harvey's measurments (6) it appears that, on the average, at equal concentrations, NH_4OH penetrates cells ninety to one hundred times more rapidly than NaOH; while in a purely chemical action, such as the saponification of esters, the activity of NaOH may be as much as two hundred times as great as that of NH_4OH (measured by the amount of ester saponified).

If the activation of a sense organ by alkali be proportional to the extent of chemical change induced by the stimulating agent, then we might expect the amount of receptor material transformed to be proportional to the chemical activity of the base and inversely proportional to the difficulty experienced by the base in penetrating the surface of the cell. Assuming the receptor cells not to differ greatly from the generality of cells so far as their penetrability is concerned, we might expect in the present case to find NaOH two to three times as effective as NH_4OH. The extrapolated stimulating powers of unit concentrations of NaOH and of NH_4OH, that is, the "mass action constants" in figure 2, are in the ratio 2.4:1. This is consistent with the assumptious above made, since NH_4OH diffuses very rapidly into the surface layer of the cell, while the action of NaOH must be restricted to the very outer surface of the receptor (6), (2)—hence slow diffusion processes do not hinder the clearness of the result.[2] The

[2] Harvey's data (7) on the penetration of cells by strong hydroxides suggest that the speeds of penetration (neutral red method) are nearly proportional to the square of the concentration. The actual time-intervals involved are long, and probably intracellular diffusion complicates the phenomenon.

whole process of stimulation must take place at, and in, the surface of the cell, since the time of action is so brief.

SUMMARY

The chemically sensitive surface of the earthworm is acted upon by NaOH and NH_4OH in such a way that for each alkali the degree of activation, measured by its effect upon the resulting movements of the worm, is directly proportional to the concentration of alkali. Reasons are given for regarding this fact as evidence that a chemical reaction with some portion of the surface of the receptor elements is the essential feature of stimulation. From this point of view an increase in the permeability of the receptor cell surface, if it occurs,[3] is to be regarded as a consequence of activation, and not the essential determiner of stimulation.

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[3] According to Gray (4, p. 496), ammonia may penetrate sea urchin eggs, and give visible evidence of interaction with the cell pigment, although the conductivity of the eggs remains unchanged.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. NO. 82

SENSORY ACTIVATION BY ACIDS. I

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Received for publication December 14, 1917

I. This paper deals with experiments undertaken to determine in a quantitative manner the relative effects of different acid solutions upon organs of chemical sensitivity. In order to secure some idea of the method of sensory activation by acids, comparisons are made with observations regarding the penetration of cells by acids. The animal used in the experiments upon the activating effects of acids was a common earthworm, Allolobophora sp., obtained in heaps of manure and decaying vegetable material. The method of experimentation has been described previously (6). The stimulation figures obtained in the present tests have to do with the activation of the posterior end of this worm and are not directly comparable with such data as those previously given (4) for somewhat similar tests made upon the anterior end of the worm. Detailed work has been limited to posterior stimulation, because in this way a uniform type of response forms the basis of measurement, and the special sensitivity (to osmotic differences, for example) of the prostomium and of the semi-protrusible buccal epithelium is at the same time eliminated from consideration.[1]

The assumption is made, in interpreting the measurements of time occupied by the retraction of the posterior half of the worm stimulated by immersion in acid, that the reciprocal of the average "retraction-time," which varies systematically with the concentration of acid, is directly proportional to the degree of activation of the worm. By "retraction-time" is meant the average observed time (in seconds) required for retraction from the stimulating solution, corrected by the subtraction of a figure representing the "impedence" of the worm,—

[1] The earthworm and the foot of the spinal frog are perhaps the most favorable for quantitative experiments of this nature, but it is necessary to point out that for the interpretation of results from these two sources somewhat different considerations are required.

the mechanical resistance to, or disadvantage of, its method of progression. The "correction" is obtained from experiments designed to show the minimum time required for the fastest possible retraction of worms of the constant size used in the stimulation experiments.[2] The correction was found = 1.2 to 1.3 seconds, at the temperature of these experiments (27°).

In using this method it is not possible to study the action of solutions which lead to retraction-times greater than would, on the average, be due to the normal locomotor speed of the worms under the given conditions, about 65 seconds,—although it is, of course, possible that under some conditions, such solutions should stimulate. The minimum working retraction-time is therefore about 2 seconds, the maximum about 60 seconds. But between these limits certain characteristic features of activation by the different acids used are sufficiently made clear.

Two series of acids were considered. Certain other series will subsequently be reported upon. In the first set of experiments the chloroacetic acids were compared with acetic and with hydrochloric, and in the second, the activities of monobasic fatty acids were compared.

TABLES

NOTE: In each of the following tables, excepting Table 5, *Conc.* signifies concentration × 10^3N; *R.T.* means the corrected average retraction time, in seconds.

TABLE 1

Monochloroacetic

CONCENTRATION	*R. T.*
59.8	0.7
29.9	1.2
25.0	1.4
15.0	2.2
12.0	4.5
10.0	6.9
6.0	21.7

TABLE 2

Dichloroacetic

CONCENTRATION	*R. T.*
29.0	1.0
14.5	1.3
8.7	5.5
7.3	4.8
5.8	5.7
4.4	25.4
2.9	45.0

TABLE 3

Trichloroacetic

CONCENTRATION	*R. T.*
13.0	1.6
10.4	1.2
7.8	3.7
6.5	4.0
5.2	4.1
3.9	28.0
2.6	40.0

[2] This minimum time is not always identical with the quickest retraction time observed with increasing concentrations of acid; the retraction time *vs.* concentration curves frequently pass through a minimum point, the lengthening retraction periods at higher concentrations representing the incidence of new types of response, such as writhing movements; very toxic solutions also retard the speed of movement, and frequently result in autotomy, (10).

TABLE 4

HCl

CONCENTRATION	*R. T.*	$C \times R.\ T.$	$C^2 \times R.\ T.$
26.6	3.8	101	
22.1	5.2	115	
17.0	6.0	111	
16.6	7.1	118	
14.8	7.4	110	
11.0	9.3	102	
8.3	12.5	104	
8.0	13.4	108	
6.6	17.6	118	(768)
5.5	25.7	(141)	780
4.4	40.8	(179)	792
3.7	57.0	(211)	781
Mean		110±5	(784)

II. Tables 1 to 4 and 7 contain a summary of the results obtained with the chloroacetic acids, hydrochloric, and acetic acid. These data (excepting that for acetic) are plotted in figure 1. The procedure consisted in finding by trial the highest concentration of each acid which would produce normal retraction without entailing immediate toxic consequences. Dilutions were then made from this concentration, and analyzed by titration. The retraction-times listed in the tables are each the average of twenty-five independent observations on twenty-five different worms.

There is a certain unevenness in the data for di- and trichloroacetic, which is greater than that found with the other acids, and has repeatedly appeared in other series of tests not here recorded. This may be due to the very rapid increase of stimulating power with increasing concentration of acid (tending to magnify errors of the experiment), or may be directly due to the complex nature of the stimulation process with these substances. The curves are, however, sufficiently separate to show that the general order of stimulating efficiency is

$$\text{acetic} < \text{mono—} < \text{di—} < \text{trichloroacetic.}$$

This order is not preserved, but becomes significantly irregular, when solutions of equal hydrogen ion content are compared (fig. 2).

Over a considerable range the stimulating power of the chloroacetic acids increases more rapidly than the square of the concentration.

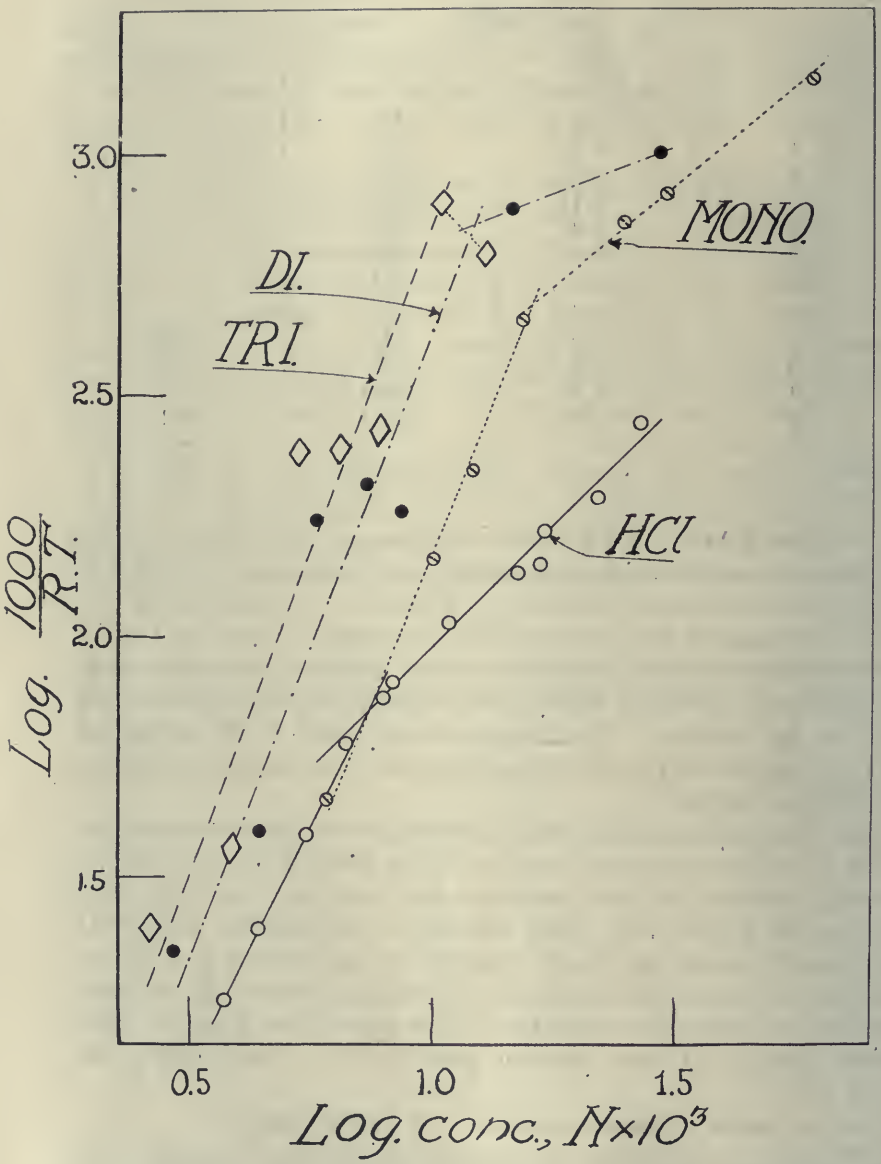

Fig. 1. Showing the relation between the reciprocal of the "retraction-time" of earthworms from solutions of the chloroacetic acids and HCl, and the concentration of acid.

In this respect they differ from acetic acid, and from the more concentrated solutions of HCl. The latter curves indicate, as in the case of bases (6), that chemical combination with some receptor constituent may be at the basis of activation. In the more dilute solutions of HCl the stimulating power is proportional to the square of the concentration.

It may be suggested that in the chloroacetic series several factors are involved in stimulation. Some light is thrown upon the nature of these factors by the following considerations.

a. From the quantitative study of cell penetration by acids it has been found that the speed of penetration of an acid is proportional to a fractional power of the acid concentration up to a certain concentration, beyond which the speed of penetration increases very rapidly. When plotted in the form

$$\log\left(\frac{1}{P.T}\right) = \frac{1}{n}\log C - \log K, —$$

where $P.T$ = the (corrected) penetration-time, C = the concentration of acid, and K is a constant for each curve (or part of the curve),—the resulting figures are, for each acid, characteristically composed of two intersecting straight lines.[3] The concentrations at which the acids begin to penetrate tissue with greatly augmented velocity are, in the case of HCl and the chloroacetic acids, significantly correlated with those at which they produce maxima in the viscosity curves of protein solutions. According to Pauli, the concentrations at which these acids produce maxima in the viscosity curves of albumen solutions are as given in the last column of table 5 (quoted from Ostwald (26)). Similar relations hold for other proteins. The second column of table 5 shows the concentrations at which an abrupt increase occurs in the speed with which these acids penetrate an indicator-containing tissue (Chromodoris). The first column of this table lists the maximal concentrations at which these acids could be used to stimulate earthworms; at concentrations higher than these, toxic effects resulted in less than 1 second, so that the worm did not escape from the acid solutions.

[3] The correction factors involved in this treatment are obtained directly (without assumptions) from the empirically determined penetration curves. A discussion of this matter will appear elsewhere. Some of the data are contained in previous papers (4), (5), but a good deal is as yet unprinted. The equation given above is derivable from the well known formula for adsorption at constant temperature; but it does not refer to the *adsorption* of acid, in the penetration experiments.

The parallelism in these series of figures is on all essential points complete, and demonstrates the implication of cell proteins in the process of activation by these acids.

b. The operation of at least two factors in sensory stimulation by these acids is seen in the way according to which the stimulating power is related to the hydrogen-ion concentration. The hydrogen-ion content of the acid solutions used, calculated from standard conductance data, is plotted, in figure 2, against the "retraction-time;" formic acid (observations in table 6) is included in this figure. It is found that for a given "retraction-time" (which is a reciprocal measure of the activating power) the hydrogen ion concentration required decreases in the following order:

$$HCl > di - > tri - > monochloroacetic > acetic$$

TABLE 5

Showing the maximal concentrations for stimulation of the earthworm (Stimulation), the concentration at which a rapid increase is observed in the speed of cell penetration (Penetration), and the lowest concentrations above which, according to Pauli, the ionizations of the corresponding protein salts are diminished (Effect on proteins). All concentrations = 10^3N.

ACID	STIMULATION	PENETRATION	EFFECT ON PROTEINS
Acetic	>100	100	>50
Monochloric	63	40	>50
Dichloric	31	23	20
Trichloric	13	8.3	10
Hydrochloric	28	24	16

The facts to which attention is directed in *a* and *b* can be understood upon the assumption that that characteristic of the chloroacetic acids which determines their capillary activity coöperates with the hydrogen-ion concentration in effecting stimulation, and that (in part, at least) this stimulation concerns proteins of the receptor surface. According to the ideas developed especially by Langmuir (12), the capillary activity and lipoid solubility of these acids are together and simultaneously determined by the nature and orientation of the component parts of the acid molecules at the surface of their (aqueous) solutions. That surface effects are primarily concerned, is indicated by the very brief time required for the process of excitation (cf. 4), as well as by the relative activities of the monobasic fatty acids (fig. 3) which are subsequently discussed.

It is conceivable that the effects here considered have reference to the complex construction of the cell surface, which is independently indicated by a great variety of considerations (1, p. 129); (19); cf. also (28). In this connection use may be made of the relative speeds of cell penetration by the acids. There is a uniformity in the results

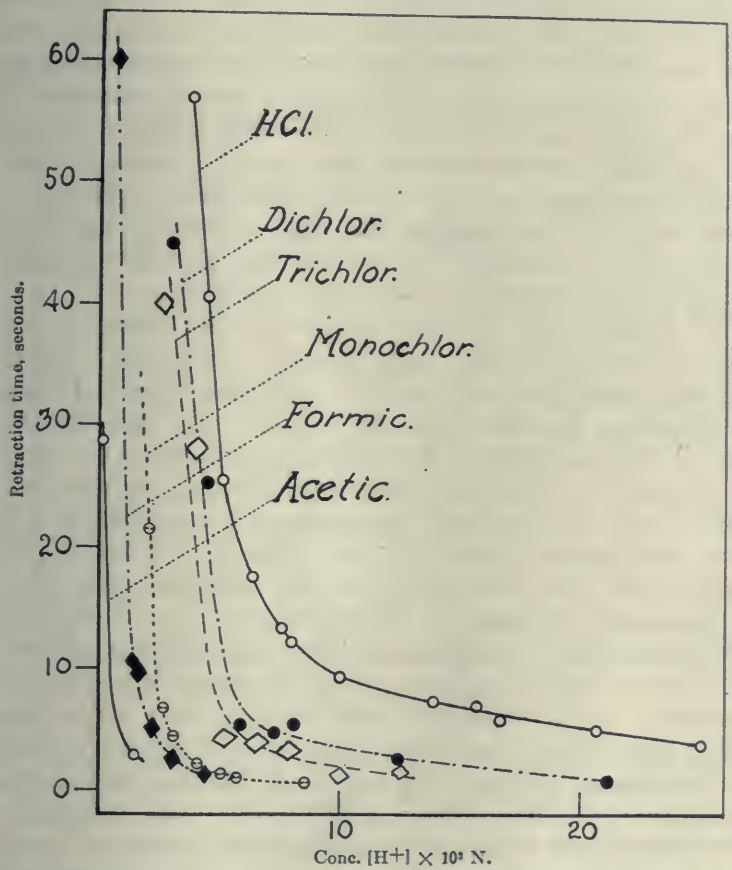

Fig. 2. Showing the relation between retraction-time and the (calculated) hydrogen-ion concentration of certain acid solutions.

which different observers have arrived at by this method (cf. (4), (9), (7)) which can only have reference to some general fact concerning protoplasmic organization. It is held that the penetration data afford a means of analyzing the surface composition of the cell, and that, according to the results of this method, both lipoids and proteins are

present at the cell surface. Hence it is possible that both the hydrogen ions *and* the remaining portions of the chloroacetic acid molecules are concerned in stimulation, and act upon both lipoids and proteins. The effect of these acids upon the surface tension of water, and their affinity for—solubility in—non-polar fatty substances depends, according to Langmuir and Harkins, upon the orientation of the molecules at the water surface in such a manner that the less active atomic groups are turned away from the aqueous phase. The surface activity of the chloroacetic acids increases in the same sequence as their ionization, so it is, then, not surprising to find that trichloroacetic acid, with highest ionization and highest surface activity, is (at equal hydrogen-ion concentrations) more efficient as a stimulating agent than dichloroacetic. In this way it can also be seen why the chloroacetic acids are more active in stimulation than is HCl, and why the stimulating power should increase very rapidly with increasing concentration.

This view requires that part, at least, of the activation process should include chemical action upon proteins. Preliminary experiments on the temperature coefficient of stimulation indicate for the chloroacetic acids a Q_{10} value (20°−30°) of 2 +, and the same for HCl; whereas in measuring the effect of temperature upon the speed of cell penetration by acids the coefficients obtained are about Q_{10} (20° − 30°) = 1.9 − 2.0, provided one considers that part of the acid curve where penetration is rapid, so that intracellular diffusion may be discounted,—at lower concentrations the coefficients are of the order of magnitude for diffusion or fluidity (Q_{10} = 1.1 − 1.7) (Cf. 22).

III. The comparative stimulating powers of the lower monobasic fatty acids, from formic to caprylic,[4] reveal interesting but not unexpected relations. Aside from formic acid, which is more active than valeric, these acids follow in general the order of their capillary activity and lipoid partition with water. Precisely these relations are seen also in the penetration of cells by these acids (4), (5). The figures contained in tables 6 to 12 are plotted logarithmically in figure 3.

It is seen that for each of these acids, excepting the lower concentrations of caproic and caprylic, the product (*Conc.*) × (*Retraction-time*) is essentially constant. With lower concentrations of caprylic and caproic acids the stimulating power is more nearly proportional to the square of the concentration. This may be the result of some "error" in the experimental method or it may be concerned with the

[4] The *normal* acids were used, except in the case of valeric, where the *iso*-acid was employed.

nature of the activation process itself. The phenomena in dilute caprylic and caproic solutions are therefore more comparable, it is believed, to that in dilute HCl solutions (cf. fig. 1) than to the curves of the chloroacetic acids. The squared-concentration effect appears in very dilute solutions, and does not appear in more concentrated solutions of other fatty acids of even much lower stimulating efficiency. One may therefore hold that the characteristic action of the weak acids is depicted by the constants derived from the $(C) \times (R.T)$ rela-

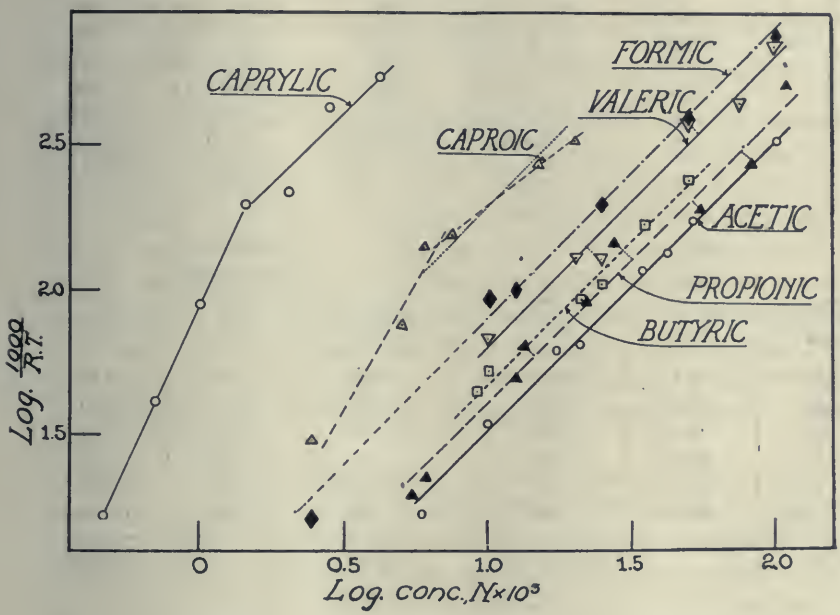

Fig. 3. Showing the relation between the reciprocal of the "reaction-time" of the earthworms from solutions of fatty acids and the concentration.

tions, or, in other words, by the portions of the curves (fig. 3) which slope at an angle of 45°.

These constants vary in a systematic manner with the effects of these acids upon the surface tension of water. There is, however, no ground for the supposition that surface tension changes, as such, are primarily implicated in stimulation. The behavior of HCl and of formic acid is significant at this point, as is also the fact (6) that NaOH is more active than NH_4OH. There is no indication that adsorption plays a part in the activation of the worms, as the stimulation curves

TABLE 6

Formic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
100	1.25	125
50	2.5	125
25	5.0	125
12.5	9.8	12.3
10.0	10.5	105
2.5	60	(150)
Mean		121±6

TABLE 7

Acetic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
103.5	3.0	310
52	5.8	302
42	7.4	311
34.6	8.5	294
21.	15.2	319
17.3	16.0	277
10.0	28.8	288
(6.0	60.0	336)
Mean		300±12

TABLE 8

Propionic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
110	1.9	209
82.5	3.6	297
55	5.2	276
27	6.8	184
22	10.8	240
13.5	15.4	208
12.5	19.8	248
6.1	44	268
5.5	59	(324)
Mean		241±31

TABLE 9

Butyric

CONCENTRATION	*R. T.*	*C.* × *R. T.*
50	4.1	205
35	5.8	203
25	9.4	235
21	10.6	229
10	18.8	188
9.2	22.1	203
Mean		210±13

TABLE 10

Valeric (iso-)

CONCENTRATION	*R. T.*	*C.* × *R. T.*
100	1.4	140
75	2.2	165
50	2.6	130
25	7.7	192
20	8.9	178
10	14.6	146
Mean		155±18

TABLE 11

Caproic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
20	3.0	60.0
15	3.6	54.0
7.5	6.4	48.0
6.0	7.0	42.0
5.0	(16.3)	
2.5	(32.5)	
Mean		50.8±6.0

TABLE 12

Caprylic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
41	1.8	7.38
2.8	2.3	6.44
2.0	4.5	9.00
1.42	5.0	7.10
1.0	(11.1)	
0.7	(25)	
0.5	(60)	
Mean		7.48±0.6

(fig. 3) are not of the proper shape and indeed give evidence of a contrary significance. If adsorption (surface condensation) of acid upon the receptor organs were a deciding process in stimulation, we should expect to find that some function of the form $(C.^{1/n}) \times (R.T.)$ would be constant for different concentrations of any one acid; because in the commonly used equation

$$\frac{x}{m} = KC^{1/n}$$

m (the adsorbing surface) is by the method of experiment made constant, and because the degree of activation, measured by $\frac{1}{R.T.}$, would be proportional to x (the amount adsorbed),—or to a logarithmic function of x, in case Weber's rule were to apply. This is not found to be the case. It is doubtful if the ordinary adsorption equation can legitimately be applied to a matter involving such brief time-intervals, but in any event some similar expression, involving a fractional power of the concentration, would be expected; whereas in fact the amount of activation appears to be proportional to the concentration, or to the square of the concentration, or increasing even more rapidly than this. According to Lillie (14), (15), the activation of starfish eggs by butyric acid follows the same law as that found here in a case of sensory excitation.

Recent work on surface tension has demonstrated that the "capillary activity" of the fatty acids, as well as their effect upon the interfacial tension in such a system of immiscible phases as benzene-water, depends upon the orientation of the molecules and their orderly arrangement with respect to the surface of the water phase (Langmuir, (12) and Harkins, (8)). With the dilute solutions here studied the concentration of acid at the interface between water solution and the receptor cell, while higher than that within the body of the solution (or rapidly becoming so soon after the formation of this interface by the immersion of the worm), may nevertheless be considered proportional to the formal concentration of acid. The surface-activity of these acids, at corresponding concentrations, increases by a constant amount for each addition of CH_2 to the molecule, because "each CH_2 group, in these solutions, forms a part of the surface," and the potential energy of the surface is therefore correspondingly increased by a constant amount (cf. Langmuir's discussion of the data of Trauble and others, (12, p. 1885 et seq.). The stimulating power of these

acids should increase by a constant amount for each addition of CH_2 to the acid molecule (neglecting the effects of isomerism, which are relatively unimportant). Figure 4 shows that this expectation is realized. In curve *A* the abscissas represent the number of CH_2 groups in the molecule, from formic (0) to caprylic (7), the corresponding

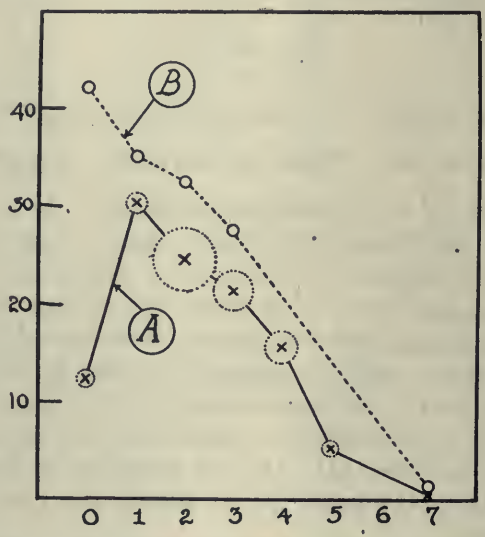

Fig. 4. *A*. The ordinates are the concentrations ($N \times 10^3$) required to effect a definite amount of stimulation (such that $\log \frac{1000}{R.T.} = 2.00$). The abscissas are the number of CH_2-groups in the molecules of the monobasic fatty acids, from formic 0 to caprylic 7. The dotted circles are drawn with a radius equal to the average deviation calculated from individual observations (cf. tables 6 to 12).

B. Against the same abscissas as in *A* there are plotted the times required to effect membrane formation in 60 per cent of the eggs of the sea urchin, according to experiments tabulated by Loeb (15), in acid solutions at 0.001 N concentration. The unit of ordinates is here = 0.1 minute (see text).

ordinates being the concentrations required to effect a definite amount of activation (such that $\log \frac{1000}{R.T.} = 2.00$ — see fig. 3). This curve shows that, aside from formic acid, the amount of each fatty acid which is required to bring about a constant degree of activation decreases in regular manner (within the limits of error of the observations) according to the number of CH_2 groups in the acid molecule.

The exceptional behavior of formic acid is due to its much stronger ionization.[5] It is probable that here the hydrogen ion is also concerned in activation. These acids may, in other words, combine with the surface of the cell, *a*, through the affinity of the carboxyl group for water or protein; and, *b*, through the affinity of their hydrocarbon chains for lipoids. When *b* is relatively much larger than *a*, owing to the orientation of surface molecules of acid, it alone appears to be the controlling factor in stimulation, as in the fatty acids other than formic. Hence there is no evidence among these acids that their ionization affects their stimulating ability, as in that event some disturbance of the straight line relation depicted in figure 5 would be found. Careful work may show that such deviation does in fact exist in some cases, and in human taste it is known that although the sourness of different acids depends directly on their tendency to ionize (when the penetration of the receptor is taken into account), the acids weaker than acetic nevertheless produce a more powerful sensory effect of another kind (and probably upon the same taste cells).

IV. Relations similar to those just described are found in comparing the penetration of cells by these acids. In referring these effects to interactions with cell lipoids, there are several objections to be considered.

a. It might be conceived that sensory cells specialized for chemoreception are merely more permeable, to acids, for example, than most cells appear to be. There is no good reason favoring this belief, and the reverse is equally likely to be the case. Low concentrations of acid do not behave in stimulation as high concentrations do in the penetration of cells in general. The chemoreceptors of the earthworm have long modified distal extremities, which project, cilia-like, through openings in the cuticula of the worm, and they must therefore be rather dense and rigid. (It has been suggested that these processes may contract upon stimulation, but it does not appear that surface tension forces thus brought into play are the determiners of activation.)

Chambers (2) states that a resistant surface film of a specialized kind is present only upon protozoans and germ-cells. The histological appearance of sensory cells suggests that they also have very specialized outer surfaces. It is, then, of interest to compare the effects of these

[5] Formic solutions have effects like those of HCl, and unlike those of the weaker acids, when their toxicity is considered. Formic acid is much more efficient in destroying the sensitivity of the earthworm's chemoreceptors than are the weaker acids.

acids upon other cells which do undoubtedly possess a highly specialized surface; such cells are found in the mature eggs of the sea urchin. The time required by solutions of the monobasic fatty acids, at constant concentration (0.001 N), to bring about activation (membrane formation) in 60 per cent of the mature eggs of the sea urchin is plotted in figure 4, curve *B*. The figures were obtained by graphic interpolation from data given by Loeb (17, p. 134). The ordinates for this curve are in units of 0.1 min. The ordinates for curves *A* and *B* are in different units, but are directly comparable because in the stimulation figures the concentration is inversely proportional to *R.T.*, which is itself inversely proportional (by assumption) to the intensity of stimulation; hence the "concentration" and "time" in the two sets of experiments have similar meaning. The behavior of formic acid is quite different in the two cases, but otherwise the effects are qualitatively identical. This suggests a difference in the superficial composition of the two kinds of cells which are compared.

b. Weak acids and weak alkalies have a more powerful action upon proteins than their H^+ or OH^- concentrations would theoretically warrant. In this respect there is a certain parallelism with the results of stimulation experiments, which might be regarded as evidence against the idea that lipoids are concerned in the stimulation effects. There is, however, independent evidence favoring the presence of lipoids at the surfaces of cells (cf. 19), and it seems unlikely that the relative activities of the several fatty acids could be accounted for on this basis.

On the other hand, Langmuir has shown (12, p. 1883) how the hydrogen ion may act to make thin oil (or lipoid?) films more mobile, and there are other physical effects of acids upon lipoids which might be considered significant. But the explanation of activation by the strong acids without reference to their action on proteins would ignore the curious parallels which have previously been pointed out. In addition, the stimulating power of HCl is greater than that of NaOH, which is also favorable to the idea of action upon proteins (cf. 29). Taking into account the very different behavior of the mineral and fatty acids in producing toxic results at high concentrations, and the differences in their behavior in penetrating cells, it seems most reasonable to refer otheir effects in stimulation to reactions with different components of the sensory cell-surface. Further experiments are being made to test this idea.

V. One view of the process of stimulation endeavors to reduce all

forms of activation to a common basis of increase in the permeability of the cell surface. A widely entertained theory of this nature, notably developed in the writings of R. S. Lillie, considers that the essential act in stimulation has to do with the depolarization of the cell surface, which is supposed to exhibit in its resting state a polarization resulting from differential permeability toward oppositely charged ions, and particularly from its impermeability for anions. This idea of the origin of bioelectric potentials is, however, confronted by peculiar difficulties of its own (cf. 18), and the general conception that stimulation (activation) is brought about by agents operating to increase permeability is further opposed by the fact that substances which (so far as we know) act primarily to decrease permeability are very efficient in stimulation. This matter can best be studied by means of measurements of the stimulating powers of various substances for organs of chemical sense, where the problem of excitation by external influences has some primary significance. It is furthermore of interest to note that in the case of the earthworm the sensory cells which are probably concerned in the effects here described, are neurones of the first order, and that the ganglion cell is in all probability stimulated directly by the dissolved substances.

Strong acids (HCl) produce a decrease in permeability toward ions, which is followed by an increase only after the elapse of a relatively considerable interval (at the concentrations we are dealing with); (cf. 24). The general and specific parallelisms between the behavior of cells composing tissues of the most varied origin on the one hand, and the sensory receptors of the earthworm on the other, in penetration and stimulation experiments respectively, makes it unnecessary to assume the existence of fundamentally exceptional structural conditions at the receptor surface. Hence it is improbable that acid has here an exceptional effect upon surface permeability for ions. If, however, the hydrogen ion should *hinder* stimulation by inducing an increase in surface polarization or by otherwise decreasing permeability, then we should expect to find that acids would be specifically less efficient as excitants in proportion to their ionization. In figure 2 it will be seen that apparently this is in part true; for example, at a given stimulating power the [H·] values decrease in the order *acetic* < *formic* < *HCl;* the explanation, of course, lies in the fact that in these acids the stimulating property does not concern merely the H·.

There is also the fact that HCl, which leads to an increase in permeability only after a pronounced decrease, is specifically more ener-

getic as an excitant than is NaOH, which produces only an increase in permeability (23). Figure 5 shows that at all corresponding concentrations of [H·] and [OH⁻] HCl is a more powerful excitant than is NaOH. (The data for NaOH are taken from the preceding paper; the two series of experiments were strictly comparable). This is equally true at dilutions where Na· and Cl⁻ are quite inoperative in pure salt solutions, and where indeed if they were significantly con-

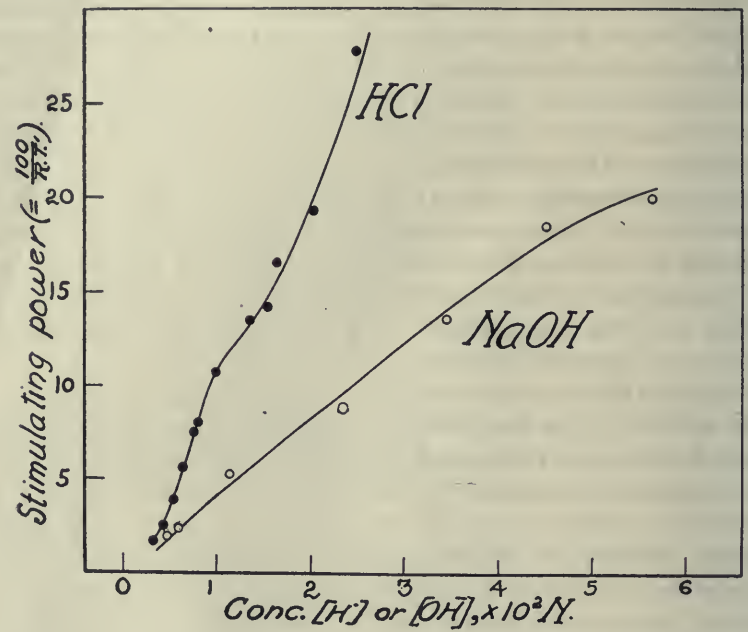

Fig. 5. The relative stimulating powers of solutions of HCl and of NaOH at corresponding concentrations of $H^{\cdot}$ and OH^{-}.

cerned a reverse order of stimulating capacities would be expected, since Na· is more powerful than Cl⁻ in activating these worms. HCl, moreover, is active at a lower threshold than is the alkali. These relations obtain also for the comparative toxic effects of H· and OH⁻, and are significantly seen also in taste excitation. The limiting dilutions at which HCl and NaOH are perceptible upon the tongue are respectively at [H·] = 0.0011 ± and [OH⁻] = 0.007 ± (11). According to Parker (27) the relative effectiveness of HCl and NaOH for

stimulation in lower vertebrates is of the same order.[6] It is significant that in Osterhout's experiments (23), (24) NaOH at 0.001 N concentration has practically no effect on permeability, although HCl 0.001 N has a pronounced effect in decreasing permeability. The limiting concentrations in the earthworm experiments were found to be: HCl < 0.0037; NaOH = 0.005 ± (at the *anterior* end of the worm the absolute dilutions are greater, although the relative proportions are about the same). The changes in permeability measured by Osterhout are due to chemical reactions with surface constituents of the protoplasm.

Moreover, the concentration of HCl which in Osterhout's experiments with Laminaria induces a rapid increase in permeability (following the preliminary decrease) is between 0.015 and 0.02 N; that is, the extent of preliminary decrease in penetrability for ions reaches its maximum value in solutions about 0.015 N, and thereafter becomes smaller as the concentration of acid is made greater This is essentially the order of magnitude of the maximal concentration with which the earthworms in the present experiments may be stimulated and yet quickly recover. So there is reason to believe that, although the changes induced in specialized sensory cells by acids and alkalies are much more rapidly brought about than in ordinary tissue elements (and this is possibly the primary expression of their specialization), the essential nature of these changes is nevertheless identical in all cases (or in nearly all cases, excepting perhaps egg cells).

The results of experiments dealing with the activation of the earthworm seem, then, to be opposed to the idea that stimulating agents universally produce their effects by virtue of a permeability-increasing action. They are, however, favorable to the idea that in chemoreceptor activation there occurs some union, essentially chemical in nature, between the activating agent and some one or more constituents of the receptor surface (which may itself vary in composition according to the habitat of different worms of the same species). The amount of stimulation depends upon the character and extent of this combination. It is assumed that the "all or none" principle does not apply to these effects (which does not mean its lack of applicability to a single propagated impulse), since when large areas of the worms used are exposed to stimulation the intensity of activation is not pro-

[6] At higher concentrations the alkali is sometimes more stimulating; this is probably due to secondary influences, as different types of response may be concerned.

portional to the number of sense organs involved; in another direction it can be pointed out that human taste buds detect a wide range of differences in, for example, sourness.

These experiments are in agreement with a conception of stimulation which seems first to have been formulated by Loeb ((16) and earlier papers), which holds that ion-protein (or ion-soap) compounds control the ratios between free ions in the cell, and by variations in their composition or physical state determine in this way the propagation of impulses. Whether or not the specific form of this general theory elaborated by Macdonald (cf. 20) is applicable here, seems doubtful. In his theory a local colloidal condensation (or precipitate) at the point of excitation, decreasing the extent of local surfaces available for the adsorption of ions, results in a freeing of ions for diffusion. From these earthworm experiments it seems possible that either precipitation or the reverse may serve equally well for the initiation of excitation.

SUMMARY

When earthworms are stimulated by acids according to a method which gives quantitative results connecting the concentration of the excitant with the amount of stimulation induced, it is found that the acids stimulate as if by simple chemical combination with one or more constituents of the receptor surface. There are striking quantitative paralellisms between the powers of different acids to penetrate cells and the peculiarities of their relations in stimulation. This does not mean that they stimulate by mere diffusive penetration, but that similar combinations with cell materials are fundamental to both processes. Independently of assumptions made for purposes of quantitative treatment, the results of these experiments are inconsistent with the idea that activation is induced by surface depolarization of the cell, and with the associated, but unduly generalized, conception that stimulation involves in all cases an increase in cell permeability to ions. This conclusion is obviously not opposed to the idea that stimulated cells may in many cases become more permeable, but it does imply that an increase in surface permeability is not the determiner of activation. It does favor the idea that alterations in the condition of materials at the surface of the cell are instrumental in determining the diffusion of ions within the cell.

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X. DIFFERENCES IN THE BEHAVIOR OF SEGMENTS FROM DIFFERENT PARTS OF THE INTESTINE

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Received for publication December 26, 1917

The writer has shown in a previous paper that, roughly speaking, the rate of rhythmic contraction in excised segments of small intestine varies inversely as the distance from the pylorus (1). Since then a better technic has been worked out. Five segments are now used in the same beaker of Locke's solution, and all are kept at a constant temperature of 38°. The rabbits are killed by a blow on the head; they are opened immediately and segments 15 cm. long are removed from the first portion of the duodenum, from the first portion of the jejunum, from the middle of the small intestine, from the lower ileum opposite the tip of the appendix and from the colon where it parallels the duodenum. These segments are kept in iced Locke's solution and shorter pieces, 2.5 cm. long, are cut as required. Long heart levers have been made with the arms more nearly equal, so that the magnification will be less and the five records will go on the drum. The measurements are 18.5 cm. from fulcrum to writing point and 12 cm. from fulcrum to thread. The lever is weighted just enough to keep the thread taut. The segments are held by small wire serrefines. The beaker contains 400 cc. of Locke's solution through which air bubbles.

Differences in tone. Differences in tone were observed in cutting the segments. The duodenal loops shortened a great deal after cutting and the ends rolled backwards, forming wide cuffs. The jejunal pieces contracted somewhat and showed narrower "cuffs." The middle pieces contracted still less and showed very little cuff formation. The ileum lengthened after excision and it showed poor tone. The colon showed even more tone than did the duodenum. It retained a very firm grip on the scybalae, and segments shortened to perhaps half their original lengths. These differences had to be taken into account so that the records would fit on the standard drum. The ileal segment had to be cut 1.5 to 2.0 cm. long and the duodenal and colonic ones

3.5 cm. long, so that they would be of equal length after immersion in the warm Locke's solution.

The greater tone of the duodenal and jejunal segments was shown also by the way in which they shortened after they began to beat rhythmically. The middle and ileal segments generally improved as regards amplitude of contraction, but the base line rarely rose. It generally remained very constant, probably because the muscle had relaxed until it was checked by the connective tissue along the mesenteric border. After attachment to the levers, the most pronounced rise in tone was almost always shown by the jejunal segment. The duodenal rise might have been even greater if the segment had not already contracted so much before immersion in the beaker. The colonic segments also shortened a good deal, generally after from ten to twenty minutes.

Comparative rhythmicity. The duodenal segment was generally the first to beat well. Sixty-eight records were examined as to this point and the different segments were credited with one, two, three or four points according as they began to beat first, second, etc. When two began to beat so nearly at the same time that no difference could be made out, they each received the same number. When the figures were averaged, the following data were obtained:

Duodenum	1.53
Jejunum	2.38
Middle	2.64
Ileum	1.70

The duodenum beat first forty-six times; the jejunum, thirteen; the middle, six; and the ileum, thirty-four. The great rhythmicity of the duodenal segments is the more striking when it is remembered that they seem to suffer most from trauma. They must be handled more carefully than the ileal segments. They apparently recover somewhat from the trauma of cutting during their stay in the cold Locke's solution because when attached to the levers immediately after removal from the animal, they were slower in starting up. The great vulnerability of this region probably accounts for the fact that in eight out of the sixty-eight experiments, the duodenal segment remained practically motionless. Even in sickly rabbits the other segments always showed some activity. Perhaps the high rhythmicity of the segments from the lower ileum is due partly to their comparative immunity from injury in cutting and handling.

The first few centimeters of the duodenum always beat very poorly with a small, variable amplitude. This agrees with the x-ray findings

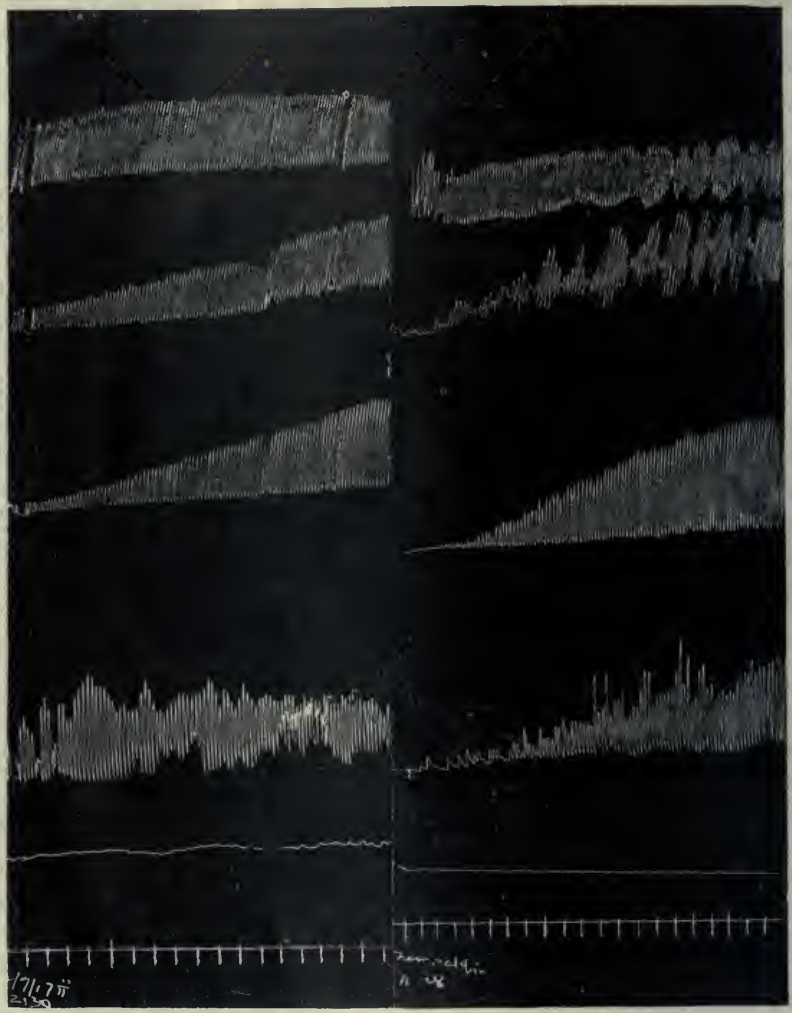

Fig. 1. Two typical beginnings. From above downward the tracings are from duodenum, jejunum, upper ileum, lower ileum and colon. The time record represents thirty seconds.

in man where the first portion of the duodenum ordinarily remains filled and shows little activity. The food naturally is delayed in this quiet region situated between two active ones.

The greater tendency to rhythmic contraction in the upper end of the tract is suggested strongly in figure 2. The segments were first poisoned with pilocarpine and then atropin was added. The first to escape was the duodenal segment and this was followed in order by the middle, ileum and colon. A similar graded escape from inhibition has

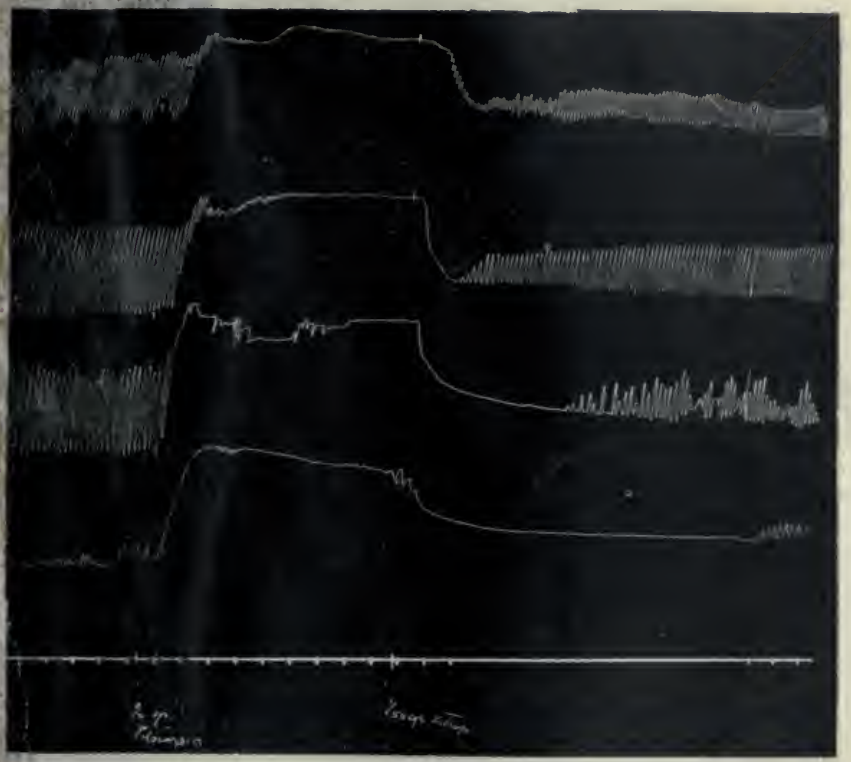

Fig. 2. To show the graded recovery from pilocarpin after adding atropin. From above downward, the records are from duodenum, upper ileum, lower ileum and colon. Time record represents thirty seconds.

been observed with some other drugs such as adrenalin and magnesium sulfate.

The colonic segments were so slow in beginning to beat that it was found best to cut them first and to leave them in the warm solution while the other segments were being prepared. After these were fastened to the levers, they were all lowered together into the solution.

Even with this head start, the colon often took an hour to get going well and it generally beat better on the second day after excision than on the first. The greater sluggishness of the colonic muscle was noticed also in pharmacological studies. Drugs which depressed the duodenum for thirty seconds often kept the colon paralyzed for five minutes or more. In some cases the drug caused a short drop and rise and then a permanent drop in the duodenum, but only the final drop in the colon. The contractions were very different, the rhythm irregular and the tone variable. The rate varied from 2 to 12 per minute. Unpublished experiments show also that the latent period of the colonic muscle is longer than that of the muscle in the small intestine. One cannot escape the impression that we have to deal in the small and large bowels with two very different types of muscle. The muscle in the colon acts more like the smooth muscle of a cold-blooded animal. The muscles in the small and large intestine, again, behave differently from the muscles in the body and antrum of the stomach.

Segments from sickly animals. The segments from sickly animals or animals heavily infected with parasites, generally beat poorly and irregularly. Sometimes they would begin beating well, or the first set would beat normally, but they generally became weak and erratic after a while. Sometimes segments from flabby looking intestines beat surprisingly well, with a very wide amplitude. The wide amplitude is probably a sign of poor tone (2). Ordinarily the duodenal and jejunal segments seemed to suffer most from the depression. Sometimes the duodenum would not beat at all even when the animal appeared to be pretty healthy. In some of the animals, short stretches of bowel were found to be flabby and filled with gas, while the rest of the gut appeared to be normal. It was interesting that when segments were excised from these peculiar regions they often failed to beat well, although the other segments behaved normally. Segments from rabbits whose abdomens were full of cysticerci generally beat well if the animal was well nourished and active. The colon did not seem to be much affected by the general depression and frequently it was the only segment that would contract normally. Segments of colon were markedly depressed in some animals with a tendency to diarrhoea. This agrees with x-ray studies in man which generally show the diarrhoeic colon unsegmented and flabby. The segments from the sickly animals often reacted poorly to drugs.

In one animal an inflamed Peyer's patch was noticed in the lower ileum. The inflamed segment, when excised and put into Locke's

solution, did not beat well and its rate was about normal. The segment just above, however, beat 21.5 to 25 times per minute. This

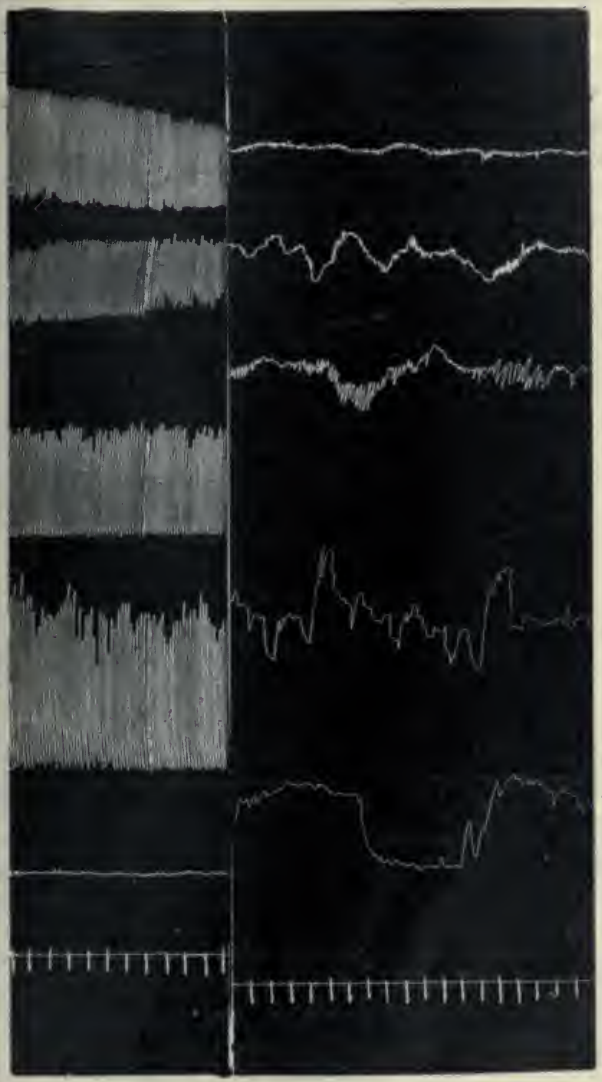

Fig. 3. Records from healthy and from diseased animals.

is not only twice the normal rate for the ileum but it is faster than normal for the duodenum. This shows what the writer has long sus-

pected, that inflammatory lesions can alter the gradients in the tract. These gradients may also be upset by the unevenness of the effects of disease toxins; that is, the duodenum and jejunum may be almost paralyzed while the ileum and colon remain active. Such a reversal was observed while studying the latent periods in different parts of the stomachs of distempered dogs (3). These observations may explain many of the digestive upsets in thin, run-down, nervous women; upsets for which no anatomical explanation can be found, but which straighten out promptly under over-feeding and rest.

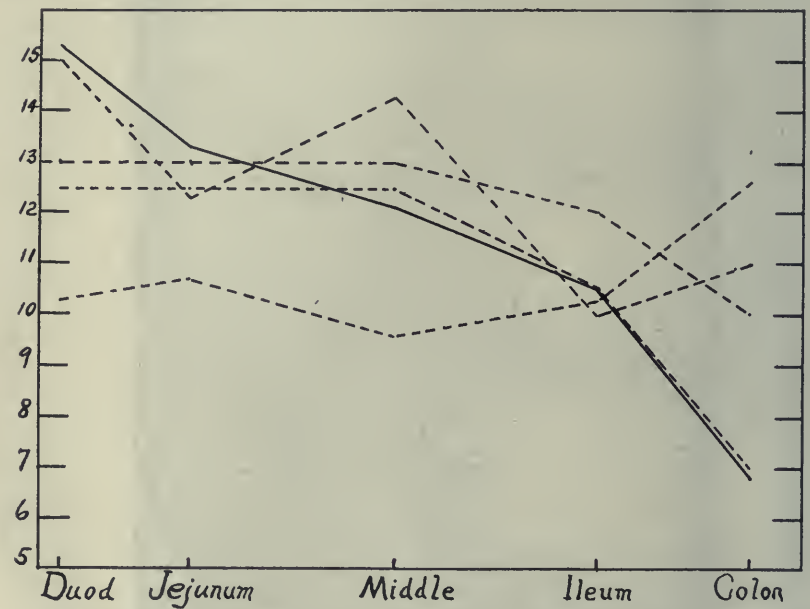

Fig. 4. Ordinates represent rates per minute; abscissae represent the segments at varying distances from the pylorus. The solid line represents the average for fifty-three animals. The broken lines represent data from sickly animals.

Gradient of rhythm. The rate of contraction has been counted in one hundred and seventy-six places on records from fifty-three rabbits. These animals were in good condition and the segments contracted well. The averages are as follows:

	per minute
Duodenum	15.3
Jejunum	13.3
Upper ileum	12.1
Lower ileum	10.5
Colon	6.8

These data have been plotted as a heavy line in figure 4, in which the ordinates represent rates per minute and the abscissae distances from the pylorus. Unfortunately, these abscissae can be only approximately correct.

It is interesting that in these fifty-three animals there was no instance in which the average of two or three readings on any one segment gave a figure higher than the average for the segment just above. The gradients were not so even, however, in the sickly animals. Samples of these are shown as broken lines in figure 4. These upsets in gradient have been found to be even more marked in the intact bowels of sick animals studied under salt solution (4).

Three animals were starved for three or four days (without muzzles). Their segments beat with a poorer amplitude than normal but no characteristic changes in rhythmicity or in gradient could be made out.

Behavior after twenty-four hours. It is a remarkable fact that after twenty-four hours in Locke's solution between 5° and 10°C., the segments beat faster and the gradient of rhythm was retained. This will be observed in the following three protocols:

	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Duodenum	15.0	18.5	14.8	16.5	12.3	16.3
Jejunum	13.7	16.5	12.6	15.0	11.0	16.0
Upper ileum	11.9	14.0	11.0	12.0	8.9	14.3
Lower ileum	11.3	14.0	9.2	8.0	7.4	14.0
Colon	7.0	5.0			6.5	3.0

The strength of the contractions suffered and the segments often became fatigued quickly. They were also less sensitive to drugs although on two occasions they reacted typically with 1 part of adrenalin to 8,000,000 of the solution.

This evidence fits in with a great deal more (5) which it seems to me has proven that the rhythmicity is initiated in the muscle itself and not in the nerve-net. The differences in rate found normally in the different parts of the gut are due probably to differences in some phase of the metabolism in the muscle.

SUMMARY

Five segments excised from different parts of the rabbit's intestine have been studied under identical conditions in warm aerated Locke's solution.

The segments of duodenum and jejunum have greater tone and contract more after cutting than do the ileal segments. The colon also has a high tone.

The duodenal segment is generally the first to begin beating well. The tendency to rhythmic activity is graded from duodenum to ileum. The first few centimeters of duodenum, corresponding to the duodenal cap in man, does not beat well.

The colon is very slow in starting up and it differs greatly from the small intestine in its behavior.

The duodenum suffers more from trauma and from adverse conditions than do the other segments.

Segments from sickly animals beat poorly and become fatigued early. These changes are often more marked in some segments than in others so that the gradation of rhythm is changed.

The gradation of rate of contraction from duodenum to ileum is remarkably constant in normal animals.

In one case the gradation was upset by the presence of an inflamed area in the ileum. The bowel in that region contracted 21.5 to 25 times per minute, or twice as fast as normal.

After twenty-four hours, the segments beat at a faster rate and maintain the gradient. They continue to react normally to adrenalin and atropin.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

I. Relation of Oxygen Concentration and the Rate of Intracellular Oxidation in Paramecium caudatum

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Received for publication January 2, 1918

So far as the writer is aware no attempt has ever been made to study with quantitative methods, the oxygen consumption of any unicellular animal. There are only two papers, Vernon (1) and Barratt (2) which attempt to give anything like measurements of CO_2 production by a protozoan cell.

The purpose of the present series of papers will be *a*, to describe methods which yield accurate and reproducible results on O_2 consumption and CO_2 production, and *b*, to show as far as may be, what the conditions are and how they affect the magnitude and rate of O_2 consumption and CO_2 production in these unicellular animals, using for this purpose to begin with, Paramecium caudatum. In this paper results are given to show what relation the concentration of oxygen has to the rate of intracellular oxidation.

METHOD

Preparation of Paramecium for experiment. The material for all the work was a pure line of Paramecium caudatum grown in large mass cultures in boiled hay infusion. The only other organisms present in the cultures were bacteria of various types commonly found in such infusions, which served as food for Paramecium; occasionally small amoebo-flagellates would occur which then served as part of the food supply for Paramecium. When the cultures were in their height of development the clear supernatant liquid containing the Paramecia was siphoned off carefully to avoid introducing any bacterial zoögloea. The organisms were then concentrated by use of the centrifuge at as slow a speed as possible. In this way injury to the animals was

avoided. The removal of the Paramecia to clear tap water, which whenever necessary had been sterilized by boiling, was accomplished gradually by repeatedly diluting the concentrated suspension with sterile or ordinary tap water at room temperature and then centrifuging. This transfer and washing of the Paramecia must ordinarily be gradual in order to allow time for the animals to adjust themselves to the new chemical and osmotic conditions. Transfer to pure tap water and washing was usually extended over a period of fifteen to twenty-five hours. In this way, with care, it is possible to wash Paramecia perfectly free from the native medium and to reduce the bacterial content of a Paramecium suspension to that of ordinary tap water or tap water which has been previously boiled. Hargitt and Fray (3) have shown by a series of careful tests that it is possible to sterilize a Paramecium by washing it five or six times in different portions of five to ten drops of sterile water. For the purposes of the experiments in this and following papers the small bacterial content of tap water and boiled water has no significance since if all the Paramecia remain alive, there will then be no pabulum in which bacteria in sufficient numbers to disturb the results will grow. Controls which rule out all effects from bacteria were always carried out whenever conditions demanded. These conditions will be referred to at the proper time. When Paramecia are washed in this way they are under starvation conditions. The food reserve of the protoplasm is gradually depleted as shown by the gradual decrease over a number of days in cell lipoids and increase in transparency of the protoplasm to light. But Paramecium will often live for as long as ten to fourteen days in tap water without food, which shows that the food reserve of the protoplasm may be sufficient to meet its expenditure of energy over this period of time. Cell division stops in a pure line population after the Paramecia have been removed to starvation conditions in tap water for twenty-four hours. Hence it is readily possible to obtain *suspensions of cells which do not divide, that is, where the number of cells remains constant.* It is always well to keep the Paramecia in a comparatively large volume of water before using, for high concentration of suspensions leads sooner or later, depending on conditions, to death of some of the organisms which serve as a food supply for bacteria that may happen to be present and also for other Paramecia which when starved become "hungry" feeders.

Just before using, the washed Paramecia were concentrated at a low speed of the centrifuge so that in 1 cc. of suspension there were

from two thousand to one hundred thousand individuals depending upon the concentration desired for the particular type of experiment. Equal volumes of this suspension were gently withdrawn by use of a 1 or 2 cc. volumetric pipette which had a large smooth opening. Stimulation and injury to individuals by the pipette is often brought about by too rapid suction on a pipette with a small opening and sharp edges. The question arises: Is it possible by this method to obtain equal numbers of Paramecia in different 1 cc. volumes drawn successively by the volumetric pipette? The answer is found by (1) a comparison of the quantities of oxygen absorbed by different 1 cc. samples of Paramecia from the same suspension, and (2) by actual counts of the number of Paramecia in such samples of equal volume. Both of these methods have been used and as will be shown in this and subsequent papers, are trustworthy criteria for determining the number of individuals in unit volume. The error from this source falls within the limits of experimental errors from other sources.

Use of Winkler's method for determining dissolved oxygen. The reagents used for Winkler's method were made up as given by Treadwell and Hall (4) except that 5 cc. instead of 3 cc. of concentrated HCl was used. The thiosulfate solution was standardized at intervals against known weights of freshly resublimed iodine according to Treadwell and Hall (4, p. 645). For simplicity the tables give the oxygen equivalent in cubic centimeters of thiosulfate.

The tap water used for an experiment was kept in carboys and allowed to stand at room temperature. A stream of air was then passed through it for several hours in order that the oxygen in the water might come into equilibrium with that of the air at room temperature. Bottles of equal volumes (137 cc.) were then filled with the tap water from the carboy. The degree of uniformity of oxygen content in such a series of bottles is illustrated by the figures in tables 4, 5 and 6. The variation is on the average less than 0.1 cc. of thiosulfate per 137 cc. volume and where the average of a number of bottles is taken the error in filling of bottles and analysis can be reduced to less than 1 per cent of the oxygen content of 137 cc. of water at atmospheric pressure and a temperature of 20°C.

After filling the bottles 1 or 2 cc. of the concentrated Paramecium suspensions were added and the bottle tightly stoppered. After varying periods of time the water in the bottles containing Paramecia and the blanks without animals were analyzed. A small amount of the liberated iodine is adsorbed by the dead Paramecia. The amount

varies with the number of animals in the bottle and also with the concentration of liberated iodine, but in all cases where the number of Paramecia is not more than five thousand, it is small and practically constant. The error due to adsorption of iodine was eliminated by analyzing at the beginning of the experiment control bottles, one set being blanks without Paramecia and the other containing the same number of Paramecia as was used in the experimental bottles. The difference between averages of these two sets of bottles gave the amount of iodine in cubic centimeters of thiosulfate which was adsorbed by the cells, and in the results are applied as corrections (see tables).

Heilbrunn (5) has pointed out the source of error due to the taking up of iodine by sea urchin eggs and possibly by the secretions liberated by eggs into sea water. This objection does not apply in any measurable degree to this work on Paramecium since, (1) Paramecia do not liberate substances into the tap water which interfere with the analysis, and (2) since the number of Paramecia which are used in each bottle is small in comparison with the number of sea urchin eggs which would utilize an equal quantity of oxygen and (3) the slight loss of iodine that does occur is corrected for as given above. The above method entirely avoids the error which is met with when the water in the bottles containing the eggs or other organisms is siphoned off into a smaller bottle for analysis. Since bottles of equal volume and O_2 content with a practically equal number of cells in each can be obtained, then by computing the average oxygen content of a number of bottles the errors can be reduced to a small value.

When the oxygen consumption of such cells as sea urchin eggs, blood cells or yeast is determined there is no definite index by means of which one can determine when one or more of the cells die and therefore it becomes difficult to know definitely in just what physiological condition the cells are at any particular time. This great experimental disadvantage is practically entirely avoided with Paramecium because one can readily determine by the use of a hand lens, or if necessary under the binocular, when the organisms are abnormal either in shape (approaching cytolysis) or locomotion. Paramecium therefore serves as an ideal organism for accurate quantitative work on intracellular oxidation.

Any experimental procedure which differs from that described above will be given in connection with the particular experiments.

EXPERIMENTAL

Oxygen concentration and the rate of oxygen consumption. Different concentrations of oxygen in tap water were obtained by passing water from the tap through a small heated copper coil into carboys, thereby removing practically all the dissolved air from the water. The water was allowed to cool to room temperature. The desired concentrations of dissolved oxygen were obtained by slowly bubbling compressed oxygen from a tank through the water and analysing samples from time to time in order to determine when the desired concentration of oxygen was obtained. Shaking with air was also convenient. The experi-

TABLE 1

Preliminary experiment. Paramecia left in clear native hay infusion for twenty-four hours, then washed three times in sterile tap water and centrifuged. Volume of bottles 137 cc. Temperature 25±.1°C. 1 cc. thiosulfate = 0.158 cc. O_2 at N. T. P.

BOTTLE	CONTROLS [ANALYZED AT ONCE		ANALYZED AT END OF 6 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	2.00	1.8	1.15		All normal at end of 6 hrs.
2	1.94	1.8			
3	1.94				
Average	1.96	1.8	1.15	*0.65*	
Iodine adsorbed by Paramecia				0.16	
B. High O_2 concentration					
1	13.30	13.00	12.40		Some dead, others dying at end of 6 hrs.
2	13.20	12.90			
3	13.15				
Average	13.21	12.95	12.40	*0.55*	
Iodine adsorbed by Paramecia				0.26	

TABLE 2

Preliminary experiment. Paramecia washed in sterile tap water three times and starved several hours: centrifuged. Volume of each bottle 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2 N. T. P. Temperature 25± .2°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 6 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed in 6 hrs.	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.60	1.58	1.16		All alive and active
2	1.57	1.50			
3	1.70	1.60			
Average	1.62	1.56	1.16	*0.40*	
Iodine adsorbed by Paramecia				0.06	
B. High O_2 concentration					
1	14.6	14.45	14.34		Only a few living at end of 6 hrs. Most of these were injured as shown by movement and shape
2	14.7	14.59	14.35		
3	14.8				
Average	14.7	14.52	14.345	*0.175*	
Iodine adsorbed by Paramecia				0.18	

mental bottles were then immediately filled. The first and the last bottle filled were included in the control blanks in order to detect any perceptible difference in oxygen content of the water drawn first and last.

Tables 1 and 2 are results from two preliminary experiments where the manipulation was not as accurate as in the later experiments. The average amount of oxygen consumed by 1 cc. Paramecia in a bottle during six hours is given in column 4. This is obtained by subtracting the average number of cubic centimeters of thiosulfate per bottle in column 3 from the average number of cubic centimeters per bottle in column 2. It will be noted (table 1) that although the con-

centration of oxygen in one set of bottles, B, was over six times that in the other set, A, nevertheless the average quantity of oxygen consumed in the high oxygen concentration was slightly less than that consumed in the low concentration. The animals in table 1, set A, were all living and normal at the end of six hours. A few of those in B were dead and many were abnormal at the end of six hours. In table 2 where the oxygen concentration in B was about nine times that in A, the oxygen consumed was less than half that consumed in A. This is due to the early death of many of the cells in B caused by the high oxygen concentration. *These results indicate that when the cell is killed by high concentration of oxygen intracellular oxidations stop.*

The remaining experiments given in tables 3 to 6 are an intensive search for any slight effect of oxygen concentration on the rate of intracellular oxidations. The source of error in tables 1 and 2 due to death of cells was carefully eliminated. The results in tables 4, 5 and 6 show the uniformity and accuracy obtainable by Winkler's method with Paramecium in tap water.

In order to detect any slight effect of concentration of oxygen on the rate of intracellular oxidation which might occur, two types of experiments were tried. The first type is represented in table 3 and consisted in determining the time rate of oxygen absorption beginning at a concentration which is near the lower limits of the oxygen concentration in which Paramecium can survive. The cells were allowed to begin the consumption of a quantity of oxygen equivalent to an average of 1.476 cc. thiosulfate per 137 cc. volume. The amounts of oxygen consumed in successive periods of time were determined in order to see if the amount of oxygen consumed per hour differed as the concentration became less. The results are given in table 3 and are shown in the curve, figure 1. At the end of seven and one-half hours the first Paramecia in the bottles were just beginning to die. Hence the results up to and including the first seven and one-half hours are free from sources of error due to death. During the period from seven and one-half to ten hours an increasing number of deaths took place but the proportion of dead to living cells was small hence the decrease in the amounts of oxygen absorbed due to death is not noticeable from the figures. With the exception of the first four hour period, the oxygen consumed per hour is practically the same even down to the concentration which is unable to support the life of the cell. During the first four hour period slightly more oxygen was absorbed per hour than in the period from four to seven and one-half hours. This, as was

discovered later, is very probably due to the progressive difference in the nutrition of the cells under conditions of starvation. This point will be dealt with in a subsequent paper. From this experiment the rate of intracellular oxidation seems to be quite independent of the concentration of oxygen, even at very low concentrations, yet the cell is unable to absorb the oxygen (at a sufficiently rapid rate?) below a certain minimal concentration.

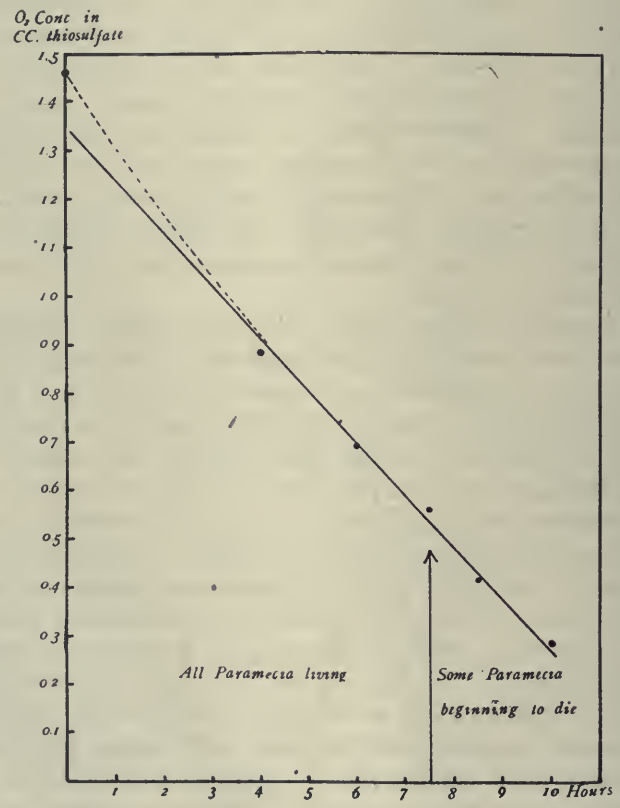

Fig. 1

It is evident that this minimal oxygen concentration varies for different individual cells, for most of the Paramecia were still normal and active at the end of ten hours or at a concentration equivalent to 0.41 cc. thiosulfate per 137 cc. of water, while others died at a concentration equivalent to 0.56 cc. of thiosulfate per 137 cc. of water. Evidently different cells differ in their ability to absorb oxygen at certain

low concentrations. What factors determine what this minimal concentration shall be for each cell?

The second type of experiment was like that used in tables 1 and 2 except that a larger number of bottles was used and the error due to death of Paramecia was carefully avoided by stopping the experiment before or when first signs of injury or death of cells were noticed. Greater care was also employed in manipulation and preparation of the Paramecia. Table 4 gives the results of an experiment lasting

TABLE 3

Showing the rate of oxygen consumption by Paramecium in low oxygen concentration. Paramecia not starved but removed directly to sterile tap water, washed three times and then used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 25±.1°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 4 HRS.		ANALYZED AT END OF 6 HRS.		ANALYZED AT END OF 7.5 HRS.		ANALYZED AT END OF 8.5 HRS.		ANALYZED AT END OF 10 HRS.	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	1.48	1.45	0.90		0.72		0.60		0.40		0.27	
2	1.40	1.45	0.86		0.68		0.55		0.46		0.23	
3	1.55	1.47	0.89		0.65		0.57		0.44		0.32	
4			0.90		0.72		0.53		0.37		0.33	
Average.....	1.476	1.456	0.887	*0.569*	0.692	*0.764*	0.563	*0.894*	0.417	*1.039*	0.287	*1.169*
Iodine adsorbed by Paramecia.		0.02										

two hours and thirty-five minutes. At the end of this time the first signs of abnormal movement occurred in those animals in high concentration of oxygen. The difference in the quantitites of oxygen used by the two sets of cells lies within the limits of error of the experiment.

In table 5 are given results of an experiment which differs chiefly from that of table 4 in that the duration of the experiment was much longer (thirteen hours). The oxygen concentrations also only differ by an amount which is often met with by the organisms in nature.

Semi-starvation of the animals previous to the experiment took place in clear native medium for twenty-four hours. They were then washed and starved for a second period of twenty-four hours in tap water, before using. The protoplasm of the cells was clear. They had

TABLE 4

Paramecia starved fifteen hours in sterile water, then washed three times in sterile tap water before using. Volume of bottles 137 cc. 1 cc. thiosulfate= 0.158 cc. O_2. Temperature 25 ± .1°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 2 HRS. 35 MIN.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
	A. Low O_2 concentration				
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	2.68	2.65	2.50		All perfectly normal and active at end of 2 hrs. and 35 min.
2	2.76	2.65	2.42		
3	2.73	2.65	2.38		
4	2.75	2.70	2.45		
5			2.40		
6					
Average.....	2.75	2.66	2.42	*0.24*	
Iodine adsorbed...				0.09	
	B. High O_2 concentration				
1	11.50	11.35	11.25		At 2 hrs., 35 min. many swimming slowly, a few deformed but none were dead
2	11.65	11.40	11.14		
3	11.55	11.45	11.25		
4	11.70	11.45	11.05		
5			11.10		
Average.....	11.60	11.41	11.16	*0.25*	
Iodine adsorbed...				0.19	

therefore entered upon a stage of "acute" starvation unlike those in the experiment of table 3. The oxygen consumption was independent of the concentration of oxygen. This experiment was repeated with the same results. It should be noticed in this experiment where the

animals were severely starved, that all the Paramecia in low oxygen concentration were still alive at an average concentration of 0.13 cc. thiosulfate per 137 cc. water, while in the experiment in table 3 where the animals had not been starved previous to the experiment, they

TABLE 5

Paramecia partly starved for twenty-four hours in clear native medium, then for twenty-fours in tap water, washed twice then used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 25° ±.2°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 13 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
	A. Low O_2 concentration				
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.57	1.55	0.10		All perfectly normal at end of 13 hrs. None dead.
2	1.58	1.50	0.20		
3	1.65	1.60	0.10		
4			0.10		
5			0.15		
Average.....	1.60	1.55	0.13	*1.42*	
Iodine adsorbed...				0.05	
	B. High O_2 concentration				
1	4.40	4.35	3.01		All perfectly normal at end of 13 hrs. None dead.
2	4.45	4.40	2.99		
3	4.45	4.35	3.02		
4			2.93		
5			2.90		
Average.....	4.43	4.366	2.97	*1.396*	
Iodine adsorbed...				0.064	

began to die at a concentration of oxygen equivalent to about 0.56 cc. of thiosulfate per 137 cc. This suggests that the nutritive condition of the cells is one of the factors which determines the minimal concentration of oxygen at which oxidations in the cell and life can proceed.

All of the previous experiments were carried out at a temperature

of 25 ± .1 or .2°C. It was thought that if the temperature was lowered and the duration of the experiment was increased that possibly any slight effects of differing oxygen concentration on the rate of oxidations might appear. Table 6 gives the results of an experiment carried on

TABLE 6

Paramecia starved twenty-four hours in diluted native medium. Washed three times in sterile tap water before using. A very concentrated suspension of Paramecia was used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 13.5 ± .2°C.

BOTTLES	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 20 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
	A. Low O_2 concentration				
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.80	1.65	0.80		All alive and active at end of 20 hrs.
2	1.80	1.65	0.87		
3	1.85	1.60	0.87		
4		1.67	0.90		
5		1.65	0.85		
Average.....	1.81	1.66	0.86	*0.80*	
Iodine adsorbed...				0.15	
	B. High O_2 concentration				
1	7.10	6.70	5.90		All alive and active at end of 20 hrs.
2	7.05	6.85	5.95		
3	7.00	6.80	5.90		
4		6.80	6.00		
5			6.00		
Average.....	7.05	6.79	5.95	*0.84*	
Iodine adsorbed...				0.26	

at 13.5°C. In this experiment there were at least three times as many Paramecia in 1 cc. as in any of the previous experiments; this accounts for the large amount of iodine adsorbed by the Paramecia. The difference in the average amounts of oxygen consumed in twenty

hours was 0.04 cc. thiosulfate. This lies within the limits of possible error for the experiment so that no evidence was found for an effect of oxygen concentration on oxidations at this low temperature.

Concentration of oxygen and rate of intracellular oxidations in other animals. It has generally been agreed among physiologists, on the basis of results from experiments on mammals, that the rate of intracellular oxidations is widely independent of the concentration of oxygen in the medium surrounding the cells. These animals are obviously poorly adapted for experimentation where accurate quantitative data on such questions as the rate of oxidations in the cell under normal conditions are desired.

In experiments on certain invertebrates Thunberg (6) found that by placing Lumbricus in 96 per cent oxygen the oxygen consumption was 44 per cent greater than that in air. For Limax similar results were obtained, and by lowering the concentration of oxygen the rate of oxygen consumption by Tenebrio and Limax decreased. Later Henze (7) found that the quantity of oxygen absorbed per hour by the coelenterates Actinia, and Anemonia, and the worm Sipunculus was influenced very largely by the concentration of dissolved oxygen. In other animals such as the crustacean Carcinus, the molluscs Aplysia and Eledone and the bony fishes Coris and Sargus, the rate of oxygen consumption was quite independent of the concentration of oxygen. Thunberg interpreted his results to mean that the rate of intracellular oxidations was determined by the mass of the reacting substances according to the mass law, when the oxygen concentration was below a certain value. Above this concentration of oxygen no increase in oxygen consumption was to be expected because for all practical purposes it could be considered infinite in amount and therefore had the same relation to the reaction velocity and equilibrium conditions of the oxidation reaction as the concentration of water has to the reaction velocity and equilibrium in, for example, the inversion of cane sugar.

Henze interprets his results in essentially the same way as Thunberg does but suggests that in cells where the actual rate of oxygen consumption due to oxygen deficiency is below the maximum, anaerobic processes supplement the aerobic processes and as a result the cells do not die because of too low oxygen concentration. To sum up, the results so far, on lower animals indicate that the relation between the concentration of oxygen and the rate of intracellular oxidations differs in different animals, in particular with respect to the oxygen concen-

tration at which an effect on the oxidative processes is noticeable. Much greater accuracy in the methods for quantitative studies on intracellular oxidation in multicellular animals is necessary before a satisfactory statement in detail of the conditions for these forms can be made.

SUMMARY

1. Paramecium serves as an ideal organism for accurate quantitative studies on intracellular oxidations.

2. In Paramecium the oxidations stop when the cell is killed by too high oxygen concentration.

3. In a pure line of Paramecium from the same culture different cells differ in respect to the minimal oxygen concentration in which they can continue to live.

4. The rate of intracellular oxidation in Paramecium is independent of the concentration of oxygen. The concentration of oxygen may vary from a minimum of about 0.04 cc. of O_2 at N.T.P. per 137 cc. to 2.2 cc. O_2 at N.T.P. per 137 cc. or fifty-five times the minimal concentration, without affecting the rate of oxidations. This is true for a temperature of 13.5° as well as 25°C.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

II. The Rate of Oxidations in Paramecium caudatum and its Independence of the Toxic Action of KNC

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Received for publication January 9, 191

Most of the recent work on the problem of the nature of the effects of anesthetics and narcotics on cells seems quite definitely to support the conclusion that these substances do not exert their distinctive effects upon cells by primarily inhibiting intracellular oxidations. Where a lowered rate of oxygen consumption or carbon dioxide production as the result of injection or immersion in solutions of alcohol, ether, chloral hydrate and the urethanes has been observed, it can usually be interpreted as an indirect effect, due for example to lowered muscular tone or death of cells (1), (2), (3), (4).

In striking contrast to these experimental results stand those from the cyanides. It is very generally agreed that the toxic action of the latter is due to their specific power of inhibiting intracellular oxidations. Any cell which can be isolated and subjected to direct observation of its structure and visible processes under experimental conditions has often, for obvious reasons, many advantages over complex cell aggregates; consequently some of the most direct and convincing evidence for this specific inhibitory action by the cyanides has been obtained from experiments on sea urchin eggs (5), (6).

On the basis of this and other evidence for specific inhibitory action by cyanides on intracellular oxidations, hypotheses and conclusions have often been arrived at by others which if proven to be correct are of importance. Potassium cyanide has been used in studies on the action of the respiratory center in mammals, its effects being attributed to its inhibitory action on the oxidations in the nerve cells of the respiratory center (7). Child (8) and his colleagues have made extensive use of the susceptibility of organisms to the toxic action of the cyanides

in studies on morphogenesis. They assume that the rate of oxidations serves as the most satisfactory available measure of the rate of (total?) metabolism, and that the rate of metabolism determines the course of morphogenetic processes, such for example as localization of body axis in regenerating pieces of planaria and various coelenterates. If the cyanides do have a specific inhibitory effect on oxidations in general they naturally offer interesting possibilities for experiment.

The purpose of the present paper is to show that in Paramecium caudatum the rate of intracellular oxidations is entirely independent of the action of KNC even in concentrations which injure and finally kill the cell by cytolysis.

RATE OF OXYGEN CONSUMPTION BY PARAMECIUM IN SOLUTIONS OF KNC IN TAP WATER

The procedure for oxygen determination and preparation of Paramecia has been described in a previous paper (9). Table 1 gives the results of a preliminary experiment carried out before the methods described in the first paper of this series had been fully worked out, therefore several sources of error such as that in the filling of the bottles, adsorption of iodine and the drawing of samples of Paramecia from the suspension were not eliminated as fully as in the remaining three experiments. It is given because in spite of some non-uniformity in oxygen concentration in the bottles the results show very clearly that KNC does not inhibit the oxidations to any noticeable degree even in the concentrations which killed some or nearly all of the Paramecia.

It will be seen from column 7 that in those bottles where a large number of Paramecia were dead at the end of fifty-one hours, the total oxygen consumed was in general a little less than in the bottles where all the Paramecia were living. The average quantity of oxygen consumed which is equivalent to 2.32 cc. thiosulfate per 150 cc., was practically the same in the bottles containing Paramecia without KNC (column 4), as in those bottles indicated by the bracket in column 7 where it was equivalent to an average of 2.43 cc. thiosulfate per 150 cc. The lower average oxygen consumption in column 7 is entirely accounted for by the death of Paramecia which occurred during the experiment in the higher concentrations of KNC. In spite of the evident errors due to non-uniformity in conditions for each bottle the fact is clear, from a glance at the figures in columns 2, 4 and 7, that intracellular oxidations were not inhibited to any marked degree by

the cyanide either in the weak or in the strong concentrations, and that an average of almost 3.00 cc. thiosulfate equivalent of oxygen was con-

TABLE 1

Preliminary experiment. Bottles were filled from tap by rubber tube. The Paramecia were centrifuged, washed twice in tap water and used immediately without starving. The KNC solution was first added, then 1 cc. Paramecium suspension was added and the bottle stoppered and shaken. Temperature 21 ± 2°C.

BOTTLE	CONTROLS NO KNC ADDED				KNC ADDED AND 1 CC. PARAMECIA. ANALYZED AT END OF 51 HOURS			CONDITION OF PARAMECIA AT END OF 51 HOURS
	1 cc. Paramecia added. Analyzed at once		1 cc. Paramecia added. Analyzed at end of 51 hours					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	Volume of bottle	O_2	Volume of bottle	O_2	N/10 KNC	Volume of bottle	O_2	
	cc.	*cc. thio*	*cc.*	*cc. thio*	*cc.*	*cc.*	*cc. thio*	
1	155	6.05	160	3.05	3.0	151	3.55	Nearly all dead
2	157	5.75	137	2.02	2.5	149	3.43	About $\frac{1}{4}$ living
3	147	5.05	163	3.00	2.2	143	3.50	About $\frac{1}{3}$ living
4	139	5.30	160	2.50	2.0	149	3.20	About $\frac{1}{2}$ living
5	153	6.25	146	2.40	1.8	142	2.27	More than $\frac{1}{2}$ alive
6	154	5.82	156	2.10	1.6	160	3.70	More than $\frac{1}{2}$ alive
7			145	1.85	1.4	154	2.35	Some dead
8			142	1.75	1.2	150	2.40	Very few dead
9			149	2.23	1.0	155	2.45	None dead in any of these bottles
10			157	2.45	0.8	153	2.75	
11			139	2.05	0.6	154	..75	
12			153	2.40	0.4	152	2.55	
13			164	2.90	0.2	150	2.65	
14			156	2.32	0.2	147	2.15	
15					0.1	166	2.65	
16					0.05	138	1.80	
Average cubic centimeters of thio. per 150 cc.		*5.67*		*2.32*			*2.74*	

sumed by the Paramecia in the KNC solutions. Is the rate of oxidations in Paramecium completely unaffected by cyanide? For an answer to this question greater degree of accuracy is necessary.

In the following experiments the bottles were of equal volume and contained nearly equal amounts of oxygen, the degree of accuracy is greatest in experiments the results of which are given in tables 2 and 3. Controls for determining the average amount of iodine adsorbed by Paramecia, are given in columns 1 and 2. This is equivalent to 0.43 cc. thiosulfate, a relatively large amount due to the large number of Paramecia in each bottle (table 2). The Paramecia in bottles containing 3 and 2.6 cc. N/10 KNC would probably not have lived more than a few hours longer, for a number in each bottle were dead and many were deformed and showed abnormal swimming movements. In bottles containing less than 1.4 cc. M/10 KNC the cells were perfectly normal and active and would undoubtedly have lived much longer. In other experiments similar to this one the Paramecia lived in concentrations of 0.05 cc. up to 0.4 cc. N/10 KNC in 137 cc. tap water for several days, until nearly all the oxygen was consumed or death occurred as a result of starvation. The average amount of oxygen in cubic centimeters of thiosulfate remaining after sixteen and one-half hours in the first six bottles of column 5 is 4.42. That of the last six bottles is 4.54 cc. thiosulfate. No animals had died in any of the bottles although the concentrations of KNC in the first three bottles were sufficient to kill many of the Paramecia had they been left for twenty-eight hours as shown in column 7. Evidently a lethal concentration of KNC in this case accelerated the oxidations or else was entirely without effect. The first three bottles (column 7) show a slightly lower oxygen content than the remaining ones. The only way I can account for this is by an accelerating effect or increased oxygen consumption due to stimulation of the organisms by the cyanide or more probably by errors in filling bottles.

A final experiment (table 3) was performed in which the greatest care was taken to avoid errors in manipulation. The range of variation of the cyanide concentrations—5 cc. to 0.05 cc. N/10 KNC—was greater than in previous experiments. It happened that the resistance to KNC of this lot of pure line Paramecia was greater than that of those used in any of the previous experiments so that at the end of twenty-nine and one-half hours relatively very few Paramecia were dead, even in the bottle containing 5 cc. N/10 KNC. Results of an analysis of the conditions for variability in resistance to KNC by Paramecium will be given elsewhere.

At the end of ten hours all Paramecia were alive in all the bottles. Those in the bottles containing 5 cc. KNC were moving more slowly

than the others and a few were abnormal in shape, an indication that death was approaching. At twenty-nine and one-half hours a few were

TABLE 2

Paramecia starved in clear native medium for 48 hours, centrifuged, washed in clear tap water twice, then used. All bottles had a volume of 137 cc. Temperature 16°C.

BOTTLE	CONTROLS ANALYZED AT ONCE			ANALYZED AT END OF 16½ HOURS		ANALYZED AT END OF 28 HOURS		REMARKS
		1 cc. Paramecia added		1 cc. Paramecia added		1 cc. Paramecia added		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	Blanks	n/10 KNC	O_2	n/10 KNC	O_2	n/10 KNC	O_2	All living at 16½ hours
	cc. thio	*cc.*	*cc. thio*	*cc.*	*cc. thio*	*cc.*	*cc. thio*	
1	5.9	3.0	5.45	3.0	4.18	3.0	3.96	At 28 hours few dead, many deformed
2	5.5	2.0	5.05	2.6	4.35	2.6	3.80	At 28 hours few dead, some sluggish
3	5.7	1.0	5.30	2.2	4.55	2.2		
4	5.7	0.8	5.35	1.8	4.55	1.8	3.90	All had nearly normal shape and were active
5	5.7	0.6	5.15	1.4	4.44	1.4	4.07	All normal and active
6		0.2	5.35	1.2	4.46	1.2	4.13	All normal and active
7				1.0	4.70	1.0	4.10	All normal and active
8				0.8	4.45	0.8	4.22	All normal and active
9				0.6	4.70	0.6	4.50	All normal and active
10				0.4	4.50	0.4	4.12	All normal and active
11				0.2	4.37	0.2	4.20	All normal and active
12				0.1	4.55	0.1	4.50	All normal and active
13				0.05		0.05	4.25	All normal and active
Average.....	5.70		5.27		4.48		4.14	
Iodine adsorbed..			0.43					
Average O_2 consumed.....					*0.79*		*1.13*	

dead in the remaining bottle containing 5 cc. KNC. Blisters were present in many and movement was slow. In 4 cc. KNC a very few were dead, many moved slowly. In the remaining bottles there was

an increasing number of normal Paramecia until in the bottle containing 3 cc. KNC no injurious effect on form or movement could be seen. One cubic centimeter of Paramecium suspension contained relatively few individuals in this experiment as shown by the small amount of iodine adsorbed which was equivalent to only 0.13 cc. thiosulfate.

TABLE 3

Paramecia starved in native medium for 24 hours, washed twice and starved in tap water for 12 hours, then used. All bottles 137 cc. Temperature 22°C.

BOTTLE	CONTROLS KNC ADDED ANALYZED AT ONCE			ANALYZED AT END OF 10 HOURS		ANALYZED AT END OF 29½ HOURS		REMARKS
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	KNC N/10	Blanks O_2	1 cc. Paramecia added O_2	KNC N/10	1 cc. Paramecia added O_2	KNC N/10	1 cc. Paramecia added O_2	At 10 hours all were living
	cc.	cc. thio	cc. thio	cc.	cc. thio	cc.	cc. thio	
1	5.0	5.40	5.35	5.0	4.22	5.0	3.40	29½ hours a few dead
2	4.5	5.45	5.20	4.5	4.20	4.5	3.20	
3	4.0	5.40	5.20	4.0	4.35	4.0	3.30	29½ hours, all living
4	3.5	5.35	5.15	3.5	4.33	3.5	3.20	
5	3.0		5.30	3.0	4.32	3.0	3.30	29½ hours, all living
6	2.5		5.30	2.5	4.30	2.5	3.14	29½ hours, all living
7	2.0		5.25	2.0	4.40	2.0	3.20	29½ hours, all living
8	1.0	5.40	5.35	1.0	4.30	1.0	3.40	29½ hours, all living
9	0.5	5.30	5.20	0.5	4.35	0.5	3.10	29½ hours, all living
10	0.05		5.20	0.05	4.25	0.05	3.20	29½ hours, all living
Average.....		*5.38*	*5.25*		*4.30*		*3.24*	
O_2 consumed...					0.95		2.01	

As a further control for this experiment the average amount of oxygen per bottle absorbed by the same number of Paramecia in tap water *without* cyanide was determined and found to be the same as the average amount of oxygen absorbed by the Paramecia in the cyanide solutions. This control was a part of another experiment carried out for a different purpose but with the same Paramecium suspension and at the same time as the experiment in table 3. It is not included in the table.

The foregoing experiments therefore demonstrate beyond doubt that the rate of oxidations is entirely independent of the toxic action of the cyanide. They further indicate that after cytolysis has occurred no more oxygen is used up. To further test this interesting point the following experiment was carried out.

Sixteen bottles of equal volume (137 cc.) were filled with tap water and divided into four sets, indicated below in table 4 by A, B, C and D. One cubic centimeter of Paramecium suspension was then added to each bottle and immediately thereafter the quantities of cyanide given

TABLE 4

Same Paramecium suspension as used in experiment in table 3. Volume of bottles 137 cc.; M/1 KNC was used. Temperature 21°C.

BOTTLE	CONTROLS				ANALYZED AT END OF 25½ HOURS 1 CC. PARAMECIA ADDED		REMARKS	
	Anaylzed at once			Analyzed at end of 25½ hours 1 cc. Paramecia added, no KNC	M/1 KNC added	O_2	20 minutes after beginning experiment	4½ hours after beginning experiment
	1 cc. Paramecia added, no KNC	1 cc. Paramecia added, M/1 KNC added	1 cc. Paramecia added, O_2					
	A		B	C		D		
	cc. thio	*cc.*	*cc. thio*	*cc. thio*	*cc.*	*cc. thio*		
1	5.35	5	5.30	3.9	5	5.20	All dead	All dead
2	5.30	4	5.25	3.80	4	5.70(?)	Most dead	All dead
3	5.30	3	5.38	3.80	3	5.08	Many dead	All dead
4	5.38	2	5.12	3.90	2	4.60	None dead	Many dead
Average....	5.33		5.26	3.85		5.39		

in the table were added to the bottles of sets B and D. The concentration of the KNC solution was N/1 or ten times greater than that used in the previous experiments, in order that the Paramecia might be killed quickly. Any oxygen consumed by the cytolysed cells could then be detected. Sets A and B were analyzed at once, serving as controls. Sets C and D were analyzed at the end of twenty-five and one-half hours. Records of the death rate of the animals were taken at frequent intervals.

All the Paramecia in all the bottles containing 5 and 4 cc. KNC were dead at the end of fifty minutes. All the Paramecia in the remaining bottles died within eight hours from the beginning of the experiment.

The oxygen content at the end of twenty-five and one-half hours, in

the bottles containing 3 and 2 cc. KNC shows that some oxygen had been consumed; this is to be expected since many of the Paramecia remained alive for from one hour in the bottle containing 3 cc. KNC to about seven hours in the bottle containing 2 cc. KNC. This experiment with the previous ones clearly shows that the oxidations stop when the cell is killed by KNC.

Does KNC inhibit oxidations which might go on in cytolysed cells of Paramecium in the absence of KNC? The answer is that there are in all probability after cytolysis of the cell, no oxidations to be inhibited by KNC, for it has been shown by Warburg (6) that when fertilized sea urchin eggs are killed by mechanical disintegration the oxidations practically cease. It was further shown in a previous paper (9) that intracellular oxidations in Paramecium stop when the cell is killed by too high oxygen concentration. In other words it appears that the oxidations stop regardless of the methods used for bringing about cytolysis. Warburg (6) attributed this disappearance of oxidations in fertilized sea urchin eggs to disappearance of the structure of the protoplasm during cytolysis. In other words that maintenance of the structure of protoplasm was a necessary condition for continued intracellular oxidations. There are reasons for believing that the conditions for intracellular oxidations which are related to the ordinary respiratory process in cells, for example in the muscle cell, are different from the conditions in such types of oxidations as occur in extracts of cells, for example the oxidation of tyrosin in potato juice. For the muscle cell the observations of Fletcher and Hopkins (10) indicate that the oxidation of or more probably the oxidation leading to replacement of lactic acid is conditioned by the existence of a normal cell structure, and that when the cells are mechanically disintegrated the conditions for oxidation or replacement of lactic acid are largely removed. The oxidation of tyrosin by means of the oxidase tyrosinase continues in aqueous extracts of the cells of various fungi (11). These and other facts indicate that biological oxidations of carbohydrates and lipoids and their physiological derivatives which are concerned with transformation of energy from oxidations into mechanical work may occur under quite different conditions than those which occur in solutions of tissue extracts. Perhaps one should therefore not wonder that enzymes which facilitate oxidations of sugars and fats in presence of free oxygen have not been found. Further work on these questions is necessary before any satisfactory conclusions can be drawn.

SUMMARY

1. The rate of intracellular oxidations in Paramecium caudatum is entirely independent of the toxic action of KNC.

2. Inferences based on supposed similarity of action by KNC on different cells in respect to intracellular oxidation are not strictly permissible unless it is shown by direct measurement on each type of cell, or in some other way, that KNC inhibits the oxidations.

3. Intracellular oxidations stop when Paramecium undergoes cytolysis in KNC solutions. But this stopping of the oxidations is not necessarily correlated with the toxic action of KNC for oxidations also stop when the cell is killed by oxygen in too high concentrations.

4. If no. 3 is true for other cells, then a necessary condition for proof that cyanides inhibit oxidations is that the cells treated with cyanide must be shown not to be cytolysed by the cyanide.

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A QUANTITATIVE STUDY OF THE EFFECT OF RADIUM RADIATIONS UPON THE FERTILIZATION MEMBRANE OF NEREIS

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Received for publication January 3, 1918

Existing studies of the effects of radium radiations[1] upon protoplasm fail to yield quantitative data of sufficient accuracy to form a basis for an investigation of the nature of the processes involved. A search has consequently been made for a physiological reaction to these radiations which could be measured with precision. A membrane is formed about the egg of the marine worm, Nereis limbata, when it is fertilized. If the egg has been exposed to the radiations from radium before fertilization occurs, the membrane will be abnormally thick when it is formed. The volume of this structure is a function of the amount of radiation which the eggs have received. Because the magnitude of this change is great—several hundred per cent—and because it may be produced by relatively small quantities of radiation a very precise and convenient test object is available, which may prove of value in solving many problems in the physiology of radioactivity.

The cortical changes which accompany fertilization of this egg have been described by Lillie (1). Upon fertilization the *vitelline membrane* of the unfertilized egg becomes separated from the underlying protoplasm by a layer of fluid, the *perivitelline space*. It cannot be stated at present which of these structures is altered by radiation. The word membrane is used in this paper to denote the optically homogeneous, colorless layer limited by the outer surface of the egg and by the "granular," yolk-laden protoplasm.

The increase in volume of the membrane is not accompanied by any diminution in the volume of the "granular" protoplasm as the meas-

[1] We are indebted to Dr. William Duane for placing a supply of radium belonging to the Cancer Commission of Harvard University at our disposal.

urements in table 1 indicate. The changes produced by radiation cannot be considered to depend on the *secretion* of an unusual quantity of material from the egg. Rather it is due to the absorption of an abnormal amount of sea water, or some of its components.[2]

The swelling of the membrane of radiated eggs takes place gradually during a considerable interval of time. A consideration of figure 1 will show that the rate of swelling decreases as the process progresses. By waiting until the process is approaching completion errors due to slight variations in the time of measurement are minimized and the percentage error in observation decreased. On the other hand eggs which have cleaved cannot be measured with precision because of the unequal thickness of different parts of the same membrane. It was

TABLE 1

September 11, 1917. Nereis eggs exposed to radium emanation for fifteen minutes

NUMBER OF EGGS MEASURED	INTENSITY	MEAN TOTAL DIAMETER	MEAN THICKNESS OF MEMBRANE	MEAN DIAMETER OF GRANULAR PROTOPLASMS
	millicurie centimeters	μ	μ	μ
12	0	131.5	3.5	124.5
11	9.0	147.5	9.0	130.0
11	22.5	152.0	10.7	130.5
11	100.8	156.0	14.0	127.0

found advisable to measure the membranes fifty or sixty minutes after fertilization during the warmer parts of the summer; as much as an hour and a half could be allowed to elapse in cooler weather. In every case each lot of eggs was measured after the same period in any single experiment.

The influence of the time elapsing between radiation and fertilization upon the thickness of the membrane has been determined. Several lots of eggs from a single female were exposed to a glass tube 12 mm. long containing 37.5 millicuries of radium emanation at a distance

[2] The phenomenon here described should not be confused with the observation of Packard (2) that some radiated eggs are larger than unradiated eggs. This condition which apparently occurs only when the egg is radiated more severely than in the present experiments, is due to a failure of the egg to secrete its jelly layer at once. I have not observed any alteration of the jelly secretion at the doses considered in this paper. Packard's phenomenon differs also from the swelling of the membrane in persisting only eighty minutes after fertilization. The change in the membrane is apparently irreversible.

of 7 mm. for ten minutes. One lot of these eggs was fertilized at once and other portions fertilized after increasing intervals of time. Measurements were made of the thickness of the membranes of eggs from

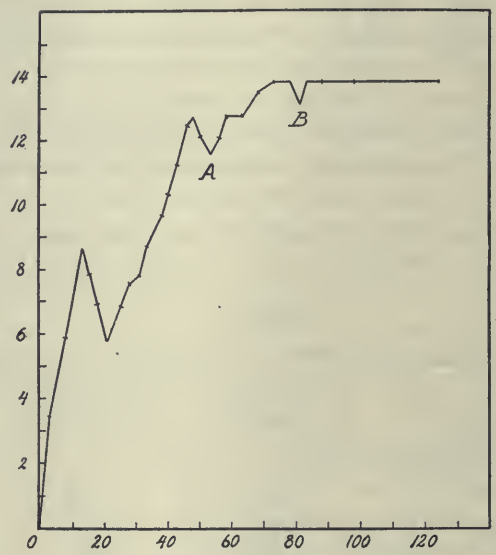

Fig. 1. Process of swelling of the membrane of a single Nereis egg. Time elapsing after fertilization measured in minutes along the abscissa. Thickness of membrane measured in micra along the ordinate. At *A* polar bodies were formed. At *B* the first cleavage occurred.

TABLE 2

MINUTES BETWEEN RADIATION AND FERTILIZATION	NUMBER OF EGGS MEASURED	MEAN THICKNESS OF MEMBRANE IN MICRA
0	10	8.5 ± 0.4
16	10	7.3 ± 0.4
30	11	7.6 ± 0.3
45	12	6.1 ± 0.5
60	12	9.5 ± 0.2
75	10	8.0 ± 0.2
90	10	7.9 ± 0.3
Unradiated control		3.1 ± 0.1

each portion between fifty and sixty minutes after fertilization. In table 2 the average measurement for each lot of eggs is tabulated. No significant recovery of the eggs occurred. Within the time limits

of the experiment the change produced by radiation is irreversible. Consequently the time which elapses between radiation and fertilization was disregarded in subsequent experiments.

Measurements were made according to the following routine. A female Nereis which had been caught the preceding night was cut open in a dry watch glass. A few drops of eggs were placed under the tube of radium emanation for the desired interval. At a convenient time thereafter—in practice never more than fifteen minutes later—the eggs were fertilized and placed in 8 or 10 cc. of sea water. At a definite time (depending upon the temperature) after fertilization a few eggs were placed in a hollow ground slide, of sufficient depth to prevent compression by the cover glass, and measured with the aid of a

TABLE 3

September 13, 1917. Nereis eggs exposed in successive lots to 34.9 millicuries of radium emanation in tube 10.2 mm. long, 6.3 mm. distant, for ten minutes (564 millicurie centimeter minutes). Measured ninety to one hundred minutes after fertilization

NUMBER OF EGGS MEASURED	MEAN VOLUME OF MEMBRANES $10^5\mu^3$
13	7.05 ± 0.18
19	6.85 ± 0.30
18	6.70 ± 1.80
18	$6.85 \pm .46$
19	7.05 ± 0.43
18	6.85 ± 0.55

high power objective and ocular micrometer. One egg after another was measured as rapidly as possible during five or ten minutes. Ten to twenty-five eggs from each lot were measured, their average taken and from these figures the volume of the membrane was computed in cubic micra, taking the diameter of the granular protoplasm to be 128 μ. In practice it was found possible to measure the membrane—which varied under the conditions of experiment from 0.8 to 7.0 divisions of the ocular scale (2.2 μ to 20 μ) to within 0.2 of a division of the scale (0.56 μ). Examination of the data will show that the probable error of the mean for a series of measurements rarely exceeds 5 per cent of the whole. In order to test practically the importance of uncontrollable sources of variation several lots of eggs from a single female were radiated successively with the same tube of radium, at the

same distance and for the same time. The dosage was selected so as to produce a membrane of such a magnitude that slight differences in treatment could be most readily detected. Measurements were then made of each lot of eggs at a uniform time after fertilization. The values obtained are recorded in table 3. It is clear that variation due to uncontrollable causes is very slight indeed.

The relation between the quantity of radiation and the amount of effect produced upon protoplasm is of the greatest importance, not only because of its obvious bearing upon the question of dosage in radiotherapy, but because its establishment will enable many theoretical and practical problems in the physiology of radioactivity to be attacked. The quantity of rays falling upon a given area from a radioactive source depends upon the intensity of radioactivity and the time during which it acts. Intensity in turn depends upon the quantity of radioactive substance present, and its distance from the area under consideration. In the present paper a notation derived by Dr. Alexander Forbes from the formula of Wood and Prime (3) will be employed to express the quantity of radiation. As a standard the intensity of rays emitted by 1 millicurie of radium emanation (1 mgm. element) located at a *point* at a distance of 1 cm. is taken as the *intensity unit* and designated 1 millicurie centimeter. The quantity of radiation emitted by 1 millicurie centimeter in one minute is taken as the *quantity unit* and designated 1 millicurie centimeter minute. Under actual conditions the radium emanation is not confined to a point but is distributed through the length of a slender glass tube. Consequently the intensity does not vary inversely with the square of the distance, but according to the formula

$$I = \frac{Q \cdot \theta}{a \cdot b}$$

when I = intensity in millicurie centimeters
Q = quantity of the radium emanation in millicuries
θ = the angle measured in radians between a perpendicular line drawn from the midpoint of the tube to the point under consideration, (i.e., the radiated cell) and a line drawn from the end of the tube to the same point.
a = perpendicular distance in centimeters from tube to point under consideration.
b = one-half length of tube in centimeters.

It follows that the quantity of radiation is expressed by

$$I \cdot t \quad \text{or} \quad \frac{Q \cdot \theta \cdot t}{a \cdot b}$$

The variables to be studied then are intensity and time.[3]

A series of experiments was performed to determine the relation between the degree of swelling of the membrane of the fertilized Nereis

TABLE 4

September 5, 1917. Nereis eggs radiated for various periods at an intensity of 29.1 millicurie centimeters. Measured sixty to sixty-five minutes after fertilization

$A = -1.6$ $\quad c = 8.12$

NUMBER OF EGGS MEASURED	TIME OF RADIATION	VOLUME OF MEMBRANE OBSERVED $10^5\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^5\mu^3$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	minutes			
11	6	5.19 ± 0.18	4.72	+0.47
12	10.5	7.13 ± 0.19	6.70	+0.43
12	17.5	8.89 ± 0.19	8.50	+0.39
11	21.5	8.80 ± 0.19	9.22	−0.42
11	26.0	9.75 ± 0.25	9.89	−0.14
11	31.0	11.10 ± 0.14	10.52	+0.58
12	36.0	10.54 ± 0.38	11.03	−0.49
12	41.0	11.55 ± 0.13	11.50	+0.05
12	46.0	12.09 ± 0.34	11.90	+0.19
12	52.0	12.05 ± 0.27	12.32	−0.27
12	56.0	13.80 ± 0.86	12.60	+1.20
12	60.0	12.40 ± 0.35	12.82	−0.42
6	0	1.60 ± 0.06		

egg and the length of radiation: the intensity being kept constant. Several drops of eggs from a female Nereis were placed under a tube 12 mm. long containing 13.24 mc. radium emanation at a distance of 6 mm. At intervals a small drop of eggs was removed, fertilized and measured. Table 4 indicates the result.

A consideration of these data shows that as the period of radiation is increased the volume of the resulting membrane is also increased.

[3] The glass tube was sufficiently thick to absorb the alpha rays. The effects are due consequently to the beta and gamma rays. The distance between the tube and the tissue never exceeded 2 cm. The absorption of rays by this thickness of air probably does not introduce a significant error into these experiments.

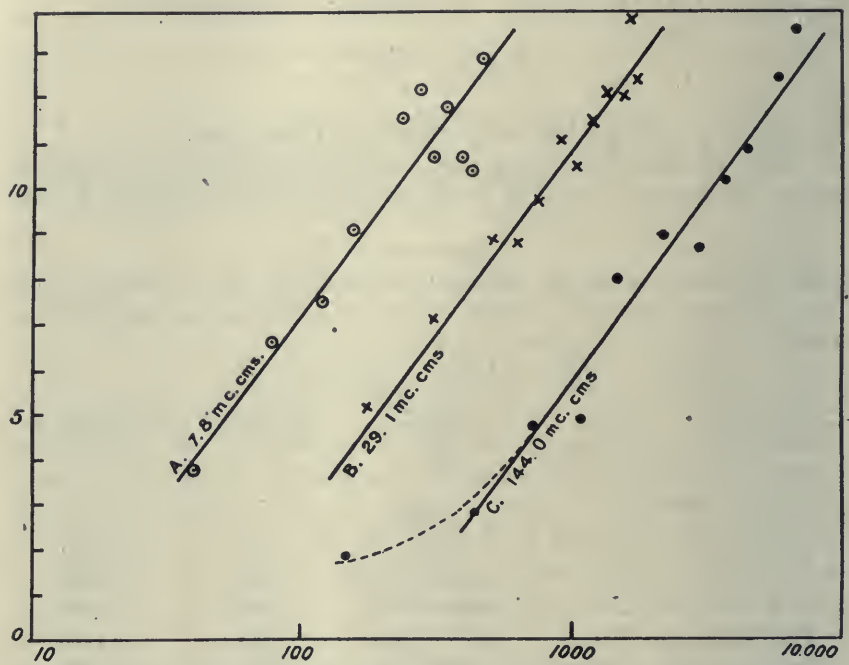

Fig. 2. Curves illustrating the influence of intensity of radiation upon the amount of swelling of the membranes of Nereis eggs produced by equal quantities of radiation. Quantities of radiation in millicurie centimeter minutes are measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

A. (September 13, 1917) represents the effect of radiating eggs from a single female for various periods of time with an intensity of 7.8 mc. cm.

B. (September 5, 1917) illustrates the data presented in table 4. Intensity 29.1 mc. cm.

C. (September 6, 1917) represents data from a similar experiment made with an intensity of 144 mc. cm.

Longer periods of radiation are however *relatively* less effective than shorter periods. The volume of the membrane varies directly with the logarithm of the time of radiation. The relationship may be expressed by the equation

$$V = A + c \log t$$

where V is the volume of the membrane resulting from radiation for a given time t, and A and c are constants depending on the experimental conditions, e.g., intensity of radiation, temperature, time elapsing between fertilization and measurement of membrane, etc. Values

for V calculated from these data, taking $A = -1.6$ and $c = 8.12$, are included in table 4. These values are in good agreement with the observed values. In figure 2, B is a curve based upon these data.

TABLE 5

Nereis eggs radiated for periods which varied inversely with the intensity employed
September 8, 1917. Measured sixty to seventy minutes after fertilization
$a = -0.02$ $b = 1.128$ $c = 3.75$

NUMBER OF EGGS MEASURED	INTENSITY	TIME	INTENSITY X TIME	VOLUME OF MEMBRANE OBSERVED $10^5\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^5\mu^3$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	millicurie centimeters	*minutes*	*millicurie centimeter minutes*			
25	8.94	64.8	578	8.40 ± 0.19	7.84	+0.56
25	24.4	34.3	837	6.60 ± 0.19	7.29	−0.69
25	26.7	23.4	625	5.98 ± 0.08	6.72	−0.74
25	37.3	15.0	560	6.65 ± 0.12	6.16	+0.49
25	71.5	7.9	565	5.13 ± 0.13	5.43	−0.30
25	172.0	3.4	585	5.17 ± 0.14	4.49	+0.68
September 10, 1917. Measured ninety to one hundred minutes after fertilization $a = 0.068$ $b = 0.67$ $c = 4.03$						
25	6.25	72.5	453	7.80 ± 0.25	8.10	−0.30
25	11.7	40.0	468	6.80 ± 0.09	7.24	−0.44
26	16.0	27.5	440	6.60 ± 0.26	6.57	+0.03
25	28.0	16.0	448	5.42 ± 0.16	5.34	+0.08
13	47.7	8.9	424	5.45 ± 0.43	5.01	+0.44
25	71.6	5.4	387	4.10 ± 0.16	4.25	−0.15
25	120.5	3.6	434	4.68 ± 0.20	3.73	+0.95
September 12, 1917. Measured ninety to one hundred minutes after fertilization $a = 0.017$ $b = 0.895$ $c = 3.674$						
25	12.4	55.0	682	7.84 ± 0.12	7.45	+0.39
25	23.1	29.4	682	6.06 ± 0.13	6.65	−0.59
16	36.2	19.3	698	6.62 ± 0.17	6.14	+0.48
25	54.9	12.4	681	6.05 ± 0.19	5.59	+0.46
25	95.8	7.05	675	3.62 ± 0.09	4.91	−0.29
25	162.5	4.20	683	5.27 ± 0.11	4.29	+0.98
23	251.0	2.72	683	3.22 ± 0.13	3.76	−0.54

One might expect the effect of radium radiations upon protoplasm to be a linear function of the product of intensity and time; that is, to be proportional to the number of rays striking the cell irrespective

of their distribution in time. That this relation is not true is clearly indicated by the subsequent data. In table 5 three experiments are recorded in which the time of radiation and its intensity were so varied as to keep the product of the two approximately constant. On the above supposition the volume of the membranes of the eggs in each experiment should have been the same. This result was not realized, the membranes being more voluminous with long exposures to low intensities than with short exposures to high intensities. The same condition is indicated by the data illustrated in figure 2. Here the volume of the membranes resulting from three experiments made with three different intensities of radiation are plotted against the logarithms of the quantity ($I.\ t$) of radiation. If the volume were a function of the product of intensity and time these curves should be superimposed. This is clearly not the case; the curve, A, made at the lower intensity indicates a much greater volume for a given quantity of radiation than do the curves, B and C, made at higher intensities.

A consideration of additional data has suggested that the equation relating intensity, I, and time, t, of radiation with the resulting volume, V, has the form

$$V = a + b \log I + c \log t$$

in which a, b and c are constants and b *is less than* c.

The best representative value for these constants can be determined for any given set of data. From the figures recorded in table 5 the membrane volume to be expected for each combination of intensity and time has been calculated. Although the deviation of the observed values from the calculated is considerable, in some cases exceeding 10 per cent, the deviations fall at random above and below the calculated values. Deviations of such magnitude are readily accounted for by errors in measuring the distance between the radium and the eggs. An error of 0.5 mm. in this measurement would cause an error of 5 per cent when the intensity was lowest and of 20 per cent when the intensity was highest.

A further test of the equation is afforded by measuring the volume of the membranes which result from exposing the eggs to various intensities of radiation for a uniform period of time. Such data are recorded in table 6 and in figure 3. Here a and $c \log t$ remain constant and V varies with $\log I$. The membrane volumes to be expected for the intensities employed have been calculated. Although this value agrees with the observed values in a quite satisfactory way the exact

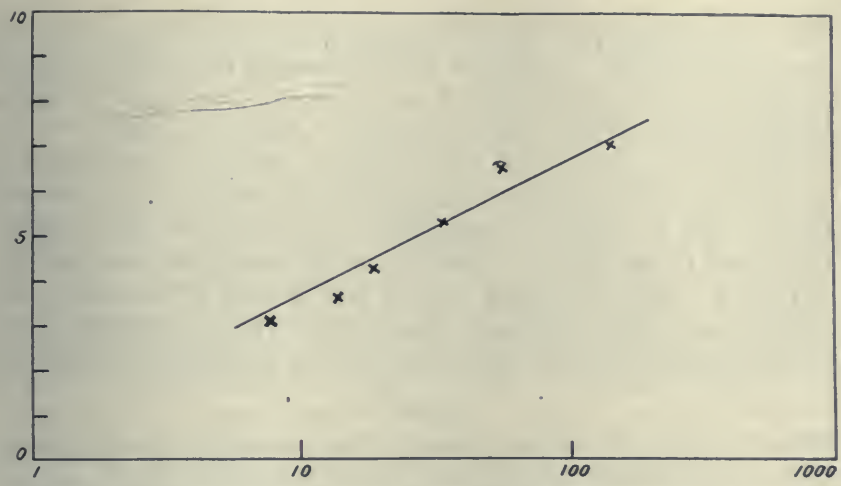

Fig. 3. Curve illustrating the data in table 6. Intensity of radiation in millicurie centimeters is measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

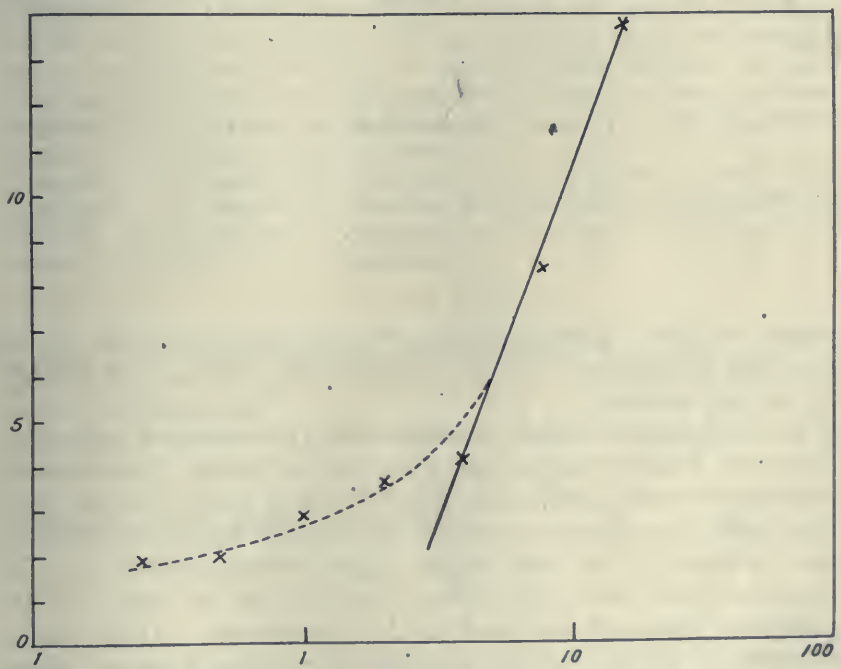

Fig. 4. The deviation in the lower range of the curve is indicated by data obtained by radiating Nereis eggs with 85 mc. cm. for short periods of time (September 12, 1917). Periods of radiation in minutes are measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

form of the expression relating intensity to the rest of the equation is somewhat doubtful. Extensive data confirming the results recorded in table 6 are lacking, while errors in determining the intensities employed discount the value of such data which are at hand.

One fact stands out clearly from all data. *The value of b is less than the value of c.* This relation is indicated by the slope of the curves in figures 3 and 2 which are proportional to the value of *b* and *c* respectively, and by the values of these constants determined from the data in table 5. This relationship tells us that *quantity of radiation in the physical sense is physiologically meaningless.* The physiological action of radium radiations upon these cells does not depend simply upon the

TABLE 6

September 9, 1917. Nereis eggs radiated for fifteen minutes with varying intensity. Measured seventy to eighty minutes after fertilization

$a + c \log t = 0.68 \qquad b = 3.0$

NUMBER OF EGGS MEASURED	INTENSITY	VOLUME OF MEMBRANE OBSERVED $10^5\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^5\mu^2$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	millicurie centimeters			
25	7.54	3.15 ± 0.05	3.31	−0.16
25	13.78	3.65 ± 0.09	4.11	−0.46
25	18.70	4.30 ± 0.06	4.50	−0.20
25	33.70	5.35 ± 0.07	5.26	+0.09
25	57.52	6.55 ± 0.16	5.96	+0.59
25	145.2	7.05 ± 0.20	7.16	−0.11
13	0	1.80 ± 0.17		

number of rays or particles striking the cell; their distribution in time must also be considered. In this consideration time is a more effective factor than intensity.

In conclusion the range through which the equation holds good within the limits of experimental error may be defined. Experimental values of the volume of the egg above eleven or twelve hundred thousand cubic micra fluctuate considerably, but without any tendency in one direction. Below three or four hundred thousand cubic micra, on the other hand, there is a distinct tendency for the volume of the membrane to exceed that predicted by the equation. This tendency is clearly indicated in figure 4 and in the lower point in curve *C*, figure 2. In the lower range of the curve the values of *a*, *b* and *c* are no longer constant. Above this range the equation may be considered a

satisfactory approximation of the relationship of intensity and time to the swelling of the fertilization membrane of Nereis eggs.

The equation $V = a + b \log I + c \log t$ suggests that the change in the membrane is due to a process commencing after a latent period which is represented by the value of t when $V = 0$. The considerations of the preceding paragraph indicate, however, that the equation does not hold true at the beginning of the process. At this time the reaction evidently proceeds more slowly, as indicated by the slope of the curves, than the constants of the equation demand. A considerable time must elapse before the rate of change reaches a maximum. In this way a latent period which probably does not exist is suggested. The initial acceleration of the process finds a striking analogy in the phenomenon of photochemical induction which is said to occur in practically all photochemical reactions. Serious consideration of the nature of the processes in question should await, however, more complete demonstration of the relationship of the factors involved.[4]

[4] Striking, though perhaps superficial, resemblances exist between the action of light upon the photographic plate and the phenomenon here considered. Radiations from radium produce in the egg of Nereis a "latent image" which does not manifest itself until the egg is "developed" by fertilization. Moreover the change in the membrane is no more effected by the time elapsing between radiation and fertilization than the photographic negative is altered by the time elapsing between exposure and development.

Hurter and Driffield (4) have expressed the relation between the intensity, I, and time, t, of exposure of a photographic plate and the resulting density, D, of the negative—which is "directly proportional to the amount of silver deposited per unit area"—by the equation

$$D = \gamma \log \left(\frac{I\ t}{i}\right)$$

in which γ is a constant depending on the time of development and i is the "inertia" of the plate, "measuring those properties of the film which together constitute its sensitiveness."

If we let d represent the difference between b and c, then $c - d = b$ and we may rewrite our equation

$$V = a + (c - d) \log I + c \log t.$$

Rearranging we get a form of the equation,

$$V = a + c \log \left(\frac{I \cdot t}{\frac{d}{I^c}}\right)$$

which is strikingly like that of Hurter and Driffield. The density of the photographic plate after periods of *underexposure* also deviates from the expectation raised by their formula in a manner similar to the deviation of eggs radiated with small doses of radium.

Study of the subsequent history of radiated Nereis eggs indicates that a close parallelism exists between the abnormality in development and the change in the fertilization membrane. The data in table 7 show that similar quantitative relationships connect the degree of abnormality and the intensity and time of radiation. Although these lots of eggs have all received approximately the same quantity of radiation, those eggs which have been exposed for a long time to a low intensity have been much more affected than those exposed for a short time to a high intensity. This result suggests that a variety of cellular functions are affected in the same relative degree by intensity and time of radiation.

TABLE 7

September 12, 1917. Nereis eggs radiated for periods which varied inversely with the intensity employed. Membranes measured ninety to one hundred minutes after fertilization

INTENSITY	TIME	QUANTITY	VOLUME OF MEMBRANE $10^6 \mu^3$	CONDITION OF LARVAE AFTER TWENTY TO TWENTY-FOUR HOURS
millicurie centimeters	*minutes*	*millicurie centimeter minutes*		
12.4	55.0	682	7.84	A few irregular cleavages. No swimmers
23.1	29.4	682	6.06	Irregular cleavage. No swimmers
36.2	19.3	698	6.62	Normal cleavage. No swimmers
54.9	12.4	681	6.05	ca. 5 per cent swimmers
95.8	7.05	675	3.62	ca. 50 per cent swimmers
162.5	4.20	683	5.27	ca. 50 per cent swimmers
251.0	2.72	683	3.22	ca. 90 per cent swimmers
0	0	0	1.8	ca. 90 per cent swimmers

The establishment of a mathematical relationship between quantity of radiation and amount of swelling should enable this reaction to be used as a measure of radioactivity. Heretofore our only methods of quantitating the intensity of radiation reaching a given point have been physical. Were the radiations homogeneous in nature such measurements would do very well as a basis for physiological investigation. Not only do the radiations vary qualitatively, but radiations of any sort have various penetrating powers. There is no reason to suppose that a direct proportionality exists between the physiological effects of these different rays and their action upon the physical instruments used in measuring them. It should now be possible to

measure the intensity of those rays which alone are physiologically efficient, and thus to arrive at a rational basis for dosage in radiotherapy.

It is interesting to find, in the action of radium radiations the same logarithmic relationship as appears in the Weber-Fechner law relating stimulus and response. Davey (5) has found that a similar relationship determines the resistance of the beetle, Tribolium confusum, to death from various lengths of exposure to x-rays of a uniform intensity. Davey also observed a deviation from the expectation raised by the equation when the exposure was short. A similar deviation in the lower range of the curve is recorded by Henri et Larguier des Bancels (6) in experiments in which light was the stimulus.

SUMMARY

1. The fertilization membrane of the egg of *Nereis limbata* becomes abnormally thick if the egg has been exposed to radiations from radium prior to fertilization. This reaction is well adapted to quantitative study.

2. The change leading to this condition is irreversible.

3. The physiological effect is not proportional to the product of intensity and time. The time factor is relatively more important than the intensity factor.

4. An equation is suggested which expresses approximately the relation between intensity and time of radiation and their physiological effects.

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THE MECHANISM OF THE ACTION OF ANAESTHETICS

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Received for publication January 7, 1918

Many theories have been advanced in attempts to explain how anaesthetics produce anaesthesia. Shortly after the discovery of ether and chloroform anaesthesia, Bibria and Harles (1847) called attention to the fact that practically all fat solvents are anaesthetics and as a result of a quantitative estimation of the fat contents of the brain of a normal animal and of a narcotized one, came to the conclusion that narcosis is produced by the direct removal of the fatlike substances, or lipoids, from the brain. The difficulty of explaining the rapid recovery which follows the interruption of anaesthetization and the fact that no one has been able to confirm the observations of Bibra and Harles, seems to have rendered their theory untenable. Hermann (1866) showed that all the narcotics of the methane series hemolyze the red blood corpuscles. He attributed this property to the power of these narcotics to dissolve the lecithin of the red blood cells, and assumed that narcosis of the central nervous system, with its high lecithin and cholesterin content, was due to the dissolving of these substances by the narcotics. Richet (1895) contended, on the contrary, that the effectiveness of a narcotic stood in an inverse relation to its solubility in the water fluids. The foregoing observations suggest the modern theory as set forth by Meyer and Overton. Hans Meyer (1) and Overton (2), independently, pointed out that the intensity of a narcotic is directly proportional to its distribution coefficient between the lipoids of the nervous system and the watery fluids; that is, the more soluble the narcotic is in the lipoids, the more effective it is as an anaesthetic. According to this theory, the narcotics of the methane series produce their characteristic effect on the central nervous system by going into solution in the fatlike constituents, the lipoids of the nervous tissue, and forming a physical-chemical combination with them. It should be mentioned, in this connection, that the Meyer-Overton law holds only for the narcotics of the methane series. The

dissolving of the lipoids of the nerve cells by these narcotics is supposed to alter the function of these cells, thus producing narcosis. It will be noted that while this theory explains very satisfactorily how a certain class of narcotics, namely those of the methane series, obtains access into the interior of the red blood corpuscles and of the nerve cells, it does not explain so satisfactorily the main point at issue, namely, how the narcotic produces narcosis. The fact that there are so many anaesthetics which are not fat solvents, magnesium sulphate and nitrous oxide being conspicuous examples, would seem to indicate that the Meyer-Overton theory, after all, may explain nothing more than how the narcotics of the methane series gain access into the nerve cells.

R. S. Lillie (3) has presented evidence showing that excitation is associated with increased permeability of the cell membrane, and depression with decreased permeability. According to this hypothesis, narcotics produce their characteristic effect by decreasing the permeability of the cell membrane. Claude Bernard (1853) suggested that narcosis was due to the semi-coagulation of the protoplasm, analogous to muscle rigor produced by chloroform.

It has been recognized for a long time that oxygen deprivation or asphyxia favors or may actually produce anaesthesia. So far as I have been able to find, John Snow, in his classical work, *On Chloroform and Other Anaesthetics* (1858), was the first to suggest that narcotics may produce narcosis by limiting or interfering with the normal oxidative processes. After reviewing the different theories of narcosis, Hewitt, in his book on *Anaesthetics* (1907), states that it is not at all improbable that future experimental research may lead us to the conclusion that general anaesthetics produce their characteristic effect by limiting the normal processes of oxidation, upon which the intellectual, sensory and motor centers depend for the execution of their respective functions. Paul Bert (4) and Arloing (5), independently, showed that oxidation was decreased during chloroform anaesthesia, as was indicated by the decreased oxygen intake and carbon dioxide output. Alexander and Cserna (6) showed that oxygen consumption and carbon dioxide production, and hence oxidation, in the brain was greatly increased during the excitement stage of anaesthesia, and was decreased during the stage of deep narcosis. Verworn (7) and his pupils have furnished much evidence showing that narcosis is usually accompanied by decreased oxidation, and that deficiency of oxygen, or asphyxia, as has been recognized for a long time, produces anaes-

thetic phenomena, hence they conclude that narcosis is due to the inhibition, or interference, with oxidation. Allied to this view is that of Mansfield (8) who assumes that narcotics decrease the solvent power of lipoids for oxygen, and hence prevent or interfere with its entrance into the cells. A. P. Matthews (9) believes that anaesthetics "fix the oxygen receptors of the protoplasm into a non-irritable anaesthetic-protoplasm combination." Herter found that the oxidizing capacity of the tissues was greatly reduced during anaesthesia. Tashiro found that anaesthetics greatly diminished the carbon dioxide output of nerves.

From this brief survey of the principal theories of narcosis, it would seem that the one of Verworn, which attributes narcosis to an interference or inhibition of the normal oxidative processes, is as plausible as any, if not the most plausible. In our work (10) we have shown that when oxidation was increased, as, for example, by increasing the amount of work, by thyroid feeding, by fighting, during the excitement stage of ether anaesthesia, there was an accompanying increase in catalase, due to the stimulation of the liver to an increased output of this enzyme, and that when oxidation was decreased or rendered defective, as, for example, by decreasing the amount of work, by starvation, by phosphorous poisoning, by extirpation of the pancreas, thus producing pancreatic diabetes with resulting defective oxidation, and in deep ether anaesthesia there was an accompanying decrease in the catalase of the tissues. From these results it was concluded that catalase, an enzyme in the tissues possessing the property of liberating oxygen from hydrogen peroxide, may be involved in the normal oxidative processes of the body. If it can be shown that the different narcotics decrease the catalase of the blood and hence of the tissues parallel with the decrease in oxidation during narcosis, it would seem to render it still more probable that catalase is involved in the oxidative processes and that the cause of the diminished oxidation, which is probably responsible for the narcosis, may be due to the decrease in catalase. The narcotics used were ether, chloroform, chloral hydrate, nitrous oxide and magnesium sulphate. These widely different kinds of narcotics were chosen intentionally. The animals used were cats, dogs and rabbits. The catalase of the blood was determined by adding 0.5 cc. of blood, taken from the external jugular vein, to hydrogen peroxide in a bottle at 22°C., and as the oxygen gas was liberated it was conducted to an inverted, graduated vessel, previously filled with water. After the oxygen gas thus collected in ten minutes had been

reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. In determining the catalase of the dogs' blood, 50 cc. of hydrogen peroxide were used, owing to the low catalase content of the dogs' blood, while 250 cc. of peroxide were used with the cats' and rabbits' blood. The material was shaken in a shaking machine at a fixed rate of one hundred eighty double shakes per minute during the determinations. The results of the determinations are given in figure 1. The figures (0–360) along the abscissa indicate time in minutes; the figures (0–900) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

Curve *1* was constructed from data obtained from four cats during ether anaesthesia. The anaesthesia was produced by bubbling air through ether in a bottle, which was connected by a rubber tube to a cone adjusted to the snout of the animal. It will be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the production of anaesthesia, was 875 cc. and 870 cc.; that after fifteen minutes of administration of the anaesthetic, 0.5 cc. of blood liberated 850 cc.; after thirty minutes, 835 cc.; after forty-five minutes, 790 cc.; after sixty minutes, 720 cc.; and after seventy-five minutes, 700 cc. From these figures it may be seen that the catalase of the blood was decreased parallel with the increase in the depth of narcosis, and that after seventy-five minutes it had been decreased by 20 per cent as indicated by the decrease in the amount of oxygen liberated from 875 cc. to 700 cc.

Curve *2* was constructed from data obtained from two cats during chloroform anaesthesia. The chloroform was administered in the same manner as was the ether. It may be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the anaesthesia, was 820 cc. and 820 cc.; that after fifteen minutes of administration of chloroform, 0.5 cc. of blood liberated 640 cc. By comparing the effects of ether and chloroform, it will be noted that there was a gradual decrease produced in the catalase of the blood during ether anaesthesia, whereas there was a very abrupt decrease during chloroform anaesthesia; that is, chloroform destroys the catalase of the blood more quickly than does ether. It was also found that with equal concentrations, chloroform destroyed the catalase of the blood more quickly and extensively in vitro than did ether.

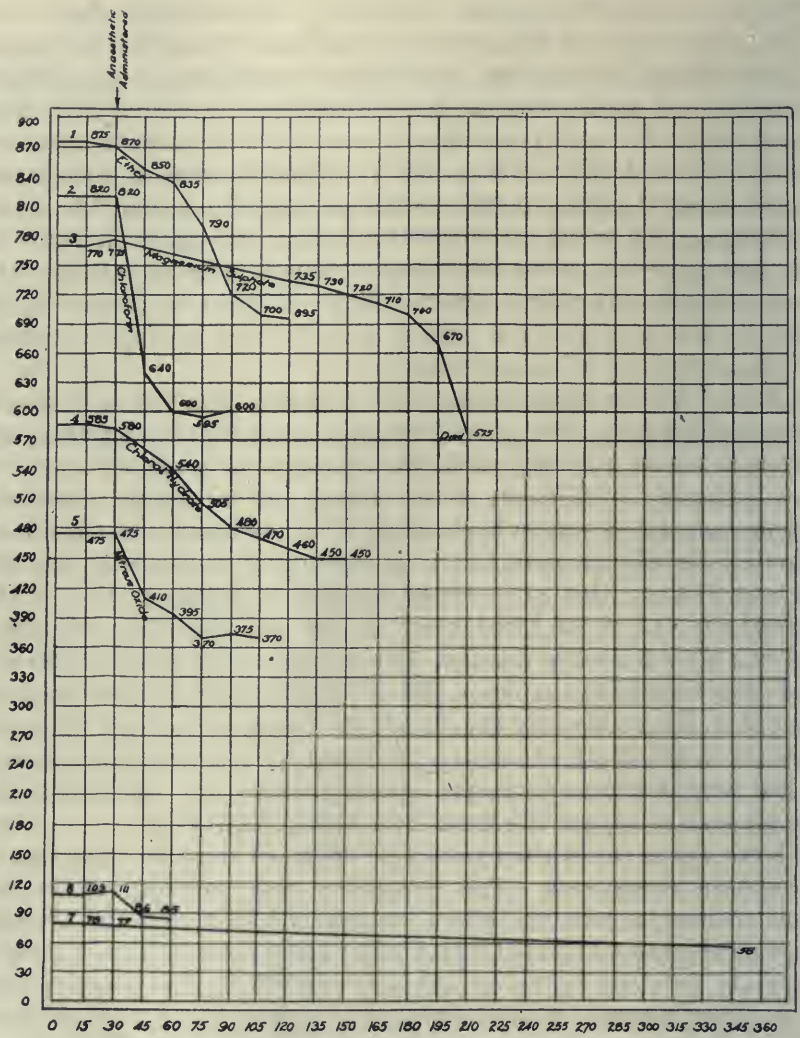

Fig. 1. Curves showing the effect of narcosis on the catalase content of the blood. The figures (0–360) along the abscissa indicate time in minutes; the figures (0–900) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

In the preceding experiments no attempt was made to administer the anaesthetic in equimolecular concentrations. Both were administered in sufficient concentrations to produce a fair degree of anaesthesia by the end of the first fifteen minute interval, and the amount administered during the remaining periods was such as to keep the animal in fairly deep but safe narcosis. We found that by choosing large, active cats with blood of high catalase content, and by forcing the anaesthetic, it was possible to decrease the catalase much more quickly and extensively than was done in the preceding experiments, but even in these cases it was found that chloroform produced a much more abrupt decrease than did ether, and the decrease, as a rule, was slightly greater with chloroform than with ether, particularly when the narcosis was continued over a period of two or three hours.

Curve *3* was constructed from data obtained from a cat during magnesium sulphate anaesthesia. The anaesthesia was produced by the subcutaneous injection of 7.5 cc. of a 20 per cent magnesium sulphate solution per kilo of body weight. It will be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the production of anaesthesia, was 770 cc. and 775 cc.; that ninety minutes after the injection of the magnesium sulphate, 0.5 cc. of blood liberated 735 cc. of oxygen; after one hundred thirty-five minutes, 730 cc.; after one hundred fifty minutes, 720 cc.; after one hundred sixty-five minutes, 710 cc.; after one hundred eighty minutes, 700 cc.; after one hundred ninety-five minutes 670 cc.; and after two hundred ten minutes, 575 cc.; when the animal died. It will be noted that the catalase was decreased more slowly during magnesium sulphate narcosis, except the abrupt decrease just preceding the death of the animal, than during narcosis produced by any of the other narcotics. It was also found that magnesium sulphate was the least effective of the narcotics used in destroying the catalase in vitro.

Curve *4* was constructed from data obtained from two rabbits, during chloral hydrate anaesthesia. The anaesthesia was produced by the introduction into the stomach of the animals of 10 cc. of a 2 per cent solution of chloral hydrate, per kilo of body weight. It will be noted that chloral hydrate decreased the catalase of the blood during narcosis more slowly than any of the other narcotics, except magnesium sulphate. It was found that when chloral hydrate was added to blood in vitro in as large quantities as was the magnesium sulphate, it de-

stroyed the catalase more quickly and extensively than did the magnesium sulphate, but less extensively than did the ether or chloroform.

Curve *5* was constructed from data obtained from two cats during nitrous oxide anaesthesia. The anaesthesia was produced by administering a mixture of nitrous oxide and oxygen in the proportion of one to five, or 80 per cent nitrous oxide and 20 per cent oxygen. It will be noted that the decrease in catalase was more abrupt with nitrous oxide than with any of the other anaesthetics, except chloroform.

Curve *6* was constructed from data obtained from a dog, during chloroform narcosis. The chloroform was administered in the same manner as it was with the cats for curve *2*. The same abrupt decrease in catalase during the first fifteen minutes of narcosis was obtained with the dog as was obtained with the cats.

Curve *7* was obtained from a dog, chloretonized in the same manner as were the rabbits for curve *4*. It will be noted that the same gradual decrease in catalase was obtained with the dog as was obtained with the rabbits.

If narcosis be due to decreased oxidation, and if this decreased oxidation, in turn, be due to a decrease in catalase, then the destructive effect of an anaesthetic on catalase should be an index to the character of the anaesthesia produced by the anaesthetic in question. It may be seen in the chart that chloroform, in keeping with its more powerful action as an anaesthetic, is more destructive to catalase than any of the anaesthetics used, and that magnesium sulphate, in keeping with its slow action, is least destructive, while ether occupies an intermediate position. Chloral hydrate does not act so rapidly as ether, chloroform or nitrous oxide, nor does it act so slowly as magnesium sulphate. It may be seen that chloral hydrate, accordingly, destroys catalase during narcosis less rapidly than ether, chloroform or nitrous oxide, and more rapidly than magnesium sulphate. It is known that a state of acidosis is more likely to develop with chloroform than with ether. It may be that the greater tendency toward acidosis in chloroform narcosis is due to the greater destruction of catalase by this narcotic, and to the injury of the liver, the organ in which catalase is formed, with the resulting decrease in oxidation.

SUMMARY

1. Narcotics of widely different constitution, such as chloroform, ether, chloral hydrate, nitrous oxide and magnesium sulphate, decrease the catalase of the blood, parallel with the increase in the depth of narcosis.

2. A very powerful anaesthetic, such as chloroform, decreases the catalase more quickly and extensively than does a less powerful anaesthetic, such as ether. Slowly acting anaesthetics, such as chloral hydrate and magnesium sulphate, decrease, accordingly, the catalase of the blood more slowly than a quickly acting anaesthetic such as nitrous oxide.

3. As a result of the experiments reported in this paper, and of work done previously on the anaesthetics in this laboratory, the theory is advanced that narcosis is due to the direct destruction of catalase by the narcotic, with resulting decrease in oxidation, while recovery from anaesthesia is brought about by an increase in catalase due to the increased output from the liver, with resulting increase in oxidation.

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THE RELATION BETWEEN GROWTH CAPACITY AND WEIGHT AT BIRTH

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Received for publication January 12, 1918

The statistical data concerning the course of human growth during the first few days of extra-uterine life are voluminous and collectively significant. The individual reports however are unfortunately complicated by a certain degree of non-recognition of the fact that human milk is the proper food for human infants, and with the consequent inclusion in the data of growth curves of infants whose nourishment has been derived from other sources.

It is a matter of common knowledge (1) that the chemical composition of the milk produced by the various types of mammals is regularly different. It is also well known that, by and large, young mammals thrive better when feeding on the milk produced by their kind. No experimental evidence has as yet been presented in support of a theory that the milk elaborated by the mother is not that biologically adapted to the needs of the young of the same species. There apparently exists in mammals a mechanism for the production of a food specifically adapted to the needs of the young of the same kind. Moreover Osborne and Mendel (2), McCollum (3) and others have shown that the nature of the food ingested is one of the very important factors concerned in growth, and any attempt to determine the fundamental biological laws of growth during this period should recognize these facts.

The factors influencing milk production are manifold. The nutritive condition of the mother (4), the maternal metabolism, health or disease (5), all contribute their quota to the value of the milk as a food supply adequate to the growing infant. Nevertheless during the first two weeks of lactation the variations in the chemical composition of human milk have a remarkably uniform tendency (5) and where the changes in the weight of the infant do not indicate supplementary feeding necessary it can reasonably be assumed that the nourishment is sufficient and characteristic.

Growth is a bio-chemical process (6), (7), (8), (9), and as such is obviously susceptible to the influence of regulatory or interfering factors. Quantity of food ingested (10), climate, nationality (11), sex (12), and a variety of other conditions serve to produce a composite picture of such apparent intricacy that as late as 1913 Kjölseth (13) considered statistical studies on growth to be so hopeless as to propose the motto: "Die Natur is nicht schematisch." Fortunately the results of the studies of a long line of investigators extending from 1716 (14) to the present (15), (16) refute this unorderly point of view. A comprehensive bibliography of the work with infants up to 1913 is given by Benestad (11). No specific attempts were made to correlate birth weight and rate of growth, any such isolated observations as were reported yielding conflicting opinions; Schäffer (17) considering—"und so leichter ist das Kind . . . . um so länger dauert es, bis dasselbe sein Geburtsgewicht wieder erricht hat," and Benestad (11) that "die kleinen Kinder erleiden einen geringeren Gewichtsverlust und beginnen ihren Zuwachs eher als die grossen Kinder. Aber von dem Augenblick an, da die Gewichtzunahme einsetzt, besteht kein nennenswerter Unterschied zwischen ihnen."

Anticipating that a detailed study of the relation between weight at birth and early growth would bring out some significant and interesting differences, and having in mind as a fundamental requisite for normal growth a generically adapted nourishment, data were collected from the records of the Boston Lying-In Hospital of the weights of five hundred and thirty-seven infants on the 1st, 3d, 5th, 7th, 9th, 11th and 13th days after birth, excluding from consideration those whose food supply was derived either wholly or in part from sources other than the maternal breasts. Due care was taken throughout as to uniformity of conditions when weighing.

The classification is as follows:

GROUP	WEIGHT	NUMBER OF SUBJECTS
	pounds	
A	5–6	100
B	6–7	100
C	7–8	100
D	8–9	100
E	9–10	100
F	10–11	37

Infants under 5 pounds could not be included in the calculations inasmuch as they invariably received feedings supplementary to the mothers' milk.

Any comparative study of the power of growth in various groups of individuals is valid only when the changes occurring are considered from a percentage point of view. The absolute variations show the direction of the change but fail to give its value in terms of the original.

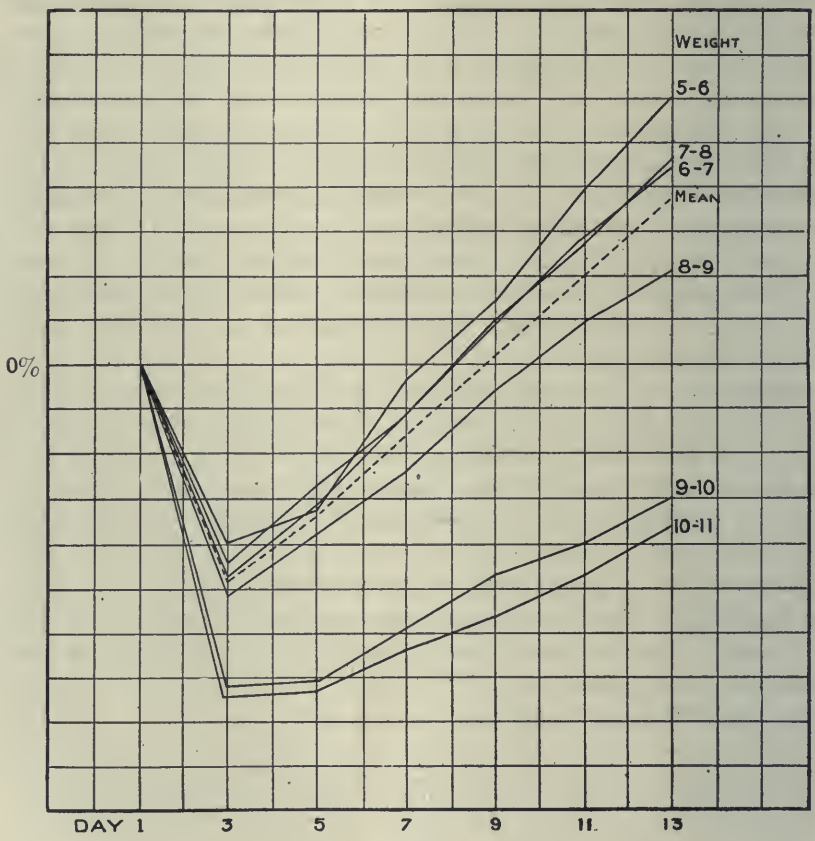

Fig. 1.

The results recorded in this paper are based on this fact and represent the per cent variations in the weights of the infants based on the weight at birth.

Table 1 gives the per cent change in body weight of the six groups of subjects during the period studied. These data have been plotted on figure 1.

There is at once made evident the division of the groups into two general classes, the members of which are governed by factors of similar intensity characteristic for the class. The groups A, B, C and D are seen to be closely alike throughout and distinct from the groups E and F. The causes of this differentiation are not at present explicable.

The post-natal decline mentioned by Quetelet (18) in a quotation from Chaussier, and the subject of much speculation, is here shown to vary in a remarkably uniform manner, according to the variation in the initial weight. The heavier the initial weight the greater the per cent drop in weight after birth. The post-natal per cent loss of weight varies directly with the weight at birth.

TABLE 1

The per cent change in weight from the first day during the first thirteen days after birth of the infants at the Boston Lying-in Hospital

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	−4.0	−3.3	−0.4	1.4	3.9	6.0
B	6–7	−4.7	−3.2	−1.1	0.9	2.8	4.4
C	7–8	−4.4	−2.7	−1.1	1.0	2.7	4.6
D	8–9	−5.1	−3.8	−2.4	−0.6	0.9	2.1
E	9–10	−7.2	−7.1	−5.9	−4.7	−4.0	−2.9
F	10–11	−7.4	−7.3	−6.4	−5.6	−4.7	−3.6

This decrease which occurs during the first two or three days after birth is the physiological response to the radical change in the methods of food assimilation and ingestion. During this period of readjustment the catabolic processes are superior to the anabolic with the resultant utilization of body tissue for maintenance. Pending the efficient establishment of a functional activity these destructive reactions overbalance the effect of the growth stimuli, if such are present at this stage, and no increase in weight occurs.

The growth catalyzers are apparently lipoidal in nature (19) and one of them has been recently isolated and named "Tethelin" (20), (21). Similarly acting substances have been found in various foods (22). It is possible to consider that those animals a part of whose development occurs in the uterus receive the necessary stimuli to growth from the maternal blood or placental secretions (23), and ample evidence has been presented that an organ may be developed to a point where

it is capable of assuming its normal function yet does not do so, or does so only at a minimum rate, until the call for functional activity is thrust upon it. Now at birth the infant is cut off from the maternal exogenous stimulation to growth, and simultaneously is readjusting itself to changed methods of nutrition. Pending this readjustment the ability to make visible growth will depend on the relative intereffect of stimulus and catabolic processes. Those organisms in which there is proportionately less substrate for activation, from whatever source, would tend to have a lesser loss of weight. Minot (12) has shown that during intra-uterine life there is an enormous loss of growth capacity. It appears to be a fact however that the first growth cycle is not quite completed at birth and that an increased growth rate occurs at or near birth (6), therefore the most effective stimulation to growth would

TABLE 2

The per cent change in weight from day to day during the first thirteen days after birth of the infants at the Boston Lying-in Hospital

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	−4.0	0.7	2.9	1.8	2.5	2.1
B	6–7	−4.7	1.5	2.1	2.0	1.9	1.6
C	7–8	−4.4	1.7	1.6	2.1	1.7	1.9
D	8–9	−5.1	1.3	1.4	1.8	1.5	1.2
E	9–10	−7.2	0.1	1.2	1.2	0.7	1.1
F	10–11	−7.4	0.1	0.9	0.8	0.9	0.9

occur in those lighter individuals who have failed to reach the normal point in intra-uterine development, and the counterbalancing effect of the catabolic processes would be diminished in an effort to respond to the greater stimulus.

With the exception of groups E and F the third day after birth marks the beginning of the pick-up to the normal rate of growth characteristic for the individual groups. The heavier infants do not begin this increase either as soon or to the same degree as do the lighter ones, this retardation effect is explained by an extension of the principles embodied in the previous discussion. From this time on the growth acceleration is practically uniform in value for any single group but diminishes with the increase in initial weight. It is significant of an underlying causative factor of growth inversely varying in intensity

of effect with the weight at birth that the various groups tend to individually attain a uniform percentage increment.

The differences from day to day of the per cent change in weight from the first day during the period of observation are given in table 2. They show the per cent additions made between the successive weighings.

The post-natal lag, extending to the fifth day, before the pick-up to the relatively uniform increment characteristic for the single groups, exhibited in groups A, E and F, at the extreme upper and lower limits of the weight at birth are indicative of a factor or factors retarding the early attainment of a normal rate of growth in these individuals. This phenomenon is not found in groups B, C or D, where although variations in the differences in the per cent increments do occur from day to

TABLE 3

The per cent recovery to or over the initial weight of the groups studied

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	19	29	50	62	75	82
B	6–7	8	24	45	60	75	80
C	7–8	12	24	39	60	74	78
D	8–7	7	17	30	49	60	70
E	9–10	2	5	15	20	30	35
F	10–11	3	3	5	8	11	20

day, yet they are relatively negligible even from the onset of demonstrable growth, and especially when compared with the marked retardation effect shown in the other groups. It is possible that a condition of instability between catalyst and substrate due to the radical change in environment of the individual as a whole is the cause of this condition in group A, and this seems more probable when we look at the subsequent variations in this value during the remainder of the period. The delay in picking up to the rate of growth normal for the group in the heavier infants is the expression of either a diminished response to the growth stimulus due to the increase in substrate, or to the preponderant effect of the catabolic processes over those initiated by the growth catalysts. The former hypothesis is the more attractive.

Continuing the inspection of this table it is seen that the variations in the per cent increment from day to day grow smaller as the weight at

birth increases. This diminution in absolute per cent increment is not sufficiently compensated for by the differences in initial weight to cause an equivalent growth acceleration in all groups. It can be stated with certainty that the heavier the initial weight the slower the rate of growth.

Camerer's (24) results seem to indicate that these differences in growth are similarly correlated with the differences in initial weight even throughout a much longer period for he found that in a series of one hundred and thirty-eight cases divided into three groups, according as they weighed under 2000 grams, between 2000 and 2750 and over 2750, and studied after weekly weighings, that the percentage increment of the subjects in the first group was 427, of the second 219 and of the third and heaviest only 195.

TABLE 4

The per cent distribution according to the weight at birth of one thousand consecutive cases at the Boston Lying-in Hospital

GROUP	WEIGHT	PER CENT
	pounds	
A	5–6	8.6
B	6–7	30.3
C	7–8	34.7
D	8–9	19.8
E	9–10	5.8
F	10–11	0.8

The extension of these facts as given by the data calculated for this paper clearly indicates that the mass or weight of the infant at birth is a determining factor in the subsequent rate of growth.

As a corollary to the foregoing, table 3 shows that the per cent of the subjects recovering or passing their initial weight after the postnatal decline is regularly influenced by the weight at birth, the retardation effect of an increased initial weight is here particularly well demonstrated.

The mean increment per cent for the six groups has been calculated and plotted on figure 1. The close approximation of this value to a straight line lends support to Osborne's (25) citation as to the applicability of Newton's first law to biological phenomena.

To obtain the figures necessary in calculating the mean, record was made of the weight of one thousand consecutive subjects and classified accordingly. Table 4 gives the per cent distribution in the six groups.

A correlation of the fact that 65 per cent of all infants weigh between six and eight pounds at birth, with the coincidental character of the growth curves of these two groups as shown on the chart, together with the fact that the curve of the mean is practically parallel with these curves leads to the idea that the normal birth weight lies between these limits and that the normal growth acceleration follows the indicated direction.

If increments of matter are used as a measure of growth it is obvious that in order to obtain a fair idea of the relative growth capacity of the various groups it is necessary to use that weight as a basis for calculation to which demonstrable additions are being made. With this point in mind there has been calculated the per cent increment in weight from the third day after birth. This is given in table 5. The third

TABLE 5

The individual growth capacity and the relative growth capacity of human infants classified according to their weight at birth

GROUP	WEIGHT	INCREMENT FROM THIRD DAY	CAPACITY	RELATIVE CAPACITY
	pounds	*per cent*	*per cent*	
A	5–6	10.0	1.818	100
B	6–7	9.1	1.400	77
C	7–8	9.0	1.200	66
D	8–9	7.2	0.847	47
E	9–10	4.3	0.453	25
F	10–11	3.6	0.343	19

column shows the per cent addition capacity of one pound of body weight at birth for the first thirteen days of extra-uterine life. The last column shows the relative growth capacity of the groups studied when group A is used as unity.

Having thus brought growth capacity to a unit basis and with the idea that the per cent increment that can be made by unit weight is an index of the capacity to grow, we find that the ability to add to the initial weight decreases with the increase in initial weight. That is to say, one pound of body weight of an infant weighing from eight to nine pounds at birth can add on a greater proportion of itself than can one pound of an infant weighing from nine to ten pounds at birth, and less than can one pound of an infant weighing from seven to eight pounds initial weight.

What is the significance of this regular variation in growth capacity? If we agree with Minot (26) that the "more rapid growth depends on

the youth of the individual" we must conclude that the weight of an infant at birth is an index of its relative physiological age. Extending this to the data presented here would give rise to the opinion that a birth weight lying between six and eight pounds is indicative of the completion of the intra-uterine growth cycle, that weights under six pounds represent physiologically younger individuals, while those over eight pounds at birth have completed and passed this cycle and are physiologically older. It is a fact that in the several groups the variability is inversely roughly proportional to the weight at birth; a correlation of this with the fact that there occurs a diminution of variability as time goes on or with senescence (12) produces additional support for the above idea.

Now growth in large measure is dependent upon the mutual intereffect of the growth stimulus, food supply and the catabolic processes of metabolism. This has been expressed in part by Friedenthal (27) who states that "ein Lebewesen wächst solange die Zunahme der Masse der lebendigen Substanz in seinem Körper den Verbrauch an lebendigen Substanz durch die Lebenschädigungen im ganzen überwiegt." It is permissible to omit from discussion the reciprocal interdependency of metabolism and growth. The diets were substantially the same for all the mothers and the metabolic processes of the infants can be considered as sufficiently uniform in nature to require no further comment. This leaves the relation of mass to catalyst as the fundamental determinant of the growth capacity of the infants studied. Now Hatai's (28) expression of the idea that "an organism tends during growth to form the greatest amount of mass with the least loss of growth capacity" does not quite coincide with these results; in fact they seem rather to lend support to Robertson's conception of growth as an autocatalyzed reaction: for it is plainly evident that growth capacity and rate of growth are increased by a diminution in the total mass at birth and decreased by an increase of the total mass at birth; this is so not only from the relative point of view but also from the standpoint of absolute increments. Enriques' (29) opinion that "das Wachstum des Stoffes wird zur einschränkenden Ursach des Wachstums selbst" would also seem to be borne out by these figures and the retardation effect on the rate of growth of the increase in weight at birth is obviously the result of the preponderance of substrate over catalyst.

CONCLUSIONS

The growth capacity of human infants during the first two weeks after birth is in a large degree dependent upon the weight at birth. It

is roughly inversely proportional to the initial weight. The ability to recover and pass the initial weight after the post-natal decline obviously varies in the same way, so that at the completion of the period studied some 82 per cent of those infants weighing between 5 and 6 pounds at birth have recovered or passed their initial weight, as compared with 20 per cent of those weighing from 10 to 11 pounds. The intermediate groups vary inversely as to their weight at birth.

Thanks are due to the staff of the Boston Lying-in Hospital and to the office force and nurses for their unfailing courtesy and assistance in making possible the collection of the data herein discussed. The kindly criticisms of Dr. John L. Bremer have done much to make this material presentable.

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THE INCREASE OF PERMEABILITY TO WATER IN FERTILIZED SEA-URCHIN EGGS AND THE INFLUENCE OF CYANIDE AND ANAESTHETICS UPON THIS CHANGE

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Received for publication January 14, 1918

I. INTRODUCTORY

The problem of the nature and conditions of cell-permeability is by no means a special or limited one but involves the whole question of the essential physico-chemical constitution of living matter. The plasma-membrane, or semi-permeable surface-layer of the cell, is not to be regarded merely as a simple passive partition separating the living substance from the surrounding medium, but rather as an integral part of the living protoplasm itself, characteristically modified in its physical properties and in its chemical composition and activities by the conditions at the cell-boundary. Some of its general peculiarities, such as the existence of a surface-tension, its greater viscosity or structural density as compared with the internal protoplasm and its difference in composition from the latter, are general properties of any surface-layer at the boundary between two immiscible fluids containing surface-active substances (especially colloids) in solution; hence the protoplasmic surface-layer has frequently been compared to a haptogen membrane. But no merely static conception of the structure and composition of this region of the cell is sufficient to explain all of its observed properties. Thus there is no doubt that its most general characteristic of semi-permeability—the all-essential insulating and diffusion-preventing property—is not merely the result of a special chemical composition and structural density, such as determine the semi-permeability of a precipitation-membrane, but is inseparable from the living condition, i.e., is actively maintained by a continual process of metabolism. The proof of this is that death—the cessation of metabolism—however caused, is invariably followed by a loss of semi-permeability, i.e., the normal state of the membrane then ceases to be maintained and the unhindered processes of diffusion lead to the disintegration of the

cell. Hence destruction of the surface-layer by artificial means—cytolytic substances, heat, extensive mechanical injury—is quickly fatal to all cells.

Since the normal properties of this layer are thus preserved by cell-metabolism, and are lost when metabolism ceases, it is not surprising to find that these properties vary with the state of metabolism, i.e., with the physiological activity of the cell. The impermeability which the plasma-membranes of most cells usually exhibit toward substances like sugar and neutral salts may thus temporarily disappear under certain conditions, and there is much evidence that this takes place especially at times of stimulation or increased functional activity;[1] the necessary entrance and exit of materials to which the membrane is at other times impermeable is thus rendered possible. Simple diffusion, however, is not sufficient to account for this interchange. We know that in the transport of substances into and out of cells in the processes of absorption and secretion osmotic work is performed, the energy of which is evidently derived from the chemical decomposition of cell-constituents; and in the normal entrance of food-substances and the exit of excretory materials in all cells similar factors are probably at work. Apparently it is by means of this physiological mechanism of transport, acting in association with temporary increase of permeability, that the normal interchange of materials with the surroundings is effected.

It is to be noted that this condition implies *intermittency* in the process of interchange, with corresponding intermittent variations in the osmotic properties of the plasma-membrane. The constant association of these variations of permeability with bioelectric variations, i.e., with electric currents passing between the cell-interior and the surroundings, suggests that processes of electrical convection or electro-endosmose are concerned in the transport. Bioelectric currents always accompany stimulation and functional activity, and must, like other electric currents passing through solid partitions, cause transport of fluid.[2] That the physiological transporting mechanism acts inter-

[1] *Cf.* the instances of secretion, activation of egg-cell, many stimulation processes. For a summary of the general evidence *cf.* my papers in this Journal, 1909, xiv, 24; 1911, xxviii, 197; 1915, xxxvii, 348.

[2] *Cf.* my recent paper in Biol. Bull., 1917, xxxiii, 135; see pp. 170 *seq.* In any bioelectric current the positive stream flows in the extracellular part of the circuit from the inactive to the active region; the flow of water in electro-endosmose is with the positive stream when the solid material composing the partition is negatively charged, as in living cells; hence the current will tend to convey water into the cell at the active region, which is also the region of increased permeability.

mittently seems certain from the above considerations; and this intermittency may at times become regularly rhythmical in character; the case of heart-muscle and other rhythmically acting tissues probably exemplifies this condition. It is possible that the wide distribution of rhythmical activities like ciliary movement is an index of a general tendency on the part of protoplasmic surface-structures to vary rhythmically in their permeability and electrical polarization.[3]

The theory that variations of permeability are constantly associated with functional activity explains the apparent paradox that the plasma-membranes of resting cells, e.g., muscle-cells, usually exhibit themselves in osmotic experiments as impermeable to just those substances which are most necessary for their continued life, viz., sugars, amino-acids and neutral salts; as we have just seen, such substances are probably transported across the cell-boundary by a physiological process acting intermittently, which is largely independent of diffusion. On this view, variations in the permeability of the plasma-membrane form an essential condition of interchange with the surroundings; and since such interchange is obviously necessary to continued metabolism, we reach the general conclusion that the control of metabolic processes depends largely upon these variations of permeability. The associated bioelectric currents may by means of their electrolytic action directly determine the chemical changes taking place at the cell-surface.[4]

On the other hand, the normal properties of the plasma-membrane itself, as of other cell-structures, are maintained by processes of constructive metabolism; these automatically replace the material which is altered or destroyed in activity or otherwise. The materials necessary for the reconstitution of the membrane-substance after breakdown are continually being synthesized and laid down as part of the organized structure; in this manner the properties of the membrane are kept constant and the stability of the living system is ensured. Of all cell-structures the plasma-membrane thus appears to be the most stable and resistant; for example, in the decrease of size incident to starvation it remains intact with unaltered properties, a fact showing that it must then maintain itself at the expense of the internal protoplasm. This fact again illustrates the special importance of the surface-film in the maintenance of the living system. There are various other indications that the *surface-metabolism* of living protoplasm is the controlling metabolism; it seems indeed probable that the prevalence of the

[3] Biol. Bull., *loc. cit.*, 169.
[4] Biol. Bull., *loc. cit.*, 172.

cellular type of structure in organisms, with the large development of surface-protoplasm which it makes possible, is ultimately to be referred to the existence of a general condition of this kind.[5]

The normal semi-permeability of the plasma-membrane thus appears to be maintained by a specific metabolic regulatory process, the precise nature of which is still far from clear but which automatically restores the semi-permeability of any region of the cell-surface whenever the latter becomes permeable to any of the essential water-soluble cell-constituents—i.e., whenever the continuity of the surface-film is interrupted. Hence this continuity tends to be regained quickly after any change involving alteration of the cell-surface.[6] On any other assumption it seems impossible to account for the characteristic insolubility of living protoplasm in the aqueous medium usually surrounding it; although typically in a fine state of subdivision (i.e., into numerous minute "cells"), protoplasm resists perfectly the solvent or disintegrative action of water, notwithstanding the high water-solubility of many of its constituents. This water-insolubility—which is shown by the existence of a sharply defined and permanent surface of separation between protoplasm and medium—constitutes, from the physico-chemical point of view, one of the most remarkable of its peculiarities. In order to account for this property it seems necessary to assume that the cell-surface consists chiefly of water-insoluble materials and also that materials of this nature are continually being formed in metabolism and deposited at the surface to replace those normally lost. The significance of the lipoid constituents of protoplasm becomes clearer on such a view; these substances have the solubilities of fats and are water-insoluble in the true sense, although readily forming colloidal suspensions or emulsions; hence they are probably chiefly responsible for the water-insoluble character of the surface-film. We may thus understand why the plasma-membrane is so effective a barrier to those water-soluble compounds (like sugar and neutral salts) which are also lipoid-insoluble, while readily admitting lipoid-soluble compounds—a general property of living cells the importance of which was first recognized by Overton.

One might suppose that a layer composed largely of water-insoluble material would also form a barrier to the passage of water, yet the general impression is that water enters and leaves living cells with

[5] Biol. Bull., *loc. cit.*, 184.

[6] *Cf.* the observations of Chambers showing rapid reconstruction of the surface-film in sea-urchin eggs after injury: this Journal, 1917, xliii, 1; *cf.* pp. 6 *seq.*

great ease. This however may be due to the large ratio of surface to volume in such minute structures as cells, rather than to a high specific permeability to water. It is noteworthy that living cells retain water with greater tenacity than dead cells, as shown by their more gradual loss of weight when exposed to evaporation;[7] and this fact indicates that the permeability to water undergoes a decided increase after death, coincidently with the general increase of permeability to dissolve substances. Bernstein's explanation is that in the living cell the electrically polarized condition of the plasma-membrane makes the outward passage of water difficult; but Höber opposes this view and suggests that the slower evaporation from living tissues is due simply to the presence of turgor; when the cells die and lose semi-permeability turgor also disappears, and water then for the first time leaves the cells readily and evaporates. There is however little if any turgor in the vertebrate tissues used in many of Bernstein's experiments; yet in these, as well as in plant tissues, the rate of evaporation is much increased by death. Why such evaporation should take place slowly through the living and rapidly, through the dead plasma-membrane seems not easily to be explained except on the view that the living membrane offers a greater resistance to the passage of water, i.e., is relatively impermeable to water. In general a high degree of semi-permeability in artificial precipitation-membranes appears to require a high degree of impermeability to water, as Morse found in his determination of the osmotic pressure of sugar solutions.[8] That the almost perfect semi-permeability exhibited by many living plasma-membranes is in reality often associated with a correspondingly high impermeability to water may readily be shown in certain cases. For example, the rate of abstraction of water from unfertilized sea-urchin eggs in strongly hypertonic sea water is surprisingly slow, as I shall describe later; and the same is true of the rate of swelling in dilute sea-water, a fact to which both Harvey and I have recently called attention.[9] It is possible that the degree of permeability of the plasma-membrane to water may be a general index of its permeability to all substances which enter and leave the cell in aqueous solution, especially if this transport is normally

[7] *Cf.* Bernstein: Elektrobiologie, Braunschweig, 1912, pp. 165 *seq.*

[8] Morse: Osmotic pressure of aqueous solutions. Carnegie Institution, Washington, 1914. See Morse's remarks on the necessity of a fine texture in the porcelaine cells supporting the precipitation membrane, p. 15; also pp. 87 *seq.*

[9] Harvey: Science, N. S., 1910, xxxii, 565; R. Lillie: This Journal, 1916, xl, 249.

accompanied by a flow of water (as is the case, e.g., in secretory processes). Evidently there can be no interchange of dissolved material between two adjacent solutions separated by a solid partition if the solvent cannot pass the partition (unless indeed the partition itself also acts as a solvent); for example, a glass bottle containing a solution shows no osmotic effects when immersed in water. In general, the rate of any osmotic process is limited by the permeability of the membrane to the solvent[10]; and in view of the importance of osmotic processes in physiology, it seems desirable that the conditions of permeability to water, as well as to dissolved substances, should receive further investigation. Hitherto little attention has been paid to this general problem; in many cells, however, the degree of permeability to water is a constant and definite character, which varies with physiological conditions and can be measured with considerable accuracy.

In certain cases a quantitative expression of the permeability of the plasma-membrane to water may be obtained by measuring the rate at which water enters or leaves the cell under a definite gradient of osmotic pressure. This is done by determining the alteration in weight or volume taking place in a given time in a hypertonic or hypotonic physiologically balanced medium of known osmotic pressure. The cases where such a method can be expected to give reasonably accurate results are perhaps not numerous. To determine at frequent intervals the weight of a tissue immersed in an anisotonic medium is a difficult and often impracticable process with many sources of accidental variation. Consistent results are possible, however, in the case of spherical cells like sea-urchin eggs, which swell slowly in hypotonic media (e.g., dilute sea-water) without change of form. In such eggs the diameter at any time can be measured rapidly by the ocular micrometer with a sufficient degree of accuracy, and the volume can be calculated on the assumption that the form is spherical. Using this method, I was able to show that in the Arbacia egg fertilization is followed by an approximately fourfold increase in the permeability to water.[11] By this means it would probably be possible to compare the relative permeability of different species of eggs to water and to study the variations of permeability in the same egg under different conditions of temperature, physiological activity, composition of medium, etc. The ratio between the rate of entrance of water under standard conditions (of osmotic pressure-gradient, temperature, composition of

[10] *Cf.* Antropoff: Zeitschr. physik. Chem., 1911, lxxvi, 721.

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

medium) and the area of the membrane would give a measure of the specific permeability of the membrane to water. In cases where this method proved applicable its simplicity would be an advantage.

II. DIRECT EFFECTS OF HYPERTONIC SEA-WATER UPON FERTILIZED AND UNFERTILIZED ARBACIA EGGS

In any hypertonic medium which is otherwise non-injurious (*i.e.*, free from toxic substances and containing the necessary salts in balanced proportions, like sea-water or van't Hoff's solution) Arbacia eggs show the usual behavior of living cells, they lose water and shrink; in a hypotonic medium they swell. In general the rate of the osmotic entrance or exit of water in any cell, after transfer from its normal medium to one of similar constitution but different osmotic pressure, varies directly (1) with the gradient of osmotic pressure between the interior and the exterior of the cell, (2) with the area of the enclosing semi-permeable membrane, and (3) with the permeability of this membrane to water. Hence if the same cell exhibits at different times definite inequalities in the rate of osmotic gain or loss of water in the same medium, the inference is that the resistance to the passage of water across the membrane has varied correspondingly,—in other words that the permeability to water is subject to change under varying physiological conditions. The Arbacia egg presents a very clear case of this kind, fertilization being followed regularly by a marked increase in the permeability of the plasma-membrane to water,—as may readily be shown by bringing the eggs into either dilute or concentrated sea-water; in the former medium they swell, in the latter they shrink, but in both cases the rate of the process is much greater in the fertilized than in the unfertilized eggs. Both swelling and shrinkage are surprisingly slow in unfertilized eggs; when these eggs are transferred from sea-water into a strongly hypotonic or hypertonic medium they exhibit little alteration of size at a time (e.g., one or two minutes after transfer) when fertilized eggs in the same solution are conspicuously swollen or shrunken (see fig. 1). This difference of behavior relates entirely to the *rate* at which water either enters or leaves the egg; the *degree* of swelling or shrinkage when osmotic equilibrium is reached does not differ appreciably in the two kinds of eggs.[12] It is clear therefore that the change in osmotic properties has nothing to do with any change which

[12] See the curves in my former article, *loc. cit.*, 255.

fertilization might be supposed to produce in the osmotic pressure of the egg-protoplasm, but is determined solely by the greater readiness with which water enters or leaves the fertilized egg.

According to my former measurements on the rate of swelling of fertilized and unfertilized eggs in dilute sea-water, the resistance to the passage of water through the plasma-membrane is decreased, as a result of fertilization, to approximately one-fourth of its former value. A second method of detecting and estimating the change in the permeability of the egg to water is to determine the relative rates

TABLE 1

CONCENTRATION OF SOLUTION	IMMEDIATE EFFECT OF SOLUTION	EFFECT OF RETURN TO SEA-WATER
1. 2.0m	Rapid and complete collapse with immediate and marked loss of pigment from the eggs	Eggs remain collapsed and do not cleave or develop
2. 1.5m	Collapse and loss of pigment are rapid, but less so than in solution 1	Most eggs remain collapsed, but a few (*ca.* 5 per cent) recover the normal water-content and cleave
3. 1.25m	Rapid shrinkage and crenation with some extraction of pigment, but less than in solution 2	Most eggs round off within 3 minutes and later cleave; the majority form blastulæ.
4. 1.0m	Eggs shrink and crenate more slowly than in solution 3; no evident loss of pigment in 2 minutes	All eggs round off rapidly and later continue cleavage and development; the great majority form larvæ
5. 0.75m	Shrinkage is slower than in solution 4 and relatively slight; all eggs are slightly crenated in one minute	All eggs form larvæ

of shrinkage in strongly hypertonic sea-water or van't Hoff's solution, and some of the possibilities of this method were investigated at Woods Hole last summer. In experiments of this kind it is important to avoid too great a degree of hypertonicity in the solutions used, since then injury results; on the other hand, if the osmotic pressure is too low the effects are not definite enough. The following experiments (table 1) will illustrate the nature of the effects observed with media of varying osmotic pressure. Fertilized eggs were placed, thirty to forty-five minutes after fertilization, in van't Hoff's artificial sea-water of the concentrations given in the table. The action of each solution upon

the eggs was observed in watch-glasses. After remaining in the solutions for ten to fifteen minutes the eggs were returned to normal sea-water, and the changes immediately following this second transfer were also observed. Some of the eggs were left in sea-water in order to observe later the effect of the treatment upon cleavage and development.

These experiments show that irreversible effects appear only when the excess of osmotic pressure approaches the order of twenty atmospheres (solution 2). The osmotic collapse in the first two solutions is permanent and is associated with a cytolytic action indicated by loss of pigment; the failure of the eggs to round off on return to sea-water shows that the semi-permeability of the plasma-membrane has been permanently destroyed; evidently some irreversible structural alteration has taken place. In the less concentrated solutions the osmotic properties of the membrane remain unimpaired, and the eggs recover their normal water-content in sea-water and continue development. Solutions having an osmotic pressure similar to that of solutions 2 and 3 were used in most of the following experiments. In most cases these solutions were made by mixing concentrated van't Hoff's solution with sea-water.

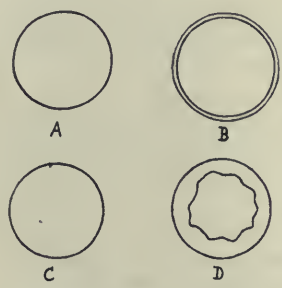

Fig. 1. *A* and *B*, outlines of unfertilized and fertilized Arbacia eggs in normal sea-water; *C* and *D*, appearance of the same eggs after one minute in hypertonic sea-water.

Differences between fertilized and unfertilized eggs. When transferred to hypertonic sea-water or van't Hoff's solution of 35 to 40 atmospheres osmotic pressure, fertilized eggs are at once seen to shrink rapidly and the egg-surface is thrown into characteristic folds and crenations; unfertilized eggs in the same medium shrink slowly and at first imperceptibly, and remain round. Any balanced medium of sufficient hypertonicity may be used to demonstrate this difference; in most of the following experiments a mixture of one volume of 2.5m van't Hoff's solution[13] *plus* three or four volumes normal sea-water was used; the former solution has an estimated osmotic pressure at 20° of *ca.* 40 atmospheres, the latter of *ca.* 36 atmospheres—as against the 24 atmospheres of the sea-water at Woods Hole. In either of these solu-

[13] Van't Hoff's solution contains the following salts in the molecular proportions: 100 NaCl, 2.2 KCl, 7.8 $MgCl_2$, 3.8 $MgSO_4$, 2 $CaCl_2$. The above solution is made by mixing 2.5m. solutions of the salts in the above proportions by volume.

tions fertilized eggs begin to crenate within fifteen or twenty seconds after transfer while unfertilized eggs show no evident change until much later. The most striking and convenient method of showing this difference is to place a mixture of equal numbers of unfertilized and fertilized eggs (the latter fertilized at least fifteen minutes previously) in hypertonic sea-water. The fertilized eggs at once shrink rapidly and undergo crenation, and within less than one minute exhibit a collapsed, shrunken and angular appearance; at this time the unfertilized eggs are apparently unaltered, so that a striking contrast is presented (see fig. 1). Shrinkage continues slowly in the unfertilized eggs and becomes well marked in the course of five or six minutes; but a curious feature in the behavior of these eggs is that they remain smooth and spherical during the entire period of shrinkage, the surface showing no sign of the folds and crenations characteristic of the fertilized eggs. It is evident that as a result of fertilization the properties of the plasma-membrane have undergone profound alteration affecting both its osmotic properties and its physical consistency, so that this simple osmotic treatment is sufficient to differentiate sharply between the two kinds of eggs.

An incidental effect of the above treatment is that the rapid abstraction of water from the fertilized eggs causes a considerable increase in their density, hence they sink in the hypertonic sea-water more rapidly than the unfertilized eggs; in fact a partial separation of the two kinds can readily be accomplished by taking advantage of this difference in the rate of sinking. The following experiment will illustrate. To a mixture of fertilized and unfertilized eggs in normal sea-water a relatively large volume of hypertonic van't Hoff's solution (1m; O.P. = *ca.* 40 atm.) was added; the eggs were then uniformly distributed in a graduate and allowed to sink. At the end of three minutes and again at the end of twelve minutes some eggs were removed with a pipette from the upper layer of the sinking mass, and the proportions of fertilized and unfertilized were determined by counting the numbers of each kind in several microscopic fields under the low power. The numbers obtained were as follows:

	FERTILIZED	UN-FERTILIZED
Before sinking	48	70
After 3 minutes sinking (sum of four counts)	46	89
After 12 minutes sinking (sum of eight counts)	17	116

It is evident that the proportion of unfertilized eggs in the surface layer increases rapidly as the eggs sink through the solution. A practical method of separating fertilized from unfertilized eggs without injury—in case such a method were required for any purpose—could no doubt be devised, e.g., by using moderately hypertonic sea-water and a centrifuge.

When eggs that have been shrunken in hypertonic sea-water are returned to normal sea-water, water reënters the fertilized eggs rapidly, the unfertilized eggs slowly. It has already been shown that shrinkage in moderately hypertonic sea-water does not impair the developmental capacity of the eggs; and correspondingly the osmotic properties of the plasma-membranes appear to be unaffected by this treatment, as indicated by the following experiment. Mixed fertilized and unfertilized eggs were placed in hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 4 vols. sea-water) and left for five minutes; at this time the fertilized eggs had the typical collapsed and angular appearance and were smaller than the round unfertilized eggs which had not yet reached osmotic equilibrium. The eggs were then returned to normal sea-water. After one minute in sea-water the fertilized eggs were again round and already distinctly larger than the unfertilized eggs—showing that even under a smaller gradient of osmotic pressure water reenters the fertilized eggs more rapidly. After two or three minutes in sea-water the difference in size was still apparent though less so than before; later, as the unfertilized eggs also approached osmotic equilibrium, the difference disappeared. It is clear that the resistance to the passage of water in either direction through the plasma-membrane is much smaller in fertilized than in unfertilized eggs. This experiment thus yields the same result as the earlier experiments in which eggs were transferred from normal to dilute sea-water—in this case also from a more to a less concentrated medium. The use of dilute sea-water in the osmotic method of determining relative permeability has the advantage that a quantitative comparison between the rates of entrance of water is possible, since the eggs remain spherical and the volumes can be calculated from the diameters; this is not possible when hypertonic sea-water is used, because of the irregular form of the collapsed fertilized eggs.

The rapid dehydration in strongly hypertonic solutions has a destructive action upon fertilized eggs (see table 1); unfertilized eggs, in correspondence with their slower shrinkage, are less injured by such solutions, as is shown by the following experiment. Equal quantities

of fertilized and unfertilized eggs in separate watch-glasses were exposed to equal volumes of strongly hypertonic sea-water (10 cc. of a mixture of 10 vols. 2.5m van't Hoff's solution *plus* 15 vols. sea-water). Within one minute the fertilized eggs turned pink in color, a sign of cytolysis, and within two minutes the solution was deeply colored with escaped pigment. After the same interval the unfertilized eggs showed no indication of cytolysis and the solution remained clear; an hour later the eggs were still uncytolyzed, although much shrunken, and there was no evident loss of pigment. Loeb and others have described experiments showing the greater resistance of unfertilized eggs to various forms of toxic action;[14] and the above observations indicate that this difference depends primarily upon the difference in the properties of the semi-permeable protoplasmic surface-layer (or plasma-membrane) before and after fertilization. The precise physico-chemical basis of this difference remains to be determined; there are, however, two definite changes in the physical properties of the plasma-membrane which are brought out clearly by the above experiments: (1) the increased permeability to water following fertilization, i.e., decreased resistance to its passage or decreased waterproof property, and (2) the simultaneous disappearance of the tension or contractile properties of the surface-film of protoplasm. The decrease in the resistance to toxic agents is apparently correlated with this change of properties in the membrane.

The latter of these two changes is remarkable. In the unfertilized egg, as already described, the surface contracts or decreases in area as water is lost, so that the whole egg remains smooth and spherical—indicating the existence of a certain contractile tendency or tension in the surface-film; i.e., the surface acts as if fluid or elastic in its properties, while in the fertilized egg even a slight decrease in volume throws the surface into folds, indicating the lack of any such effective surface-tension. Apparently the surface-film in the latter case is solid and under little or no tension; hence its area does not adjust itself to the decreased volume of the egg. It should be noted here that certain constant differences in the mechanical and other properties of the egg-surface before and after fertilization have also been observed by Chambers,[15] using the methods of microdissection. His observations, not yet published in detail, should be correlated with those above described.

The action of hypertonic sea-water upon eggs with artificial fertilization-membranes. Eggs in which artificial fertilization-membranes have been

[14] J. Loeb: Biochem. Zeitschr., 1906, i, 200; 1906, ii, 81.

[15] See the footnote on p. 264 of my former paper, *loc. cit.*

formed by exposure to butyric acid solution exhibit the same increase of water-permeability and liability to crenation as sperm-fertilized eggs; but the effects are more variable and present an interesting series of gradations. The following description of a typical experiment will illustrate:

August 17, 1917, Unfertilized Arbacia eggs were exposed for seventy-five seconds to a solution of N/260 butyric acid in sea-water (2 cc. n/10 acid *plus* 50 cc. sea-water) and then returned to normal sea-water. Twenty-five minutes later a strongly hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 2 vols. sea-water) was added to a mass of these eggs in a watch-glass (lot A). At the same time, for comparison and control, a similar quantity of sperm-fertilized eggs (fertilized twenty-five minutes previously) was similarly treated in a second watch-glass (lot B).

In lot A the majority of eggs showed well-marked shrinkage and crenation in less than one minute. The shrinkage was however on the whole less marked than in lot B and its degree was more variable; a considerable proportion of eggs in which membranes were imperfectly separated or absent shrank slowly and remained round; even after three minutes this minority (*ca.* 20 to 30 per cent) were still rounded and less shrunken than the others. In general the eggs with the most definite and well-separated membranes showed the most rapid and complete shrinkage and crenation. There was also a considerable loss of pigment from the eggs in lot A, but distinctly less than in lot B; closer examination showed that in the eggs with imperfect membranes or without visibly separated membranes the cytolytic effect was slight or absent.

In the control experiment (lot B) all eggs underwent rapid and complete collapse and the loss of pigment was well-marked.

Several other experiments of the same kind, with less strongly hypertonic solutions, gave the same general result. It is well known that artificial membrane-formation in Arbacia eggs is a highly variable process and that the membranes are typically thinner than those formed in normal fertilization and adhere more closely to the egg-surface; usually in a minority of eggs no separate membrane can be seen. In all cases it was found that those eggs which underwent the most rapid and well-marked collapse were those having well separated and definite membranes; eggs with imperfect membranes shrank more slowly and tended to remain round; while there was always present a certain variable proportion of eggs, without visibly separated membranes, which exhibited a behavior almost indistinguishable from that of unfertilized eggs, shrinking slowly and remaining quite round.

A definite correlation was thus found between the degree of membrane-separation and the degree of increase in permeability to water. The eggs with the most nearly normal membranes approach most

nearly in their osmotic behavior to sperm-fertilized eggs. It seems probable that the degree of completeness of the activation-effect is similarly determined; as a rule the results of artificial parthenogenesis in Arbacia show wide variation and only a small proportion of eggs form normal larvae. I have not yet investigated the effect of the second "corrective" treatment with hypertonic sea-water upon the water-permeability of these eggs. It is clear, however, that the membrane-forming agent, when effective, produces the same kind of effect upon permeability as the normal entrance of the sperm. This may also be demonstrated by the use of dilute sea-water; eggs with artificial membranes swell more rapidly than normal unfertilized eggs, although on the whole less rapidly and at a more variable rate than sperm-fertilized eggs.[16]

Effects of hypertonic sea-water upon Echinarachnius eggs. Experiments similar to the above were performed with the fertilized and unfertilized eggs of the sand-dollar, Echinarachnius parma. These eggs are spherical in form and much larger than those of Arbacia, having an average diameter of about 140μ;[17] they have a clear protoplasm, only slightly pigmented, and are highly favorable objects for experimental purposes. They are readily obtained in quantity and resemble sea-urchin eggs in their general properties.

Unfertilized eggs placed in a strongly hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 2 vols. sea-water) shrink gradually and remain round, while fertilized eggs in the same solution shrink rapidly and crenate. A mixture of fertilized and unfertilized eggs exhibits the same kind of contrast, after a minute or two in the solution, as Arbacia eggs. This contrast is most striking in about two minutes; fertilized eggs also sink more rapidly in the solution than unfertilized eggs.

Similar experiments with a concentrated van't Hoff's solution (1.25m) gave the same result. After one minute in this solution the fertilized eggs are collapsed and crenated and smaller in diameter than the unfertilized eggs which shrink slowly and remain round. If such mixed eggs are returned to normal sea-water, after four or five minutes' exposure to the hypertonic solution, the fertilized eggs regain water much more rapidly and within a minute are round and larger than

[16] See the curve representing the average rate of intake of water by these eggs in hypotonic sea-water on p. 255 of my former paper (*loc. cit.*).

[17] The average diameter of 18 unfertilized Echinarachnius eggs, measured with the ocular micrometer, was 139.4μ. Arbacia eggs measure about 74μ.

the unfertilized eggs. As in the similar experiment with Arbacia eggs, this difference is temporary and disappears as the eggs near osmotic equilibrium. Fertilized eggs also swell more rapidly in dilute sea-water. Experiments with artificially activated eggs have not yet been made.

Starfish eggs. Experiments with starfish eggs have been few in number as yet, but a brief report seems desirable since an entirely different type of behavior was found. These eggs are usually difficult to procure at the time of year (late August) in which these experiments were begun. Only one normal lot was obtained but these showed in hypertonic sea-water a definite behavior which is undoubtedly characteristic. Unfertilized mature eggs were found to shrink in concentrated sea-water much more rapidly than the eggs of either Arbacia or Echinarachnius, and fertilization had little or no effect upon the rate or character of shrinkage. It was also found that fertilized and unfertilized eggs swelled in dilute sea-water at about the same rate. These observations indicate that in the mature unfertilized starfish egg the permeability to water is already relatively high and undergoes little or no change as a result of fertilization. Further investigation of the conditions in these eggs is, however, desirable; and the present incomplete results are cited chiefly because of the suggestive parallel which they show to the observations of Loeb and Wasteneys[18] upon the relative rates of oxidation before and after fertilization. In the starfish egg these investigators found no significant difference; while in the Arbacia egg fertilization increased the rate of oxygen-consumption nearly four-fold, i.e., to about the same degree as the permeability to water. The absence of increase in water-permeability in the starfish egg has probably some direct relation to the absence of increase in oxidations. In both species of eggs the rate of oxygen-consumption appears to run parallel with the permeability to water.

III. TIME-RELATIONS OF THE INCREASE OF PERMEABILITY IN ARBACIA EGGS

The change of permeability following fertilization in Arbacia eggs is not sudden or rapid but begins gradually and requires a considerable time—at least twenty minutes at the summer temperature of the sea-water (20 to 22°)—to reach approximate completion. During the first few minutes after insemination the eggs, when placed in hypertonic

[18] J. Loeb: Artificial parthenogenesis and fertilization, Univ. of Chicago Press, 1913, chapter II.

sea-water, shrink slowly and remain round, like unfertilized eggs. Not until five or six minutes have elapsed is there any noticeable increase in the permeability to water, as indicated by increased rate of shrinkage; from then on the rate becomes by degrees more and more rapid and the tendency to crenation also appears and increases *pari passu* with the rate of shrinkage. Both of these effects are clearly expressions of the same change in the plasma-membrane. In about twenty minutes the process of permeability-increase is nearly complete; but usually a test with hypertonic sea-water made at thirty or forty minutes after insemination shows a distinctly greater rate and degree of collapse than at twenty minutes, indicating that the membrane continues to change slowly for some time afterwards. The condition of increased water-permeability appears to remain as a permanent property of the developing egg, and may readily be demonstrated in the two-cell and four-cell stage in the intervals between cleavages; such eggs collapse rapidly and crenate in the same manner as the uncleaved egg.[19]

The general course of the process can best be indicated by the description of a typical experiment in table 2. This summarizes a series of observations in which eggs from a single fertilized lot were tested in hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 4 vols. sea-water) at successive intervals of two minutes through a total period of sixteen minutes, beginning immediately after insemination. Each portion of eggs was examined immediately after placing in the solution and again later after nineteen minutes in the solution.

Several other similar series gave the same general result. It will be seen that the differences between the successive members of such a series are easily appreciable at first, but become less so later. Apparently the increase of permeability begins between two and four minutes after insemination and is in greater part completed during the next ten minutes. The process continues long after the complete separation of the fertilization-membrane and cannot be referred directly to the formation of this structure or to any changes in its properties after separation. Heilbrunn has shown that the fertilization-membrane undergoes an increase of permeability to salts during the first few minutes after separation;[20] but its prompt collapse in sea-water con-

[19] According to J. Gray (personal communication) the electrical conductivity of Echinus eggs after fertilization remains permanently greater than that of unfertilized eggs (*cf.* my recent paper on the present subject, this Journal, 1917, xliii, footnote p. 44).

[20] Heilbrunn: Biol. Bull., 1915, xxix, 160.

taining a little egg-albumin (which raises osmotic pressure very slightly) shows that it offers only a negligible resistance to the passage of water. Moreover this change of permeability is completed within five minutes,[21] i.e., at a time when the permeability of the egg to water is just

TABLE 2

TIME OF PLACING IN SOLUTION (MINUTES AFTER FERTILIZATION)	EFFECTS OF EXPOSURE TO HYPERTONIC SOLUTION
1. 2m	Fertilization-membranes are well separated in all eggs. All remain round without any crenation and shrink slowly. At 19 minutes all are round, shrunken and crenated
2. 4m	After 1 minute nearly all eggs are round and not evidently shrunken, but a few show traces of crenation. At 19 minutes the great majority are round and shrunken with smooth contour
3. 6m.	At 30 seconds all eggs are round; at 40 seconds a few are slightly crenated; at 1 minute most are moderately crenated but some remain round. At 19 minutes most eggs are again round, but the contours are slightly irregular in many
4. 8m.	Many eggs show slight crenation by 30 seconds; at 1 minute all are more or less shrunken and crenated. At 19 minutes nearly all show a slightly crenated or wavy contour; a few are round
5. 10m.	Most eggs show slight crenation at 20 seconds, well-marked at 30 seconds, and decided at 45 seconds and 1 minute. At 19 minutes all are more or less crenated and collapsed, but less so than in the later members of the series.
6. 12m.	Crenation is more rapid than in no. 5 and all eggs are well collapsed at 1 minute. At 19 minutes the degree of crenation is distinctly greater than in no. 5, and the eggs are collapsed and polygonal in form
7. 14m.	Crenation is rapid and pronounced in all eggs, more so than in no. 6. At 19 minutes the degree of collapse and irregularity of form appears somewhat greater than in no. 6, and rounded eggs are fewer
8. 16m.	Crenation and shrinkage are rapid and pronounced, as in no. 7, but with no evident difference. At 19 minutes the condition is the same as in no. 7.

beginning to show increase, hence it cannot be regarded as a factor of any importance in the above effects. There seems to be no doubt that a progressive change in the osmotic properties of the semi-permeable surface-layer of the egg-protoplasm (the plasma-membrane proper) is

[21] Heilbrunn: *loc. cit.*, 161.

chiefly or wholly responsible for the change in the behavior of the egg in the anisotonic medium. This change is only one of many features in the complex of reactions initiated in the egg by the entrance of the spermatozoön, and presumably it is to be referred to some special metabolic process taking place in the surface-film. It is interesting to note that the period of permeability-increase shows a general correspondence in its time-relations with the period of increased susceptibility to poisons which, as shown by Lyon,[22] immediately succeeds fertilization. At this time there appears to be a well-marked increase in the general rate of metabolism, as indicated both by the increased output of CO_2 and by the greater susceptibility to cyanide;[23] and this increase in metabolism is probably directly connected with the change in the properties of the membrane.

IV. INFLUENCE OF EXTERNAL CONDITIONS ON THE CHANGE OF PERMEABILITY

Experiments on the influence of external conditions upon the change of permeability in Arbacia eggs have shown that the process is not readily affected by increasing the calcium-content of the sea-water or by low concentrations of cyanide. Since these conditions both inhibit cleavage, a further indication is afforded that the physical or metabolic change in the plasma-membrane underlying the increase of permeability is of a special kind and different from that associated with cytoplasmic division, where also a demonstrable change in the properties of the membrane takes place.[24] On the other hand, the process is checked or arrested reversibly by higher concentrations of cyanide (M/200 and above), and also by anaesthetics. The influence of temperature has not been studied as yet.

[22] Lyon: This Journal, 1902, vii, 56.

[23] For increased output of CO_2 by Arbacia eggs after fertilization *cf.* Lyon: This Journal, 1904, xi, 52. The work of Child, in collaboration with Tashiro, indicates that the degree of susceptibility of cells to cyanide is a general index of the rate of metabolism (as measured by output of CO_2). *Cf.* Child: Senescence and rejuvenescence, Univ. of Chicago Press, 1915, chapter iii.

[24] During the formation of the cleavage-furrow the plasma-membrane loses its resistance to disruption in dilute sea-water, and the eggs then rapidly break down in this medium, recovering their resistance after cleavage is complete. There is no such decrease in the resistance to osmotic disruption immediately after fertilization—a fact indicating that the change then taking place in the plasma membrane is of a different kind from that associated with cleavage. *Cf.* Journ. Exper. Zoöl., 1916, xxi, 369; see 386.

It was expected that a decided increase in the calcium-content of the sea-water would retard or prevent the change of permeability, but this was found not to be the case. Eggs placed, two minutes after insemination, in a mixture of equal volumes isotonic $CaCl_2$ (0.35m) and sea-water were found after sixteen minutes in this solution to shrink and crenate promptly in hypertonic sea-water, showing little or no difference from eggs similarly treated after an equal interval in normal sea-water. The change of permeability thus proceeds at an unaltered rate in the presence of a high concentration of calcium.

The change is also not noticeably influenced by low concentrations of KCN, although high concentrations are effective. Eggs placed, two minutes after insemination, in sea-water containing M/1000 KCN were found to undergo rapid shrinkage and crenation on transfer to hypertonic sea-water sixteen minutes later; no difference from the control could be seen. For the complete prevention of cleavage, on the other hand, M/8000 KCN is sufficient.[25] Experiments with higher concentrations of KCN (M/800, M/400, M/200, M/100) gave a different result; eggs were placed in these solutions two or three minutes after fertilization, and after thirty to thirty-five minutes exposure were examined in hypertonic sea-water. After exposure to M/100 and M/200 KCN shrinkage was gradual and only a small proportion of eggs underwent partial crenation; with M/400 KCN shrinkage was more rapid and considerable crenation took place, indicating retardation but not entire prevention of the change of permeability; while with M/800 KCN the behavior was indistinguishable from the control. This inhibiting action of cyanide is reversible; eggs replaced in normal sea-water after the above exposures all showed thirty minutes later the typical rapid shrinkage and crenation. The replaced eggs of these lots left undisturbed in sea-water almost all developed to larval stages.

Organic anaesthetics readily inhibit the increase of permeability and the effective concentrations were found for the most part similar to those required for the prevention of cleavage in these eggs,[26] although in several instances they were somewhat higher. Experiments were made with chloral hydrate, chloroform, methyl, ethyl, propyl, isobutyl and iso-amyl alcohols, ethyl urethane and ethyl ether. In all cases

[25] *Cf.* Journ. Biol. Chem., 1914, xvii, 121; see 137.

[26] Journ. Biol. Chem., *loc. cit.* Similar concentrations of anaesthetic inhibit membrane-formation and activation in Arbacia eggs by pure isotonic solutions of neutral salts (*cf.* Journ. Exper. Zoöl., 1914, xiv, 591); they also retard the cytolytic action of such solutions on unfertilized eggs (*cf.* this Journal, 1912, xxx, 1).

eggs which were placed, two or three minutes after insemination, in solutions of these compounds in sea-water, of the appropriate concentrations, remained in the characteristic water-impermeable and slowly shrinking condition during the period of exposure to the anaesthetic, or underwent only slight and gradual change. On return to normal sea-water the permeability-increasing process was resumed and the eggs continued development, nearly all reaching larval stages.

The following description of experiments with solutions of chloral hydrate in sea-water is typical of the procedure and results with the above anaesthetics. The concentrations of chloral hydrate employed were 0.3, 0.2 and 0.1 per cent. The eggs were placed, three minutes after insemination, in the solutions, and left for thirty to thirty-five minutes; they were then transferred directly to hypertonic sea-water and the behavior was observed. In 0.3 per cent chloral hydrate the change of permeability appeared to be entirely prevented; one and a half minutes after placing in the hypertonic sea-water the eggs were still round, uncrenated and only slightly shrunken; ten minutes later shrinkage was well-marked but the contour remained smooth, as in the case of unfertilized eggs. Part of the eggs were then transferred from the anaesthetic solution to normal sea-water and thirty minutes later the reaction to hypertonic sea-water was again tested; rapid shrinkage and crenation were then found, showing that the process of permeability-increase had been resumed after the removal of the anaesthetic. Eggs that were left undisturbed in normal sea-water, after return from the anaesthetic solution, continued development and the great majority formed larvae. Similar results were obtained with 0.2 per cent chloral hydrate, but with the 0.1 per cent solution the increase of permeability, though retarded, was not entirely prevented; the eggs shrank in the hypertonic sea-water more slowly than the control unanaesthetized eggs, but more rapidly than eggs that had been treated with the two stronger solutions, and many showed partial crenation. The concentrations required for complete prevention of permeability-increase are thus higher than for the prevention of cleavage; the latter concentration is about 0.1 per cent in Arbacia eggs.

With all of the other anaesthetics results of a similar kind were obtained; the change of permeability was either prevented or markedly retarded in the anaesthetic-containing sea-water, and renewed on return to normal sea-water, and the returned eggs continued development, the great majority forming larvae. In most cases a reversible arrest or a marked retardation was found in solutions which were just

concentrated enough to prevent cleavage, but in some other cases (ether, amyl alcohol) somewhat stronger solutions were needed. The following table gives the concentrations of the solutions in which increase of water-permeability is completely or almost completely arrested without injury to the eggs. The concentrations required to anaesthetize cleavage are also given for comparison. Solutions only slightly weaker than these retard without preventing the process, or in some cases they have little or no evident action; e.g., in 0.5 vol. per cent amyl alcohol or 1 vol. per cent ether the increase of permeability takes place at almost the normal rate.

TABLE 3

ANAESTHETIC	CONCENTRATION PREVENTING INCREASE OF PERMEABILITY	CONCENTRATION PREVENTING CLEAVAGE[27]
Chloral hydrate	*ca.* 0.2 per cent	0.1–0.2 per cent
Chloroform.....	$\frac{1}{10}$ saturated (0.05 per cent)	$\frac{1}{12}$ saturated (0.06 per cent)
Methyl alcohol	8 vol. per cent	
Ethyl alcohol	5 vol. per cent	5 vol. per cent
n-Propyl alcohol	2 vol. per cent (effect is incomplete in some eggs)	2 vol. per cent
Isobutyl alcohol	1–1.2 vol. per cent	0.8 vol. per cent (for n-butyl alcohol)
i-Amyl alcohol	0.6 vol. per cent (0.5 vol. per cent is insufficient)	*ca.* 0.4 vol. per cent
Ethyl urethane	2 per cent	1.5–1.75 per cent
Ethyl ether	1.2–1.4 vol. per cent (1 vol. per cent is insufficient)	0.5–0.6 vol. per cent

The general correspondence of the above two series of concentrations shows that the process determining the permeability-increase of fertilization is anaesthetized under essentially the same conditions as that determining cell-division. In both cases a change normally taking place in the physical properties of the protoplasmic surface-layer—shown by increase of water-permeability in the one case, and by loss of resistance to osmotic disruption in the other—is prevented. The theory that anaesthetic action consists essentially in a modification in the state of the "plasma-membrane"—the general name for the water-insoluble and semi-permeable surface-film of protoplasm—is thus favored. It should however also be noted that the higher concentrations of anaesthetic required to arrest the former process in several instances, as well as its greater resistance to cyanide, indicate that the underlying

[27] Journ. Biol. Chem., *loc. cit.*

physico-chemical conditions are not identical in the two cases.[28] Apparently variations in the permeability and other properties of the plasma-membrane may take place as the result of more than one kind of change in this structure.

In general anaesthesia appears to be associated with an increased stability of the plasma-membrane and a correspondingly increased resistance to change of permeability and hence of electrical polarization.[29] The membrane preserves its semi-permeability under conditions which in the unanaesthetized cell cause temporary or permanent loss of this property; hence the greater resistance to certain forms of cytolytic action as well as the lack of response to stimulation.[30] There may also be an actual decrease in the normal resting permeability of the cell; this has been observed in several cases; for example, the electrical conductivity of plant-cells may be decreased by 13 per cent during anaesthesia, as observed by Osterhout in Laminaria.[31] I have also found that after the normal increase of permeability to water is completed in fertilized Arbacia eggs, it is possible to cause a return toward a condition of decreased permeability by exposure to the above solutions of anaesthetics. This is shown by the following experiments. The eggs were placed, about thirty minutes after fertilization, in the anaesthetic-containing sea-water, and after remaining in this solution for twenty to thirty minutes the osmotic behavior in hypertonic sea-water (1 vol. 2.5m van't Hoff's sol. *plus* 4 vols. sea-water containing the same anaesthetic in the same concentration) was tested in the usual manner. The following table summarizes the results of several observations with each anaesthetic.

These observations show that the abstraction of water from fertilized eggs by hypertonic sea-water is definitely retarded after exposure to nearly every one of the above anaesthetic solutions, in some cases markedly, in others comparatively slightly. Cyanide proved ineffective. The greatest permeability-decreasing action was shown by ethyl urethane; eggs treated with this compound remain round and uncrenated for several minutes after placing in the hypertonic sea-water, and

[28] *Cf.* footnote 24.

[29] The evidence for this general theory is summarized and discussed in my article on "The theory of anaesthesia:" American Yearbook of Anaesthesia, 1916; also in Biol. Bull., 1916, xxx, 311.

[30] *Cf.* the general article just cited; Biol. Bull., pp. 356. *seq.* also footnote 26 above.

[31] Osterhout: Science, N. S., 1913, xxxvii, 111.

TABLE 4

ANAESTHETIC AND CONCENTRATION	RESULTS OF EXPOSURE TO HYPERTONIC SEA-WATER
1. KCN, M/200	Rapid shrinkage and crenation as in the control
2. Chloral hydrate (a) 0.3 per cent	Distinct decrease in the rate of shrinkage. After 2 minutes in hypertonic sea-water the eggs remain almost round and are much less shrunken than in the untreated eggs of the control, which are all typically shrunken and collapsed in less than 1 minute
(b) 0.15 per cent	Some retardation of shrinkage but much less than in (a)
3. Chloroform $\frac{1}{8}$ and $\frac{1}{10}$ saturated°	Here there was no evident effect
4. Methyl alcohol 8 vol. per cent	Crenation and shrinkage are decidedly retarded; most eggs remain round and relatively slightly shrunken after 2 minutes in the hypertonic sea-water
5. Ethyl alcohol 5 vol. per cent	Also distinct retardation of shrinkage; after 1 minute crenation is slight and many eggs are still round
6. Propyl alcohol 2 vol. per cent	Shrinkage and crenation are retarded, but not markedly; after 1 minute the eggs are slightly or moderately crenated
7. Isobutyl alcohol (a) 1.4 vol. per cent	Shrinkage and crenation are distinctly retarded; after 1 minute many eggs remain round and only slightly shrunken; others are more shrunken and slightly or moderately crenated
(b and c) 1 and 1.2 vol. per cent	Also some evident retardation but less than in (a).
8. i-Amyl alcohol (a) 0.6 vol. per cent	Shrinkage and crenation are somewhat retarded but not markedly
(b) 0.5 vol. per cent	No evident retardation in this solution
9. Ethyl urethane 2 per cent	Rate of shrinkage is greatly decreased; after 1 minute in the hypertonic sea-water all eggs remain round and uncrenated
10. Ethyl ether from 1.2 to 0.5 vol. per cent	Some slight retardation was observed in the 1.2 per cent solution; in the weaker solutions (1.0, 0.8, 0.75, 0.5 vol. per cent) there was no evident effect

later, after shrinkage is completed, exhibit only slight crenation. In fertilized eggs thus completely anaesthetized the plasma-membrane resembles that of unfertilized eggs in its general properties although the degree of impermeability is not so great. Chloral hydrate and the alcohols also cause well-marked decrease of permeability to water, but

with ether and chloroform the effect was slight or lacking. In all cases the reversibility of the change induced by the anaesthetic was proved by subjecting the eggs to a second osmotic test about twenty minutes after returning to sea-water; the tested eggs then showed the typical rapid collapse and crenation, and eggs left undisturbed after the return to sea-water continued their development to larval stages.

It is clear that the anaesthetic modifies the permeability of the membrane to water as well as its general stability or resistance to alteration.[32] Just how this effect is produced remains for the present problematical. The anaesthetic may promote the continuity of the non-aqueous or lipoid phase of the protoplasmic emulsion at the boundary-surface of the cell, possibly in the manner suggested by Clowes;[33] or it is possible that by dissolving in this phase it may increase the relative volume occupied by the colloidal particles of lipoid and hence decrease the relative volume of the aqueous phase of the emulsion, thus making the latter a more effective barrier to the passage of water (and presumably to water-soluble substances also). In all probability the total effect depends upon a combination of several distinct actions, in which metabolic factors also enter. The plasma-membrane is undoubtedly the seat of an active metabolism of an oxidative type, and substances produced, altered or destroyed in this metabolism must influence its physical and other properties.

SUMMARY

1. Fertilized eggs of Arbacia and Echinarachnius shrink rapidly and undergo crenation in hypertonic sea-water or van't Hoff's solution (of 30 to 40 atmospheres O.P.); unfertilized eggs shrink slowly in the same solutions and remain round. The relative rates of swelling in dilute sea-water are similar. Fertilization thus results in a marked increase in the permeability of the plasma-membrane (the semi-permeable surface-layer of protoplasm) to water.

2. Artificial formation of fertilization-membranes by butyric acid causes similar though more variable effects in Arbacia eggs. Eggs with

[32] It has been mentioned that anaesthetized uncleaved eggs show greater resistance than normal eggs to the permeability-increasing action of pure isotonic salt-solutions; this fact explains why the activating effect of such solutions is prevented by anaesthetics, since activation involves increase of permeability; similarly with the inhibiting action of anaesthetics on cleavage which also is associated with a change in the plasma-membrane.

[33] Clowes: Journ. Phys. Chem., 1916, xx, 407.

well-separated membrane exhibit a well-marked increase of permeability like that of sperm-fertilized eggs; when membrane-formation is imperfect or lacking the increase of permeability is less marked or may not be evident.

3. The change of permeability is a gradual process, beginning between two and four minutes after insemination and reaching an approximate final stage in about twenty minutes (at 20 to 22°).

4. The change of permeability is arrested or retarded reversibly by potassium cyanide in concentrations of M/100 to M/400; concentrations of M/800 and lower are ineffective. This change is much more resistant to cyanide than cleavage.

5. Anaesthetics (chloral hydrate, alcohols, urethane, ether) also prevent the increase of permeability, in concentrations which are similar to but in some cases higher than the concentrations arresting cleavage in the same eggs. The effect is readily reversible. A direct influence of anaesthetics upon the permeability to water is thus demonstrable. Eggs which have undergone the normal increase of permeability following fertilization also show a reversible decrease of permeability to water in solutions of certain anaesthetics (chloral hydrate, alcohols, urethane).

AN EXPERIMENTAL STUDY OF ALTERNATING GROWTH AND SUPPRESSION OF GROWTH IN THE ALBINO MOUSE, WITH SPECIAL REFERENCE TO THE ECONOMY OF FOOD CONSUMPTION[1]

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Received for publication January 18, 1917

INTRODUCTION

Although much is known regarding the conditions under which growth may be retarded or promoted, the food consumption in relation to growth has not been studied extensively. For this reason it was thought that an investigation of the economy of food in alternating periods of growth and suppression of growth in an animal for which the diet could be controlled with accuracy would prove to be of value. The white mouse was chosen for study in the present research. It was planned to make the comparative food intake during normal, suppressed and accelerated growth the basis for a consideration of the economy of food in growth as a contribution to some of the problems of animal production. Statistics are herewith offered for the average daily food requirement, together with the curves of normal growth for both male and female mice from the age of weaning, 22 days, to the age at which average adult weight is reached, 62 days. The food consumption per day of normally growing mice has been estimated for varying body weights of from 7 to 25 grams. The daily food requirement for maintaining body weight both in an initial suppression and in repeated suppressions of growth has been compared with that for normal growth. The total food required to complete growth during a period of refeeding after suppression of growth has been compared with the food consumption of controls making the same growth from

[1] This study was aided by a grant from the Elizabeth Thompson Science Fund. The data are taken from the dissertation presented by Helen B. Thompson for the degree of Ph.D., Yale University, 1917.

the same initial weight. The total food consumed during the period of suppression of growth and the following period of refeeding has also been compared with the total consumption of the control animals for an equal number of days at corresponding ages.

It was hoped by the study indicated above to test the following points: the relation of the food intake to normal growth at different ages and at varying body weights; the food requirement per day in suppressed growth for varying periods and for successive suppressions; the economy of food in accelerated growth; the cost, in total food, of maintenance and growth when growth is completed after one or more suppressions.

PLAN OF INVESTIGATION

The mice were placed, when 20 to 22 days of age, in individual cages made of galvanized wire cloth. Absorbent paper covered with a mat of galvanized fly screen wire was used to line the bottom of the cage. The entire cage was cleaned at least once a week. Every day, while each mouse was being weighed before feeding, its cage was taken apart and the uneaten food collected, the feces removed and clean paper supplied if needed. Food and water cups were sterilized by boiling twice a week. Water cups were washed every day.

As a fairly high uniform temperature has been shown to be important in maintaining underfed animals in good health, the room temperature was kept day and night between 70° and 85°F.

The food was made up from the formula employed by Wheeler ('13) and later by Judson ('16).[2] This ration was selected because it had been demonstrated by Osborne and Mendel in feeding rats that a paste is desirable from the standpoint of economy in handling. It was essential to have a food that could be easily weighed out and from which refuse and scattered remnants could be accurately collected.

The food was made up as follows:

	grams
Skimmed milk powder	20
Casein	24
Starch	20

[2] Dissertation presented by S. E. Judson for the degree of Ph.D., Yale University, 1916. See Mendel and Judson: Proceedings of the National Academy of Sciences, 1916, ii, 692.

Salt mixture[3]	4
Butter fat	32

By analysis of a mixed sample of food prepared at several different times the composition was found to be:

	per cent
Protein (N x 6.25)	31.0
Fat (ether extract)	29.9
Carbohydrate	30.1
Ash	4.5
Water	4.5

Food was mixed fresh two or three times a week in quantities of 300 to 400 grams. It kept well without apparent fermentation or mold formation. Daily rations were weighed in grams and tenth grams; residues in milligrams.

In the early experiments it was noticed that a number of the mice ate irregularly and grew slowly. Those that did eat regularly grew at the rate described by Judson. As abnormally slow growth may indicate a low plane of nutrition brought about through failure of appetite as well as a deficiency in any food constituent, a source of vitamine in the form of 2 per cent of yeast was added to the diet without otherwise modifying the food mixture. This change was made for both the control mice and those maintained at constant weight. All animals ate much more regularly after the inclusion of yeast in the diet.

The yeast was "Torula" from the Hinckel Brewery Company, Albany, N. Y., which states that "Torula" contains on an average:

	per cent
Crude protein	46.6
Fat	0.5
Carbohydrates	32.3
Fiber	5.8
Ash	6.6
Water	8.2

With 2 grams of yeast added to 100 grams of food the estimated composition of the mixture then became:

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[3] Röhmann's salt mixture.

	grams
$Ca_3(PO_4)_2$	10
K_2HPO_4	37
NaCl	20
Na-citrate	15
Mg-citrate	8
Fe-citrate	2
Ca-citrate	8

(From Osborne and Mendel: Carnegie Inst. of Washington, 1911, Publ. 156, I, 32.)

	per cent
Protein	31.3
Fat	29.3
Carbohydrate	30.2
Ash	4.5
Water	4.6

The energy value of the food calculated from the usual factors is approximately 5.1 Calories per gram.

Mice at the age of 22 days range from 8 to 12 grams in body weight. Litters and groups for the same experiment were selected to start at approximately the same age with no greater variation in size than would be encountered in a single litter. In most cases the experiment was closed for the group when, after refeeding, a majority of the males had grown to the weight of 20 to 21 grams and the females to 18 to 19 grams, corresponding to the weights of the control animals at 45 to 50 days of age. The control animals were kept for comparison even after their weights were fairly constant. Data from all suppression tests have been included in the averages for maintenance, as stunted mice that did not respond to refeeding seemed able to maintain their weight on about the same amount of food as the other mice. Averages for renewed growth after suppression were computed for groups showing similar rates of acceleration. Slowly growing mice were included in the control curves, as in this case it was desirable to report averages from large numbers of unlike rates of growth.

NORMAL GROWTH AND DAILY FOOD CONSUMPTION OF ALBINO MICE FROM THE AGES OF 22 TO 62 DAYS

White mice reach sexual maturity and adult proportions in form at the age of about two months. The period following this age is one of very slow growth requiring, according to Judson, at least 40 days to make a gain of only 2.5 grams in weight. For this reason it was decided to include in these studies the normal growth curve to the 62d day only. The number of animals kept beyond the 54th day is rather small, but those that were allowed to live maintained average weight for 80 days or longer.

The body weights for all males included in the averages from which the curve was drawn are recorded in table 1, those for females in table 2. Mice 66, 87 and 102 made very unusual growths, but any increment which their weights might give to the averages are fully offset by the slowly growing mice 4, 7 and 36. The resulting curve is

TABLE 1

Body weight at age indicated in days. Males

MOUSE NUMBER	AGE IN DAYS										
	22	26	29	32	34	39	43	50	54	62	70
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
4	8.6	10.0	12.9	13.6	14.5	16.5	17.5	18.6	18.1		
7	7.9	10.0	11.5	13.7	14.2	16.0	17.3	21.2	22.7	24.0	24.0
19	13.4	14.0	15.9	17.9	18.5	19.5	20.6				
28	10.0	12.3	15.1	17.2	17.5	19.7	20.6	21.3	21.0	20.9	
36	7.0	9.7	9.9	9.9	11.0	15.3	16.9	19.2			
49	11.0	13.5	15.0	16.9	17.5	19.6	20.4				
57	11.7	13.7	15.5	16.2	17.6	18.0	17.3	18.0			
66	9.0	13.5	16.0	18.5	20.2						
73	9.1	12.6	15.5	17.9	19.0	19.0	19.9	20.5			
80	10.2	14.0	16.6	17.9	18.4	19.5	20.3				
87	11.2	15.1	18.9	20.7	23.2	25.7	26.4	27.1	27.2		
102	10.0	10.8	14.8	17.9	20.3	24.5	26.4	27.0			
113	10.0	12.5	13.6	15.9	17.9	21.0	22.3	21.4			
116	9.3	11.5	13.4	15.6	17.0	18.7	20.0	21.0	21.7		
117	10.6	12.9	15.3	17.0	17.3	19.5	20.5	21.4			
Average..	9.9	12.4	14.7	16.4	17.6	19.5	20.5	21.5	22.1	22.4	24.0

TABLE 2

Body weight at age indicated in days. Females

MOUSE NUMBER	AGE IN DAYS										
	22	26	29	32	34	39	43	50	54	62	70
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
5	8.4	10.0	11.5	13.0	14.0	15.2	15.6	16.0	16.5		20.2
12	8.5	11.0	13.0	14.2	15.0	16.0	17.0	17.1	17.7 (52 days)	18.8	
22	10.0	15.0	16.1	17.3	17.1	18.4	19.2	19.8	20.1		
43	8.0	10.5	12.1	14.7	16.1	18.5	19.5	20.3			
47	10.0	13.6	15.3	16.1	17.5	18.2	19.0	20.1	20.1		
56	9.6	12.2	13.6	14.4	15.1	17.5	17.9	18.7	18.9		
58	10.0	13.6	14.6	15.0	16.0	16.7	17.1	17.0	18.2	19.7	
60	8.0	10.0	11.6	12.9	13.2	14.7	16.0	16.3	16.8	17.8	
69	10.3	13.0	15.0	17.5	18.7	19.7	20.0				
94	10.7	13.3	14.9	17.0	17.9	18.2	19.0	19.7	19.9		
111	9.5	11.5	12.9	14.0	15.1	16.0	17.6				
Average...	9.4	12.2	13.7	15.1	16.0	17.2	18.0	18.3	18.5	18.8	20 3

about what it would be if both extremes of weight were excluded. The weights of the females did not show such wide variations but the average curve for this group departs more from Judson's curve than does that of the group of males.

The body weights for the normal curves which Judson discussed are given in table 3. The average weights for the fifteen males and eleven females grown as controls in the present experiment are given for corresponding days and for convenient dates between in order to describe curves that show their degree of regularity. The differences in the curves are apparent in chart I.

TABLE 3

AGE OF MOUSE	BODY WEIGHTS			
	Males		Females	
	Judson	Thompson	Judson	Thompson
days	*grams*	*grams*	*grams*	*grams*
Newborn	1.5	1.5	1.5	1.5
5	3.0	3.3	3.0	3.3
12	6.0	6.7	6.0	6.7
22	9.0	9.9	8.2	9.4
26	12.0	12.4	10.0	12.2
29		14.7		13.7
32	15.0	16.4	12.8	15.1
34		17.6		16.0
39	18.0	19.5	15.0	17.2
43		20.5		18.0
50	21.0	21.5	17.0	18.3
54		22.1		18.5
62		22.4		18.8
80	24.0	24.0	20.6	20.2
100	25.0		21.5	

Up to the 26th day the actual gain per day is the same for males and females. After this date the females gain on an average of 0.5 gram per day until the 34th day, when they drop to a lower rate. The males continue to add an increment of from 0.5 to 0.8 gram per day to their weight until about the 40th day of life. The rate of increase in body weight over the preceding day, for females, declines from 3 per cent on the 32d day to 2 per cent on the 40th day and to 0.3 per cent on the 62d day. The males grow at the rate of 4 per cent on the 32d day, 2 per cent on the 40th day, and 0.5 per cent on the 62d day.

Food consumption in relation to body weight. Males weighing from 9

to 13 grams, at 22 to 26 days of age, eat more *per gram body weight* than do the females, but there is the same rate of increase in body weight for both. After the 26th day the males continue to grow at the same rate until about the 40th day. The females change to a slower rate. This change occurs at the time that a slight reduction in the food consumption per gram body weight takes place. From the 26th day on the males have a larger daily food consumption but the food per gram body weight

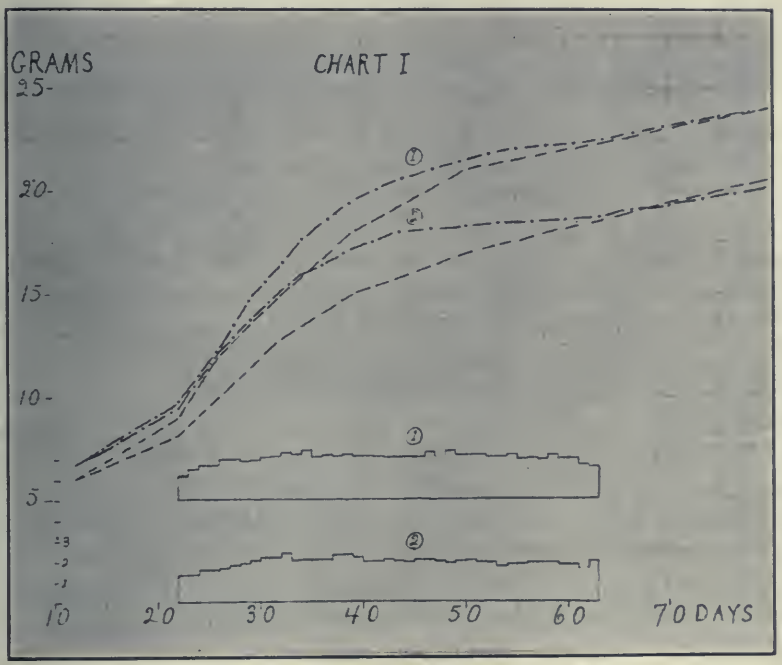

Chart I. Curve of normal growth and average daily food consumption. (1) Males (15 animals); (2) females (11 animals). - - - - - Judson, -.-.-.- Thompson.

averages the same for both sexes until about the 40th day. After the 40th day the consumption per gram body weight of the males is from 0.1 to 0.2 gram in excess of that of the females. This slight excess furnishes the material for continuing growth, at the rate indicated by the curve, until about the 60th day. From the 60th day of life the curve for the males flattens and growth proceeds at the slower rate assumed by the females as early as the 40th day.

Table 4, giving maximum, minimum and average food consumption per day of normally growing mice from the ages of 22 to 62 days, and table 5, showing the food consumption per day at varying body weights, follow.

FOOD REQUIREMENT IN VARYING PERIODS OF SUPPRESSION OF GROWTH

The methods for suppressing growth applied by Röhmann ('08), Waters ('08), Aron ('10), Osborne and Mendel ('11–'17), Wheeler ('13), Jackson ('15), Judson ('16) and Stewart ('16) include the use of qualitatively inadequate proteins, exclusion of vitamines and limitation of the protein or salts, as well as quantitative restrictions of the daily intake of an otherwise suitable ration. Osborne and Mendel have found that the growth of rats may be arrested by the withdrawal of lysine from the diet and by the use of proteins containing less than the minimum requirement of cystine. The possibility of controlling growth by the exclusion of the so-called vitamine has been demonstrated by the investigations of Hopkins ('12), Funk ('12), McCollum and Davis ('13–'15), Osborne and Mendel ('13) and their coworkers. The protein requirement from a quantitative standpoint has been studied by McCollum and Davis ('15) and by Osborne and Mendel ('15) in experiments upon rats. The value of different proteins in the growth of mice has been investigated by Röhmann ('08) and by Wheeler ('13). From these studies it has been possible to formulate dietaries containing protein limited to the amount which will provide for maintenance but suspend growth for an indefinite period. It is recognized at present that normal growth is not only dependent upon a wide distribution of inorganic salts but that in animal nutrition as well as in plant the "law of minimum" applies. McCollum and Davis ('12–'15) and Osborne and Mendel ('13) have shown that the growth of rats may be checked by a limitation of the salts. Judson ('16) used this method successfully in suppressing the growth of mice.

Statistics of daily food consumption for mice held at constant body weights for 5, 9, 18 and 27 days respectively. The tests of food consumption in suppressed growth began with mice at 20 to 23 days of age. One litter of eleven was taken from the mother at the end of two weeks and fed on milk and the experimental food until 30 days old. As they had at that time barely reached the average size of the 22-day-old mice, they were started upon suppression tests. Their subsequent growth proved that they were developing at the normal rate for their size rather than

TABLE 4

Food consumption per day of normally growing mice at successive ages. Age 22–62 days

AGE	MALES				FEMALES			
	Number of animals	Food eaten			Number of animals	Food eaten		
		Maximu	Minimum	Average		Maximum	Minimum	Average
days		*grams*	*grams*	*grams*		*grams*	*grams*	*grams*
22	9	1.5	0.6	1.2	3	1.4	1.1	1.3
23	12	2.2	1.0	1.5	9	2.2	0.9	1.3
24	14	2.5	1.0	1.7	11	2.3	0.8	1.6
25	14	2.4	1.0	1.7	11	2.6	0.9	1.6
26	14	3.1	1.1	2.0	11	2.2	0.8	1.7
27	14	2.8	1.3	2.0	11	2.5	0.8	1.8
28	14	2.6	0.8	1.9	11	2.4	1.1	1.9
29	14	2.5	1.0	1.9	11	2.7	1.5	2.1
30	15	3.0	1.3	2.1	11	2.5	1.8	2.2
31	15	3.0	1.4	2.1	11	2.9	1.9	2.2
32	15	3.2	1.2	2.3	11	3.1	1.9	2.4
33	15	3.5	1.5	2.2	11	3.1	1.5	2.1
34	14	3.3	1.6	2.4	11	2.8	1.4	2.1
35	14	2.6	1.6	2.1	11	2.9	1.0	2.1
36	14	3.1	1.6	2.3	11	2.5	1.6	2.1
37	14	3.1	1.2	2.2	11	2.7	1.7	2.3
38	14	3.1	2.0	2.3	11	2.6	1.4	2.3
39	14	2.9	1.3	2.2	11	3.1	1.6	2.2
40	14	3.2	1.7	2.2	11	2.4	1.1	2.0
41	14	3.0	0.9	2.2	11	2.3	1.6	2.0
42	13	2.6	1.6	2.1	11	2.5	1.6	2.1
43	12	2.7	1.6	2.1	10	2.6	1.4	2.0
44	11	2.4	1.3	2.0	10	2.7	1.4	1.9
45	11	2.6	1.3	2.1	10	3.0	1.5	2.1
46	11	2.8	2.0	2.3	10	3.0	1.5	2.1
47	11	2.8	1.7	2.1	10	2.8	1.4	2.0
48	10	3.1	1.6	2.4	10	2.1	0.8	1.8
49	10	2.7	1.7	2.2	10	2.6	1.5	1.9
50	10	2.6	1.5	2.2	10	2.7	1.5	2.0
51	7	2.7	1.5	2.2	9	2.3	1.1	1.9
52	6	2.7	1.4	2.1	8	2.3	1.5	1.9
53	6	2.6	1.1	2.1	7	2.2	1.1	1.7
54	4	2.5	1.7	2.2	6	2.2	1.3	1.8
55	4	2.2	1.6	1.9	6	2.2	1.3	1.8
56	4	2.6	1.2	2.0	5	2.3	1.7	1.9
57	3	2.3	1.3	1.9	4	2.1	1.8	1.9
58	3	2.4	2.0	2.2	4	2.4	1.5	1.9
59	3	2.3	1.4	2.0	4	2.3	1.4	1.8
60	3	2.1	1.8	2.0	4	2.3	1.6	1.8
61	3	2.3	1.3	1.7	2	2.2	1.1	1.6
62	3	1.9	1.3	1.6	2	2.2	1.8	2.0

for their age. Their weights were, therefore, included in the averages of those for the 9-day suppression period. The averages of all body weights and the average food consumption have been used in plotting the maintenance curves and food eaten per day for each sex in the respective groups. On refeeding it was found that the mice of both sexes fell into one of two groups, namely, those that attained the weight normal for their age before the time selected for the second suppression

TABLE 5

Food consumption per day of normally growing mice at varying body weights. Body weight 7 to 27 grams

BODY WEIGHT	MALES				FEMALES			
	Number of animals	Food eaten			Number of animals	Food eaten		
		Maximum	Minimum	Average		Maximum	Minimum	Average
grams		*grams*	*grams*	*grams*		*grams*	*grams*	*grams*
7–8	1			1.1				
8–9	2			1.2	2			1.0
9–10	6	1.7	1.2	1.5	4	1.6	1.0	1.2
10–11	12	1.8	1.2	1.5	7	1.7	1.0	1.3
11–12	14	2.2	1.3	1.7	8	1.9	1.0	1.5
12–13	14	2.3	1.4	2.0	10	2.3	1.7	2.0
13–14	15	2.3	1.5	2.0	10	2.4	1.8	2.1
14–15	15	2.4	1.6	2.1	11	2.8	1.9	2.2
15–16	15	2.9	1.9	2.3	11	2.6	2.0	2.2
16–17	15	3.0	1.4	2.3	11	2.7	1.8	2.2
17–18	15	3.2	1.9	2.4	11	2.8	1.6	2.2
18–19	14	3.2	1.9	2.4	10	2.7	1.8	2.2
19–20	14	3.0	1.9	2.5	6	2.7	1.9	2.2
20–21	10	3.1	2.0	2.5	2	2.2	2.1	2.2
21–24	4	Aver. for 10 days.....		2.8				
25–27	2	Aver. for 30 days.....		2.4				

period and those that did not. These groups are marked *A* and *B* on the charts. A comparison of the body weights and average daily food consumption of the different groups for the varying periods of suppression of growth is shown in table 6. (In the data submitted in tables 6 and 8, 5.1 Calories per gram have been used in estimating the energy value of the food.) The curves of growth may be seen on charts II to V.

TABLE 6

Body weights and average daily food consumption during varying periods of suppression of growth

NUMBER OF MICE	SUPPRESSION		PERIOD OF SUPPRESSION						FOOD CONSUMPTION				
			Initial body weight			Final body weight			Average daily food intake			Calories estimated	
	Number	Days	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Average daily	Per gram body weight
			grams	*grams*	*grams*	*grams*	*grams*	*grams*	*gram*	*gram*	*gram*		
Males													
17	1st	5	13.4	7.0	9.2	13.4	7.9	9.5	1.2	1.0	1.1	5.6	0.6
11	1st	9	15.5	8.2	10.5	14.0	8.1	10.7	1.5	1.1	1.3	6.6	0.6
8	1st	18	11.8	8.4	10.7	11.8	10.2	11.2	1.4	0.7	1.3	6.6	0.6
6	1st	27	13.4	9.7	11.2	13.5	10.0	11.8	1.5	0.8	1.3	6.6	0.6
Females													
17	1st	5	12.7	6.8	10.2	12.5	8.3	10.1	1.2	1.0	1.1	5.6	0.6
16	1st	9	14.3	6.9	8.8	13.9	8.2	9.8	1.3	1.1	1.2	6.1	0.6
8	1st	18	11.0	8.5	9.8	11.6	9.4	10.3	1.3	0.8	1.2	6.1	0.6
7	1st	27	13.1	9.6	11.0	13.4	10.7	11.7	1.5	0.8	1.2	6.1	0.5
Males													
9	2d	5	18.5	13.7	15.7	18.2	13.7	15.2	1.5	1.4	1.5	7.6	0.5
3	2d	9	17.2	10.3	12.7	16.4	10.3	12.5	1.7	1.2	1.3	6.6	0.5
Females													
15	2d	5	16.1	12.4	13.8	15.8	12.0	13.9	1.5	1.4	1.4	7.1	0.5
8	2d	9	17.1	14.4	15.2	16.2	13.3	14.8	1.5	1.3	1.3	6.6	0.4
Males													
3	3d	5	18.2	13.0	15.3	18.1	13.3	15.1	1.5	1.2	1.4	7.1	0.5
2	3d	9	14.7	11.8	13.2	14.6	12.1	13.3	1.6	1.2	1.3	6.6	0.5
Females													
8	3d	5	17.9	15.7	16.5	17.0	14.1	15.7	1.6	1.6	1.4	7.6	0.5

Reduction in the food requirement as a result of continued underfeeding. A lowered food requirement as the result of continued underfeeding has been reported for cattle by Waters ('11) and by Van Ewing and Wells ('15), and for rats by Jackson ('15). In the present experiment

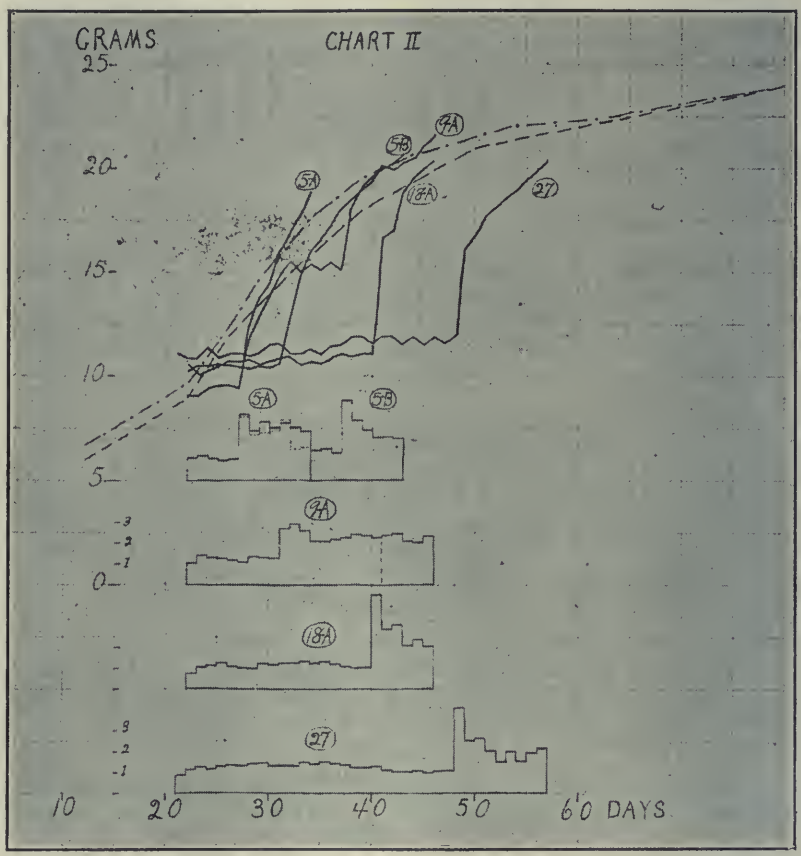

Chart II. Curves showing rapid acceleration of growth after suppression of growth for 5, 9, 18 and 27 days. Males. Normal growth - - - - Judson, -.-.- Thompson, Accelerated growth ———.

there was *in all cases of maintenance a gradual decrease in the amount of food per unit of body weight required to maintain the same weight.* In a few instances, in which the food was unchanged in amount, there is a slight rise in the curve of body weights. In the 18- and 27-day suppressions—extensive periods for such rapidly growing animals

as mice—a slight upward curve was allowed because Jackson ('15) and Stewart ('16) had found it difficult to hold rats to constant weight and keep them alive for a long period.

Changes in the food requirement in repeated periods of underfeeding. In the second and third suppressions the food consumption remained

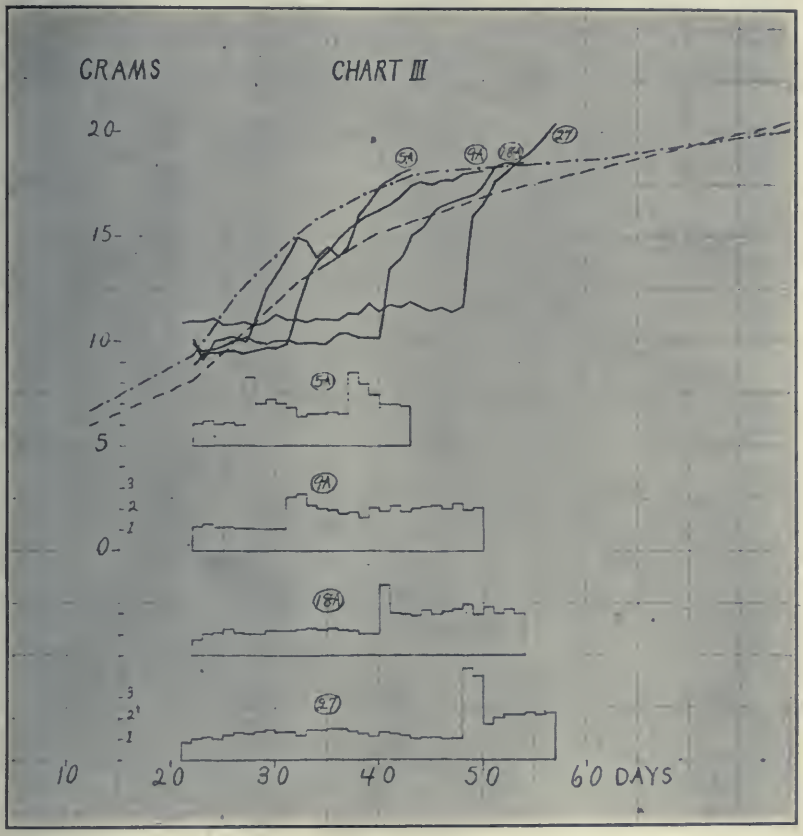

Chart III. Curves showing rapid acceleration of growth after suppression of growth for 5, 9, 18 and 27 days. Females. Normal growth; - - - - Judson, -.-.- Thompson, Accelerated growth ———.

about uniform during the period. There was an increase in actual amount eaten as compared with the first period; but it must be remembered that the mice were at a higher plane of body weight. When food is considered per gram body weight the decrease in food require-

ment in a second and third period is evident (see table 7). These results are in accord with those reported by other investigators. Howe and Hawk ('11) found, after starving the same dog twice for more than 100 days each time, that the total output of nitrogen was 7.1 per cent

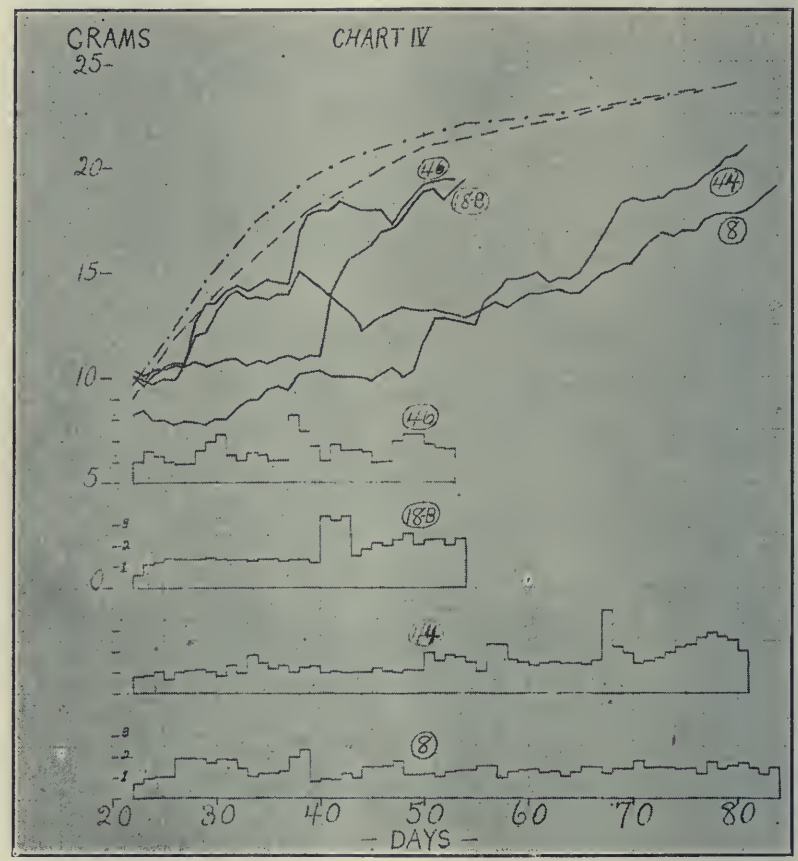

Chart IV. Curves showing moderately accelerated growth after suppression of growth. Males (46), (18-B). Repeated suppressions (44), (46). Slow growth on a small daily food intake (8). Normal growth; - - - - Judson, -.-.- Thompson. Growth after suppression ———.

less in the second fast than in the first. The loss of weight was 10.3 per cent less in the second fast and 21.7 per cent less in the second half of the second fast than in the corresponding periods of the first fast.

Stewart ('16) reported the loss of weight for two rats as slightly less in a second fast than in an initial period of about the same duration.

Comparison of food requirement in suppressed growth with that for normal growth in mice of the same weight. For normal, growing mice the average daily food intake at a size represented by 9 to 10 grams body weight is 1.5 gram for males and 1.2 gram for females. During

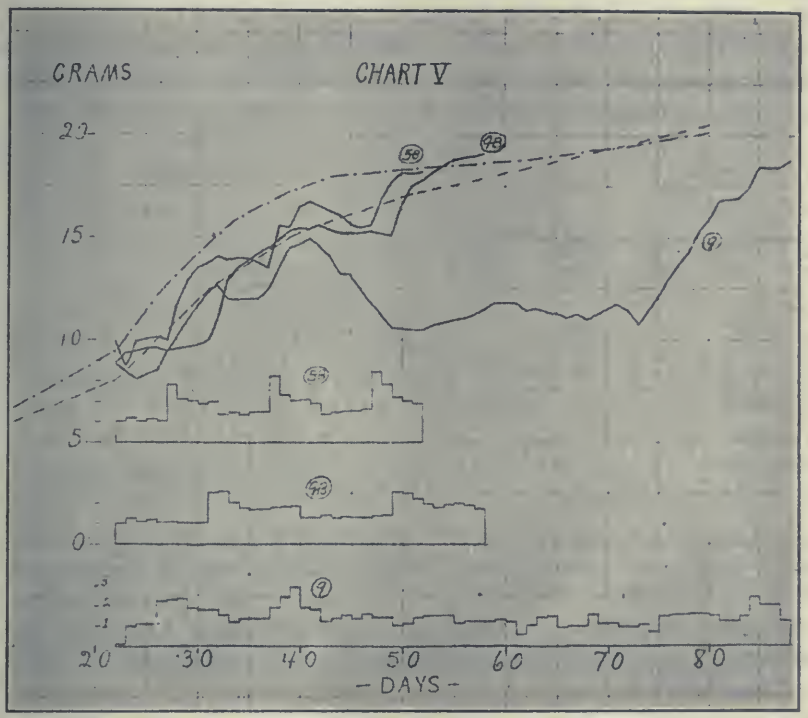

Chart V. Curves showing moderately accelerated growth after suppression of growth. Females. Repeated suppressions (5-B), (9-B). Growth after continued failure to grow (9). Normal Growth - - - - Judson, -.-.-.- Thompson. Growth after suppression ———.

maintenance without growth 1.1 to 1.2 gram is eaten. Growing males weighing 10 to 11 grams need 1.5 gram of food, of the sort here employed, per day for growth; females need 1.3 gram. Mice of the same weight are maintained without growth on 1.2 gram. An 11- to 12-gram male mouse grows normally on 1.7 gram of food; females need 1.3 gram. Thirteen-gram mice require an average food intake

of 2 grams per day for growth, whereas, for maintenance alone, 1.3 gram is sufficient. At 15 grams body weight growing mice eat 2.2 grams per day, while retarded one can maintain constant weight on 1.3 gram. From these differences it may be estimated that about 14 per cent of the food is used for growth at 9 to 11 grams body weight. After the 11-gram stage either the proportion of the food required for growth increases markedly, averaging 19 per cent for mice at 12 grams body weight, 35 per cent for those at 13 grams and 40 per cent at a weight of 15 grams; or the power to maintain the weight under adverse conditions increases considerably as the animal develops. In other words, in mice, large but still capable of gain in body weight, a relatively

TABLE 7

MALES				FEMALES				
Number of suppression	Average body weight	Average daily food intake	Food per gram body weight	Number of suppression	Average body weight	Average daily food intake	Food per gram body weight	
	grams	*gram*	*gram*		*grams*	*gram*	*gram*	
1st	9.4	1.1	0.12	1st	10.1	1.1	0.11	5-day periods
2d	15.0	1.5	0.10	2d	13.9	1.4	0.10	
				3d	15.6	1.0	0.07	
1st	10.6	1.3	0.12	1st	9.8	1.2	0.12	9-day periods
2d	12.6	1.3	0.10	2d	15.4	1.3	0.08	
1st	10.2	1.3	0.12	1st	9.9	1.2	0.12	18-day period
1st	11.8	1.3	0.11	1st	11.7	1.2	0.10	27-day period

much greater proportion of the food intake is required to produce an increment in weight, if one may judge by the comparatively small quota necessary to insure satisfactory maintenance. No account is taken in such generalization, however, of the content of water in the new tissues at the different stages of growth. There is a suggestion also in the relative number of mice responding promptly to refeeding to indicate that the smaller animals may be somewhat less capable of recovering from suppression of growth than are the larger ones.

Some physiological effects of underfeeding. In addition to lowering the food requirement for maintenance and bringing about a better endurance of a second period of restricted diet, underfeeding has been reported as causing changes in general appearance, in body proportions, in rate of growth of various organs, in sex development, in activity and behavior and in intelligence.

Weiske ('75) and Van Ewing and Wells ('14) found increased thickness of hair in cattle. Osborne and Mendel ('11), Wheeler ('13) and Judson ('16) all report changes in the hair coat of rats and mice. Increase in height in proportion to width has been observed in calves by Waters ('00) and by Falke ('10); changes in the relation of height to length were reported for dogs by Aron ('10); increased ratio of tail to body length in rats by Jackson ('15), Stewart ('16) and in mice by Judson ('16). Other features are reported by Eckles, by Jackson and Lowrey ('12), by Jackson ('13–'15), by Aron ('14) and by Stewart ('16).

With respect to bodily activity, the extreme restlessness of underfed rats and mice has been noted by Osborne and Mendel ('11), by Wheeler ('13) and by Judson ('16). Waters and Van Ewing and Wells have observed restlessness in underfed calves. These investigators also reported viciousness and retarded intelligence. In the present experiment restlessness was the most noticeable characteristic which distinguished an underfed mouse from a well nourished one.

During retarded growth the mice were thin-bodied when stretched out, but sat, when quiet, in a hunched position. The body proportions showed the changes mentioned by Judson ('16). The nose was sharp, face narrow, head large and tail length in proportion to the body increased. The fur became rough and matted in the subjects of the earlier experiments. After yeast was added to the food there were few cases of moist looking fur but more often a thicker, drier growth of hair than usual. It was noticed that control mice making very rapid gains had thick fluffed-out fur. Mouse 87 (male) which grew from an initial weight of 10.5 grams at 21 days of age to 20.6 grams at 31 days showed this unusual growth of fur. Mice which grew at about the average rate exhibited the smooth, glossy fur characteristic of normal adults.

The curvatures in the spinal column, due to the fact that underfeeding arrests the growth of the muscles and skin but not that of the skeleton, were easily felt with the fingers. Pronounced single curvatures showed in the pictures of several mice, and a double curvature in that of mouse 95. Mouse 95 (female) increased in weight from 10.9 grams on the 27th day of suppressed growth to 19.5 grams in 9 days of full feeding. At the time the second picture was taken, the curvature had almost disappeared.

To what degree, if any, the effects of underfeeding become permanent is still a matter for investigation. Disproportionate forms have been found in children by Fleischner ('06), but are reported as not occurring

in rats by Osborne and Mendel ('11). According to Waters ('08) height in calves is not altered by underfeeding unless complete retardation is continued for more than 2 or 3 months. After resumption of growth calves approach normal height more nearly than normal width. Aron ('11) thought dogs were permanently stunted in size by underfeeding, the injury being greatest in the youngest animals. He found, however, that rats were not permanently stunted in size when underfed for 150 days though he regarded the development as incomplete. Osborne and Mendel ('11) have found that rats are not necessarily undersized in later growth, even after being stunted for very long periods. They have found also that "suppression at any size does not alter the capacity to grow." By stunting rats in the nursing period Brüning ('14) prevented recovery of normal size for at least 54 days. Stewart ('16) found that in rats there is usually recovery of body weight.

Osborne and Mendel ('15) reported that the "procreative functions are not necessarily impaired by stunting before breeding is ordinarily possible." Two of their rats resumed growth at about 250 days and bore young at 310 days. "There was no damage to the maternal function." No observations were made in the present experiment upon sex development as affected by nutrition. One male and one female after enduring repeated suppressions of growth were used for breeding. The young from these animals seemed normal in every way and showed the usual marked acceleration after a preceding period of suppression of growth.

RESUMPTION OF GROWTH ON ADEQUATE DIETS AFTER RETARDATION OF GROWTH

Characteristics of renewal of growth. The most noticeable characteristics of renewal of growth are the sudden decrease in physical activity; the rapid change in the appearance of the hair coat—the fur becoming smooth and glossy in a few days; the change to normal ratio of tail to body length; and the rapid development of integument and musculature which gives the shape and proportions normal for the body weight. While a few cases of permanent stunting have been observed, the rate of growth, instead of being decreased, proves to be accelerated after suppression. This has been observed in the cat by Schapiro ('05), in the rat by Hatai ('07), in the salamander by Springer ('09) and by Morgulis ('11), in the child by Schloss ('11), by Boas ('12) and by Hess ('15), in the rat by Ferry ('13) and by Osborne and Mendel ('15 and

'16), and by others. That the rate of growth does not decrease with the age of the animal has been shown by the resumption of growth in rats fed by Osborne and Mendel at "more than twice the age at which adequate size is ordinarily reached, the growth after suppression being comparable in most cases to that of a growing rat of the same size and sex." Seland had observed as early as 1888 that rabbits and chickens enduring alternate short periods of fasting and liberal feeding grew to a weight heavier than the controls. Noè ('00) found little or no overcompensation in body weights in rats refed after repeated periods of starvation. In the salamander, in which there is probably a large absorption of water, it was found by Morgulis ('11) that the increase in body weight after refeeding may be even greater than the weight of the ingested food. Brüning ('14) failed to cause "compensatory overgrowth in rats placed on an artificial diet after being subjected, during the suckling age, to repeated periods of fasting." In investigations by Waters the food consumption and rate of gain in cattle refed after being kept on maintenance from 6 to 12 months, were about twice that of the control animals. Stewart ('16) reported that after short periods of maintenance (40 to 42 days) rats were able to overtake but not to exceed the weight of the controls.

In the present experiment most of the curves of growth resulting from full feeding after suppression of growth show noticeable acceleration. Practically all of the animals exceeded the normal curves given by Judson and most of them reached or exceeded in weight the controls grown in this laboratory. This was not true of the males suppressed 27 days although they made an average absolute gain equal to that made by the females during the 9 days of refeeding. The mice for the 27-day suppression were selected from three litters and were more alike than any other test mice. They were all 21 days old at the time the experiment began. The females were considerably larger for their sex than were the males. The fact that the females exceeded their controls in growth while the males did not might indicate, as was suggested before, that the larger the animal the less the injury from suppression. It should be mentioned that all of these mice were bred from male 8 which had endured three 5-day suppressions and finally reached a weight of 20 grams after 67 days in an experimental cage. In the 27-day group all individuals grew at rapid rates during the 9 days of refeeding. The extremes in percentage gain were 91 per cent made by mouse 96 (male) and 53 per cent made by mouse 84 (female). The heaviest weight was that of mouse 82 (female), 23.1 grams, the lowest was mouse 89 (female), 18.6 grams.

In all other periods of suppression there were at least two groups with regard to the rate of growth after refeeding. This division formed the basis of selection for the second suppression. The mice gaining most rapidly were continued on a liberal diet while the others were again put upon limited food. After a 5-day suppression the males gained a little more rapidly than did the females. However all but three were suppressed in growth again. All of the females were suppressed a second time even though many of them had overtaken their controls at the close of the first refeeding. Both males and females regained the weight normal for their ages in spite of a second or even a third suppression. In other words, even after repeated suppression of growth the growth impulse provokes a vigorous response to suitable diet, as shown by the decided acceleration of gain in weight. This corresponds with what has been observed in rats. There was a greater variation among the males with regard to the rate of resumed growth than was displayed among the females.

The 9-day group contains practically all of the earlier experiments before yeast was added to the diet. All but two males overtook their controls in 9 days of full feeding; one, 17, did not respond to a second suppression, and another, 44, started as a control and, after it had limited its own growth by inadequate eating for 18 days, it was subjected to two rather severe restrictions of diet with alternate periods of refeeding. This treatment seemed to bring about growth as well as regular habits of eating. This mouse did not, however, develop into a well proportioned adult. The abdomen was large and the body short and thick.

The mice suppressed in growth 18 days all recovered, but at two general rates of growth as in the 5- and 9-day groups. Six males made as high absolute gains in six days as the mice in which growth had been suppressed 27 days did in 9 days. The curve that flattened most was in the groups of females and represents only two individuals.

Lengthening the period of suppression might be expected to result in a more uniform rate of recovery. This is not, however, the only explanation of such a result. The males in the 18-day group and all of the mice in the 27-day groups were more uniform in weight and larger than the majority of the animals in the other tests. The curves of these three groups show the most uniformily rapid growth. There are individual exceptions to this rule, as is the case with two of the males recovering rapidly after 5 days of retardation. These two mice, 74 and 77, weighed 8.2 and 7.9 grams respectively when the test began.

It was difficult to keep them from growing. After refeeding 6 days, their weights showed an increase of 120 per cent and 125 per cent respectively. Only one other experimental mouse made as high a gain per cent and that one, 33, was larger at the beginning of limited feeding.

Statistics of daily food consumption during realimentation after periods of suppressed growth for 5, 9, 18 and 27 days. The groups of male and female mice in the different suppression tests have been arranged in table 8 according to their rate of growth during refeeding. The comparative food intake, as would be expected, runs parallel with the growth. Mice growing at greatly accelerated rate consume an average of from 2.1 to 2.9 grams per day which is more than the average for controls of the same age. Control mice weighing the same as the test mice at the beginning of refeeding consume from 1.5 go 1.7 gram per day. The maximum food eaten by some of the controls during their growth from 12 to 20 grams body weight (26 to 41 days) exceeded the average maximum per day of any animal refed after suppression of growth. The highest food consumption among the test animals was 3.1 grams per day by males in the group suppressed in growth 18 days and by females in the 27-day suppression test. The average growths in these cases were greatly in excess of the average for the controls (charts II, III). The average food consumption was approximately the same as that of the most rapidly growing controls of the same age. The growth was considerably in excess and the food intake somewhat greater than that of any controls of the same size. Mice growing at moderately accelerated rate after suppression consumed approximately the same amount as controls of their age.

A few mice proved to be capable of long continued growth and finally reached adult size on a daily food consumption only slightly above maintenance. Mice 8 and 44 (males) and 9 (female) are reported in table 8 as examples of such growth capacity. Mouse 8 reached a weight of 19.6 grams at the age of 63 days after three 5-day suppressions of growth. It ate, during the entire period of refeeding, an average of but 1.5 gram of food per day. Two grams is the average daily food intake of mice weighing 11 to 12 grams at about 26 days of age. Mouse 44 after two 9-day suppressions increased its body weight from 8.1 grams at 32 days to 20.3 grams at 62 days on an average of 1.6 gram of food per day. This mouse had maintained its weight in the first suppression on 0.9 gram of food per day and in the second period of restricted diet on 1 gram per day showing an unusually low food requirement. After the third 5-day suppression, mouse 9 was continued on a full diet

TABLE 8

Gains in weight and average daily food consumption during periods of refeeding after varying periods of suppression of growth

CHART NUMBER	NUMBER OF MICE	PREVIOUS SUPPRESSION DAYS	REFEEDING DAYS	GAINS IN BODY WEIGHT				FOOD CONSUMPTION				
				Absolute gain			Average gain per cent	Average total food intake	Average daily food intake			Calories estimated average daily intake
				Maximum	Minimum	Average			Maximum	Minimum	Average	
				Accelerated growth. Males								
				grams	*grams*	*grams*		*grams*	*grams*	*grams*	*grams*	
II, 5-A	3	5	7	10.8	7.4	9.5	105	19.2	2.8	2.7	2.7	13.8
II, 18-A	3	18	6	9.5	7.1	8.3	72	17.3	3.1	2.5	2.9	14.8
II, 5-B	6	2 x 5	11	12.9	8.8	10.6	108	27.5	2.9	2.2	2.5	12.8
II, 9-A	7	9	9–10	11.8	6.5	9.1	83	22.7	2.7	2.1	2.4	12.2
II, 27	5	27	9	9.6	6.2	8.3	70	21.0	2.5	2.2	2.3	11.7
				Accelerated growth. Females								
III, 27.....	5	27	9	9.7	7.4	8.5	73	24.8	3.1	2.5	2.8	14.3
III, 5-A...	5	2 x 5	11	9.3	7.3	8.3	81	25.6	2.6	2.1	2.2	11.2
III, 9-A...	6	9	13	9.2	6.3	7.8	82	26.7	2.2	2.0	2.1	10.7
III, 18-A...	6	18	14	8.6	5.8	7.9	74	31.4	2.3	2.2	2.2	11.2
				Moderately accelerated growth. Males								
IV, 18-B...	4	18	11–14	10.4	7.1	9.0	84	31.3	2.5	2.1	2.4	12.2
IV, 46.....	1	3 x 5	15	8.6			80	30.1			2.0	10.2
				Moderately accelerated growth. Females								
V, 5-B...	8	3 x 5	15–16	10.7	5.9	8.6	88	36.8	2.6	2.0	2.4	12.2
V, 9-B...	6	2 x 9	18	11.5	6.2	8.9	87	37.2	2.3	1.8	2.1	10.7
				Slow growth. Males								
IV, 44.....	1	3 x 9	31	7.6			73	51.0			1.6	8.2
IV, 8.....	1	3 x 5	55	9.5			89	82.4			1.5	7.7
				Slow growth. Females								
V, 9.....	1	3 x 5	57	9.2			98	75.7			1.3	6.6

47 days. During this 47-day period it grew to the average weight of females 62 days of age on an average daily food consumption of 1.3 gram.

Comparison of the average total food consumption during realimentation with food eaten by controls in the time required to make the same growth from the same initial weight. The total food required for males to make such absolute gains as are shown in the different periods of refeeding is compared in table 9 with the food eaten by control males growing with-

TABLE 9

Comparison of the average total food consumption of male mice during refeeding after varying periods of suppression of growth with food eaten by controls in the time required to make the same gain from the same initial weight

FOOD AND GROWTH CURVES CHART NUMBER	NUMBER OF MICE	GAIN IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN			GAIN IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIOD IN WHICH THE GAIN ACTUALLY OCCURRED
				Suppression period	Refeeding period	Average total	
		grams		*grams*	*grams*	*grams*	*per cent*
II, 18-A	3	8.3	6	24.8	17.3	42.1	48
II, 27	5	8.3	9	34.2	21.0	55.2	40
(Controls	15	8.3	16			31.2	26)
II, 9-A	6	9.1	9–10	11.8	22.7	34.5	40
IV, 18-B	4	9.0	12	21.6	28.3	49.9	32
(Controls	15	9.0	17			34.9	26)
II, 5-A	3	9.5	7	5.8	19.2	25.0	50
(Controls	15	9.5	16			31.6	29)
II, 5-B	6	10.7	11	12.9	27.5	40.4	39
(Controls	15	10.7	21			40.5	26)

In making these comparisons, no account has been taken of the content of water in the new tissues.

out retardation in the time necessary for them to make the same gain in body weight from corresponding initial weights. The food consumption of the controls has been estimated from table 4. The averages for food consumption during the various periods of suppression were taken from the daily records of each group. The figures differ from those

given in table 6 because of the exclusion in this instance of the animals killed for measurement at the end of the various suppression periods. Data similar to those given for the males are presented for the females in table 10.

TABLE 10

Comparison of the average total food consumption of female mice during refeeding after varying periods of suppression of growth with food eaten by controls in the time required to make the same gain from the same initial weight

FOOD AND GROWTH CURVES CHART NUMBER	NUMBER OF MICE	GAIN IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN			GAIN IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIOD IN WHICH THE GAIN ACTUALLY OCCURRED
				Suppression period	Refeeding period	Average total	
		grams		*grams*	*grams*	*grams*	*per cent*
III, 9-A	6	7.8	13	11.1	26.7	37.8	29
III, 18-A	6	7.8	14	21.6	31.4	53.0	25
(Controls	11	7.8	25			51.0	15)
III, 27	5	8.5	9	34.1	24.8	58.9	34
(Controls	11	8.5	29			54.5	16)
III, 5-A	5	8.5	11	13.0	25.6	38.6	33
V, 5-B	8	8.6	15–16	20.1	36.6	56.8	24
(Controls	11	8.5	24			50.2	16)
V, 9-B	6	8.9	18	22.6	37.2	59.8	24
(Controls	11	8.9	31			57.4	15)

In making these comparisons no account has been taken of the content of water in the new tissues.

Three males which had been suppressed in growth 5 days lived through the maintenance period and made rapid growth on less total food than the controls ate, while their weight increased by the same absolute amount. These mice, group A-5, males, include the two very rapidly growing individuals, mentioned above, which overtook their controls in 4 days. The average food eaten by the members of this group was 25 grams. Of this amount 19.2 grams were eaten during the period of accelerated growth. The gain in body weight corresponded to 50 per cent of this intake. In making the same gain, control mice ate 31.6

grams of food and retained an equivalent of 29 per cent as body weight. If mice 74 and 77 are considered without the other member of this group, it is found that their average food consumption for the period of refeeding (6 days) was 19.4 grams. During this time their average gain was 10.6 grams. Approximately 55 per cent of the weight of the food was added to the weight of the body. In calculating the gains of weight in terms of food intake, no account has been taken of the water factor. Obviously a considerable fraction of the weight put on represents water in the new tissues. Stewart reported that an average of 16 per cent of the ingested food (exclusive of water) was applied toward the increment in body weight of his test rats.

The mice suppressed in growth 9 days and group B in the 5-day test ate approximately the same amount of food as did their controls. These groups also show high gains in body weight in comparison with the total food consumption in the period of accelerated growth. *In all cases the weight gained by the experimental, i.e., stunted, animals was greater in proportion to the food eaten than that gained by the controls.*

Among the females recovering rapidly from suppression of growth, groups 5-A and 9-A show a utilization of smaller amounts of food than was eaten by the controls in making the same gain in weight. All other groups show a greater cost of growth, in terms of total food consumption, when the food intake during the period of underfeeding is also taken into account. From the results of this study it appears that, in normally growing white mice, increase in body weight, in the twenty days following weaning, is equivalent to from 20 per cent to 30 per cent of the total food eaten. In mice growing at accelerated rate after suppression of growth, the gain may be as high as 50 per cent to 55 per cent of the food eaten during the period of actual growth. The added expense of food for maintenance during the period of suppression of growth makes the *total economy* of food unfavorable when long periods are involved.

SUMMARY

From new statistics of the body weight determined daily for mice, curves of normal growth have been prepared. These indicate that up to the 26th day of life the actual gain per day is approximately the same for both sexes. After the 26th day the males continue to grow with comparative rapidity until about the 40th day, whereupon the slower, gradually diminishing rate of increment ensues. The females gain from the 26th day at the rate of 0.5 gram per day until the 34th day, when their curve flattens perceptibly.

The normal daily food consumption has been ascertained on a large scale during the period of growth between the 22d and the 62d day of life. The diet consisting of a mixture of comparatively simple food products, was selected to insure adequate proportions of all essential nutrients.

During prolonged periods of suppressed growth with a stationary body weight, the food requirement, as measured by the actual ad libitum intake, decreases after a time and remains at an apparent minimum level throughout the period of underfeeding. In second and third suppressions of growth after intervening periods of accelerated growth, the food requirement, in proportion to the body weight, is less than in the first period of retarded growth.

In correspondence with the observations of previous investigators the present experiments have shown that the resumption of growth after the suppression of growth, during periods of varying lengths at different stages of the growth cycle, ensues at a greatly accelerated rate.

The daily food intake has been determined during the period of rapid or accelerated growth which followed suppression of growth under a variety of experimental conditions. In this way it has become possible to compare the increment of body weight in relation to the food intake under widely varying physiological conditions of growth for comparable increments in individuals of the same size.

Comparisons of the economy of the food intake show that the gain of weight during the period of acceleration of growth following suppression of growth is ordinarily accomplished on a smaller intake of food than is ingested during a period of equal growth at normal rate from the same initial body weight. The advantage in this apparently better appropriation of food during accelerated growth may actually be sufficient, in some cases, to offset the added expense of the food required for maintenance without growth during a brief preliminary period of suppression. In other words, in several instances, the total quantity of food ingested during the entire period included in the failure to grow and the restitution of growth up to the normal size taken for comparison, has been no greater than that consumed by unstunted animals making the same gain of body weight at the normal rate. Ordinarily, however, the food requirement during any considerable preliminary period of maintenance without growth is sufficient to overbalance any economy in food during the period of accelerated growth.

Slow but completed growth may be accomplished even with a very small daily food intake.

Problems relating to changes in form incident to the suppression of growth are discussed. Whether permanent changes in proportions of stature actually remain after the compensatory growth following early stunting needs to be investigated more extensively.

The probable influence of the size of the animal, expressed in body weight, upon the rate of resumption of growth has been noted.

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DESCRIPTION OF FIGURES

No. 96a. Male. Initial weight, 9.7 grams. Picture taken 27th day of suppression of growth. Weight, 10 grams. Age, 47 days.

No. 96b. Male. 9th day of refeeding. Weight, 19.1 grams. A gain of 91 per cent in 9 days. Age, 56 days.

No. 95a. Female. Initial weight, 10.4 grams. Picture taken 27th day of suppression of growth. Weight, 10.9 grams. Age, 47 days.

No. 95b. Female. 9th day of refeeding. Weight, 19.5 grams. A gain of 79 per cent in 9 days. Age, 56 days.

No. 99a. Male. Initial weight, 11.8 grams. Picture taken 18th day of suppression of growth. Weight, 11.8 grams. Age, 41 days.

No. 99b. Male. 6th day of refeeding. Weight, 20.2 grams. A gain of 71 per cent in 6 days. Age, 47 days.

No. 104a. Female. Initial weight, 10.4 grams. Picture taken 18th day of suppression of growth. Weight, 10.7 grams. Age, 41 days.

No. 104b. Female. 13th day of refeeding. Weight, 19.3 grams. A gain of 80 per cent in 13 days. Age, 54 days. This mouse had reached a weight of 19 grams on the 10th day of refeeding.

No. 31. Control male. Weight, 13.0 grams. Average weight and normal form of males, 22 to 25 days of age.

No. 116. Control male. Weight, 21.4 grams. Age, 50 days. Average weight and normal form of adult males. This mouse grew at the exact rate of the average curve, chart 1.

Fig. 1 96 (a)

Fig. 2 96 (b)

Fig. 3 95 (a)

Fig. 4 95 (b)

Fig. 5 99 (a)

Fig. 6 99 (b)

Fig. 7 104 (a)

Fig. 8 104 (b)

Fig. 9 31

Fig. 10 116

OROKINASE AND PTYALIN IN THE SALIVA OF THE HORSE

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Received for publication January 22, 1918

The literature upon this subject has been rather thoroughly and painstakingly reviewed in three papers of recent publication. Palmer, Anderson, Peterson and Malcomson (1), R. J. Seymour (2) and Hayden (3) have reported upon different studies concerning salivary digestion in the horse. It is the purpose of this paper to submit data gathered more recently upon the subject and to compare the results with those reported in the preceding work. Palmer (1) and his coworkers report the existence of an enzyme, present in the buccal and possibly the lingual glands, that activates a ptyalinogen present in the secretion of the salivary glands of the horse. They conclude from the evidence of their data that neither saliva obtained from a parotid fistula nor a glycerine extract of the parotid gland digests starch. They also show that the mixed mouth secretions obtained from an esophageal fistula are more powerful in their amylolytic action than those obtained from the mouth. The name orokinase is given to the activating enzyme which activates the parotid saliva and the claim is made by them that the enzyme is present in the mouth secretions of both horse and man.

In Seymour's work mixed saliva of the horse is shown to have produced reducing sugar after eight hours digestion. The parotid saliva does the same thing with slightly accelerated action. Old and recent glycerine extracts of the salivary glands of the horse are negative up to eighteen hours. Water extracts were positive after six hours digestion with a 2 per cent corn starch solution. There is no evidence of an enzyme in the mouth secretions that activates a ptyalinogen present in the secretions of the salivary glands. The saliva of the horse, both the mixed and the isolated secretions of the parotid and submaxillary glands, contains a diastase capable of converting starch into sugar.

The diastase is extremely feeble, requiring at least five hours digestion for the conversion of starch to reducing sugar.

Hayden (3) after having digested a 1 per cent solution of soluble starch with parotid fistula saliva up to twenty-four hours obtained evidence of the presence of an enzyme capable of carrying the digestion of the 1 per cent starch solution to the maltose stage at least. The test for sugar was made with Benedict's quantitative solution.

Carlson, Greer and Becht (4) collected mixed saliva from two horses using pilocarpine as a sialogogue. They report no amylolytic action on the part of those samples. Twelve other samples of mixed horse saliva collected by them showed no power to digest starch.

Carlson and Crittendon (5) inserted a cannula into Stenson's duct. The parotid saliva collected by that method digested starch. The saliva was collected from a human subject.

M. Foster (6) in discussing the action of the saliva of man says that the parotid saliva alone digests starch and that the secretion of the submaxillary gland is sometimes more powerful than that of the parotid.

Prof. E. H. Starling (7) reports the stimulation of the flow of saliva in the dog by the use of pilocarpine with no amylolytic action on the part of the saliva.

Wiley (8) in giving an analysis of the grains says that there is in corn 71.48 per cent of starch, sugar and dextrin combined. Maize flour contains 78.36 per cent of starch and sugar. Unhulled oats contain of starch and sugar 57.93 per cent. Hulled oats contain 67.09 per cent of these substances.

Another author (9) claims that there is practically no dextrin or maltose in untreated grains.

Bradley and Kellersberger (10) report the presence of an active diastase in the seed of both mature and young corn. Jordan (11) says that sugars are formed in small quantities in the hays and in scarcely appreciable quantities in the grains. Saccharose exists in considerable proportion in field corn. Maltose exists in no quantities. Dextrose is found in maize.

Effront-Prescott (12) state that corn, rye and all other cereals contain considerable quantities of amylase and other substances that accelerate diastatic action. The action of the diastase begins during the process of milling. There is a transformation of the starch of the grains into sugar and the action of the amylase is shown at grinding. The amylolytic action takes place more readily in the presence of water.

METHODS AND RESULTS

Parotid saliva from the horse was collected through a fistula of Stenson's duct. Mixed saliva was collected both from the mouth and an esophageal fistula. The parotid saliva was collected while the animal was manger or trough feeding. The flow of mixed saliva was stimulated in various ways. Sometimes it was collected easily, other times with difficulty. Our work thus undergoes the same limitations as that of Palmer et al. (1).

Mixed human saliva was collected from a large number of persons. Parowax was chewed in each case to stimulate the flow. When this saliva was diluted the dilution was 1-50 and two drops of the dilution were used for digestive work.

The various extracts were made with 50 per cent glycerin in water. The glands and mucous membranes were extracted on the day the

TABLE 1

	STARCH	N/20 I	SUGAR
			grams
Pilocarpine crystals	0	G	0.000
Pilocarpine crystals			0.000
Pilocarpine, hypodermic	0	G	0.016
Pilocarpine, hypodermic			0.023+
Arecoline, hypodermic	0	G	0.023+
Arecoline, hypodermic			0.023+
Pilocarpine crystals plus 1 cc. parotid saliva	0	G	0.000

animal was killed and digestion was carried out the next. We have a series of four horses from which extracts have been made.

Unless exception is noted all samples were digested with 5 cc. of a 1 per cent corn starch solution for a period of two hours. Digestion was carried on in an electric incubator at a temperature of 38 to 40°.

The extent of digestion has been indicated by the physical appearance of the starch, the reaction the starch to N/20 I and the quantity of sugar produced. Most of the samples showed considerable starch at the end of the digestion period and unless the clearing was marked we have indicated the result by O. With N/20 I the achroödextrin stage has been indicated by −, the erythrodextrin by R and varying degrees of reaction by G for good, F for fair, S for slight, V. S. for very slight. Benedict's test was used to determine the quantity of sugar produced.

Palmer (13) states that pilocarpine digests starch. We used the

drug in both the crystalline form and in hypodermic tablets in previous work. In the present work we have used hypodermic tablets of arecoline in addition to the two forms of pilocarpine. Table 1 indicates typical results obtained in the use of these drugs.

One-half grain of the respective form of the drugs was dissolved in 10 cc. of water and 2 cc. of the solution used. Several samples of each drug have been tested. There is no evidence of digestive action on the part of either of them. However a considerable amount of reducing sugar is present in the hypodermic tablets of each drug.

Many samples of human saliva diluted 1-50 show a decided digestive power. One sample diluted 1-100 gave good results. Data involving the use of diluted human saliva are indicated in table 2.

TABLE 2

	STARCH	N/20 I	SUGAR
			grams
A.....	0	R-G	0.002–032
B.....	0	R-G	0.000–023
C.....	0	R-G	0.003–032

A contains 2 drops of human saliva in a dilution 1–50.
B is A plus 1 cc. mixed mouth saliva of the horse.
C is A plus 1 cc. parotid fistula saliva of the horse.

Table 2 is a summary of a very large number of samples. The starch solution was not cleared in any of them. The erythrodextrin reaction predominates in A. In B and C the reaction R–G is about even. The average amount of sugar found in A is equal to that of either B or C. From our interpretation of these results we can see no evidence that human saliva diluted 1-50 activates either mixed mouth saliva or parotid fistula saliva from the horse.

TABLE 3

	STARCH	N/20 I	SUGAR
			grams
A.....	0	– to V.S.	0.017–032
B.....	0	F-G	0.000–009
C.....	0	R-G	0.000–001

A is 1 cc. of human saliva.
B is 1 cc. of mixed mouth saliva from the horse.
C is 1 cc. of parotid fistula saliva from the horse.

The physical appearance of the starch was not a good index of digestion in this series. The human saliva changed the starch to achroödextrin in the greater number of the tests. The reaction of either B or C to N/20 I is not to be compared to that of A. The amount of sugar produced in B or C is a negligible quantity when compared with the amount indicated in A. The action of human saliva on cooked starch is much greater than that of mixed or parotid fistula saliva from the horse.

TABLE 4

	STARCH	N/20 I	SUGAR
			grams
A.....	0	R-G	0.000–011
B.....	0	R-G	0.000–007

A is 2 drops of mixed mouth saliva from the horse diluted 1–10.
B is A plus 1 cc. parotid fistula saliva.

The erythrodextrin reaction occurs but once in each series. As in the other tables a large number of samples is represented in the summary given in this table. The maximum amount of sugar in either A or B is far above the average of either. The average of each is too nearly equal to be taken as evidence of the presence of an activator in the mixed mouth saliva of the horse.

Repeated tests with corn or oats ground in a food chopper, mixed with water and filtered through cheese cloth give evidence of reducing sugar. The quantity of sugar varies from 0.000 gram in a sample of cornmeal to 0.15 gram in a sample of ground oats. The amount of sugar obtained from either of these grains after having been digested with either mixed or parotid fistula saliva from the horse does not average higher than the grains alone when mixed with water. There is evidence in the literature already quoted of sugar in these grains and of the presence of a diastase that may be activated by grinding them. The diastase is also said to be active in the grains without any mechanical change in them.

Glycerine extracts of the mucosa of the mouth, of the buccal glands, and of the salivary glands from four different horses have not shown any marked activating influence when digested with parotid fistula saliva or in different combinations with each other. We have too few of this series to come to a definite conclusion. Some of the digested products if tested with Fehling's solution alone would show a marked

reduction. Orokinase cannot be demonstrated by the use of that reagent alone. We have a large number of samples in the different phases of the work that would give some considerable reduction. When the quantity of sugar was measured it was found that it was not appreciably greater in the material in which no enzyme was supposed to have been present. Two drops of human saliva diluted 1-50 gave frequent erythrodextrin reactions when digestion was carried two

TABLE 5

Showing the amount of reducing sugar in ground corn and oats. Time of digestion, 2 hours. Sugar reported is the quantity in the whole filtrate

GRAIN	AMOUNT	AMOUNT H_2O	SUGAR
	grams	*cc.*	*grams*
Corn	20	100	0.04
Oats	20	100	0.06
Corn	10	50	0.08
Cornmeal	2	25	0.02
Oats	2	25	0.04
Cornmeal	2	25	0.000
Cornmeal	2	25	0.025
Oats	2	25	0.025
Corn	2	25	0.086
Oats	5	25	0.085
*Corn	5	25	0.019
*Oats	5	25	0.012
*Corn	5	25	0.015
*Oats	5	25	0.017
Oats	5	25	0.15
Corn	5	25	0.10
*Oats	5	25	0.10
*Corn	5	25	0.09
Oats	5	25	0.029
Cornmeal	5	25	0.019

* Indicates no digestion, samples were tested right after mixing with water.

hours. None of the samples tabulated in table 7 did save one and 10 cc. of parotid fistula saliva was used in that. No sugar could be measured from this sample. Parotid saliva in quantities greater than 1 cc. when digestion is carried up to twenty-four hours frequently give the erythrodextrin reaction and measurable quantities of sugar. The same thing has occurred with the mixed saliva and the various extracts that have been made. This seems to be in accord with the results obtained by Seymour (2).

The amount of sugar measured in these cases has been a disappointment to us. We feel that an enzyme in the mouth secretions activating the salivary secretions would lead to the production of measurably more sugar than that produced from the secretions or extracts used alone. That has not been the case in our experiments.

Our one esophageal fistula has not given the results hoped for. The amount of sugar produced in any experiment with the mixed saliva

TABLE 6

Action of saliva of the horse on ground grains. Time of digestion, 2 hours. Five grams of grain to 25 cc. of water in each sample

GRAIN	SALIVA	AMOUNT	SUGAR
		cc.	*grams*
Corn	Mixed	5	0.055
Corn	Parotid	1	0.017
Oats	Parotid	2	0.035
Cornmeal	Mixed	1	0.050
Cornmeal	Parotid	1	0.032
Corn	Mixed	1	0.067
Corn	Mixed	1	0.035
Oats	Mixed	1	0.050
Corn	Mixed	1	0.043
Oats	Mixed	1	0.057
Corn	Mixed	1	0.027
Corn	Mixed	1	0.045
Corn	Mixed	1	0.033
Oats	Mixed	1	0.046
Oats	Mixed	1	0.045
Cornmeal	Mixed	1	0.075
Cornmeal	Mixed	1	0.090
Cornmeal	Parotid	10	0.058

from that source has been small. In no wise has it been comparable with the action of human saliva on either cooked or raw starch.

Neither oats nor corn having been thoroughly masticated and passed through the fistula have given more sugar than we have obtained from the ground grains in water. Mixed saliva from this fistula did not show digestive action on these grains. The mixed saliva did not show any reducing power. Mixed saliva acting on cooked starch for twenty-four hours produced 0.008 gram of sugar. The human saliva in the dilution used produced as much or more when digestion was carried only two hours.

TABLE 7

Action of various gland extracts on starch; 0.5 cc. of extract used. Where parotid saliva is used 1 cc. is the amount. Time of digestion, 24 hours

MATERIAL	STARCH	N/20 I	SUGAR
			grams
Buccal gland	0	G	0.000
Buccal gland plus parotid saliva	0	G	0.000
Parotid saliva	0	G	0.000
Parotid saliva, 10 cc	0	R	0.000
*Buccal gland			0.000
Buccal gland plus parotid extract	0	G	0.004
Buccal gland plus submaxillary extract	0	G	0.002
Buccal gland plus sublingual extract	0	G	0.000
Lingual gland	0	G	0.000
Lingual glands plus parotid saliva	0	G	0.004
*Lingual glands			0.000
Lingual glands plus parotid extract	0	G	0.001
Lingual glands plus submaxillary extract	0	F	0.001
Lingual glands plus sublingual extract	0	G	0.002
Mucosa, mouth	0	G	0.001
Mucosa plus parotid saliva	0	G	0.002
*Mucosa			0.000
Mucosa plus parotid extract	0	G	0.000
Mucosa plus submaxillary extract	0	G	0.001
Mucosa plus sublingual extract	0	G	0.001
Parotid extract	0	G	0.002
Submaxillary extract	0	G	0.001

* Indicates no digestion.

TABLE 8

Material from esophageal fistula

	STARCH	N/20 I	SUGAR
			grams
1. Starch plus mixed saliva	0	G	0.004
2. Starch plus 0.5 cc. mixed saliva	0	S	.003
3. Oats from fistula, 1 cc. of filtrate			0.004
4. Oats from fistula, 1 cc. of filtrate 24 hours			0.003
5. Oats from fistula, 2 cc. of filtrates 0.2 HCl			0.002
6. Starch plus mixed saliva, 24 hours digestion	0	S	0.008
7. Ground corn 2 grams, 25 cc. H_2O plus 1 cc. mixed saliva			0.000
8. Oats ground 2 grams 25 cc. H_2O plus 1 cc. mixed saliva			0.001
9. 1 cc. mixed saliva, not digested			0.000

CONCLUSIONS

1. Pilocarpine hydrochloride does not digest starch. Hypodermic tablets of both pilocarpine and arecoline contain a reducing substance in comparatively large quantities but do not in themselves digest starch.

2. Two drops of human saliva diluted 1-50 carry 5 cc. of a 1 per cent starch solution to the erythrodextrin stage in a large number of cases. A measurable amount of sugar is produced as a result of that digestion.

3. Human saliva in such a dilution does not activate either mixed or parotid fistula saliva from the horse.

4. Mixed human saliva digests cooked starch much more readily than either mixed or parotid fistula saliva of the horse.

5. Two drops of mixed mouth saliva from the horse diluted 1-10 does not activate parotid fistula saliva from that animal. It does not show any appreciable digestive power when used alone in that dilution.

6. The filtrate from a solution of ground corn or oats contains a reducing sugar. The quantity of sugar does not show an average increase when the grains are digested with either mixed or parotid fistula saliva from the horse. Mixed human saliva does digest them under the same conditions.

7. Extracts from the glands and mucosa of the mouth have failed to activate parotid saliva or extracts of the salivary glands of four different horses.

8. Corn and oats passed through an esophageal fistula show no more reducing sugar than the ground grains themselves. Mixed saliva from the esophagus has not shown any marked potency.

9. The glands of the mouth as well as the salivary glands produced a small amount of enzyme that will digest starch within a twenty-four hour period.

Acknowledgments are due Dr. P. A. Fish for helpful advice and support in this work and to Dr. J. N. Frost, Dr. E. B. Hopper and Mr. G. K. Cooke for assistance given in the Department of Surgery in preparing fistulas and collecting samples of saliva.

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THE INFLUENCE OF PITUITARY EXTRACTS ON THE DAILY OUTPUT OF URINE

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Received for publication January 22, 1918

INTRODUCTORY

Since Magnus and Schäfer (1) first pointed out a possible relation between the hypophysis cerebri and renal function, a large amount of work has been done in an attempt to fix this relationship and, if possible, to point out its nature. The results obtained have been quite conflicting. The earlier investigation seemed to show that extracts of the pituitary gland when injected intravenously have a pronounced diuretic effect. The later work would indicate that such extracts give results which are exactly contrary to those obtained by the earlier investigators, namely, an antidiuretic effect.

In this investigation it was our purpose to find out first, whether the subcutaneous injection of pituitary extract will cause any quantitative variation in the daily output of urine; second, whether such injection will in any way affect the quantity of urine excreted and, if so, to find out if possible the factors involved.

LITERATURE

Magnus and Schäfer (1) working on anesthetized animals, concluded that intravenous injection of extracts of the pituitary gland causes a prolonged expansion of the kidney and a greatly increased rate of renal secretion. The diuresis was in every case of short duration, twenty to thirty minutes, while the kidney dilation continued for a longer time. These authors conclude that the extract acts directly on the renal epithelium in bringing about the increased flow of urine.

This work was later repeated by Schäfer and Herring (2) who arrived at practically the same conclusions. Their results, however, were quite inconstant. In a series of thirteen experiments on dogs, nine show

a diuretic and four an antidiuretic effect after injection. In another series on nineteen rabbits, diuresis was obtained in fourteen cases and a decrease in flow in five cases.

The theory of direct stimulation of the renal cells was also upheld by Hoskins and Means (3), who considered that the direct stimulation may be assisted by a vasodilation in the kidneys.

Houghton and Merrill (4) failed to find any direct action of pituitary extracts on the renal epithelium in the case of perfused kidneys. They conclude that diuresis is caused by an increase in the blood pressure.

Vasodilation of the renal vessels is considered by King and Stoland (5) to be the principal factor involved in the increased flow of urine which they find after pituitary extract injection.

Dale (6) working with perfused kidneys of the dog and cat found that pituitary extracts cause a vasoconstriction of renal vessels. These results were confirmed by Houghton and Merrill (4).

Pal (7) states that isolated rings of the proximal portion of the renal artery are constricted while rings from the peripheral portions of this artery are dilated by pituitrin.

The investigations of Falta, Newburg and Noble (8) indicate that diuresis generally results from subcutaneous injections of pituitary extracts.

A number of other investigators using practically the same methods as those of Schäfer and Herring have reported that diuresis results from the injection of extracts from the pituitary gland.

Within the past three years several investigators have reported that subcutaneous injection of pituitary extracts gives a diuresis of several hours duration. Pentimalli and Quercia (9) found that on the isolated kidney of the rabbit pituitary extract gave a diminished flow from both the ureter and from the renal vein. The decrease was especially marked in the flow from the vein. On the other hand Gabriels (10) reported that in the isolated kidney of the dog pituitary extract caused an increased flow of urine without vasodilation.

A large portion of the evidence in favor of the antidiuretic effect of posterior lobe extract comes from the clinical side where the extract has been used with apparent success in reducing the diuresis of diabetes insipidus. One of the first to report evidence of this nature was Farmi (11) who reported that he had been successful in reducing the diuresis in two diabetes insipidus patients by subcutaneous injections of extract of the pituitary.

Further evidence from the clinical side has been given by von der Velden (12), Konschegg and Schuster (13), Motzfeldt (14), (15) and Bab (16). Most of these investigators have checked up their results experimentally.

Meyenberg (17), working on rabbits and cats, found that subcutaneous injection produced an antidiuresis lasting for eight or ten hours.

Römer (18) found that animals catheterized every hour showed a very marked decreased output of urine after injection with pituitary extract.

The most extensive experimental work showing an antidiuretic effect from the injection of posterior lobe extract has been reported by Motzfeldt (19). Working with a large number of animals, mostly rabbits, he found that subcutaneous injection of the extract gave without exception a marked antidiuresis extending over several hours. Motzfeldt concludes that the results from his experiments on rabbits tend to show that pituitary extracts produce an antidiuretic action on account of their stimulation of the sympathetic nervous system and thus affecting the renal vasomotor system, that is, the direct cause of the antidiuresis is considered as due to vasoconstriction in the kidneys.

METHODS AND RESULTS

In all of our work we have followed rather closely the methods suggested by Motzfeldt with the important exceptions that our observations extended over much longer periods of time and the urinary output was always computed on the twenty-four hour or daily basis.

The experimental observations were made on cats and rabbits. Rabbits were found to be much more susceptible to pituitary extracts than were dogs or cats.

Three commercial extracts were used, namely, Pituitrin (Parke, Davis & Co.), Hypophyseal Solution (Squibb & Co.) and Pituitary Liquid (Armour & Co.). Practically no difference was found in the action of these preparations.

The injections were made subcutaneously in every case. The usual amount injected per day was 1 cc. for cats and 0.5 cc. for rabbits. The injections were made at the beginning of experiment at the time that the water was given by stomach tube.

Ordinary aseptic precautions were used in making the injections. No infections resulted from the repeated injections.

In order to obtain accurate data on the water intake the water was always given by stomach tube.

The animals were kept in perfectly dry cages and the nature and amount of food given were accurately noted in each case.

The urine was collected in such a way as to avoid so far as possible any evaporation or any contamination with feces.

The daily quantity and the specific gravity were the principal points noted in each experiment. Variations in the rate of output were also noted in the case of the catheterized rabbits.

Tests were made in each experiment for sugar and albumin but these were not observed. In both cats and rabbits the daily output of urine varies widely even with a constant food and water supply. On this account it was found advisable to extend the observations over several days.

In our first series of observations it was our purpose to find out whether pituitary extracts will, when injected subcutaneously, cause any variation in the daily output of urine. In table 1 we give the averages from this series of experiments. By inspection of this table it will be found that the averages for the control animals and for the injected animals are practically the same both in amount and in specific gravity. In six cases there is a slight decrease in the daily output of the injected animals but this is balanced by four cases where there is a greater increase in daily output. The variations above and below the mean were, with the exception of one case, less than 11 cc.

There is no indication that the animals established a resistance to pituitary extract after repeated injections. In practically every case the daily output of urine following the first and second injections was as high as that obtained on the ninth or tenth days of injections.

Attempts were made to study the effect of doses larger than 1 cc. per day, but these did not yield satisfactory results on account of the systemic disturbances caused. In the case of cats, vomiting was the most common result from large doses, in fact, even with 1 cc. doses a number of cats had to be rejected on account of this tendency to vomit following the injections. The vomiting may not occur until thirty or forty minutes after the water and the injections have been given. For this reason it is necessary to watch the animals closely for at least this length of time, so that one may be sure that he is not including regurgitated water in the urine measurements.

The apparent decrease in daily output of urine after injection shown in table 2 may be accounted for by the loss of water due to the increased defecation.

TABLE 1

Summary of experiments on the influence of pituitary extract on the urinary output in cats. The averages for each animal are computed from ten days of normal (control) and from ten days of daily injection with 1 cc. of pituitary extract. Each animal was given 100 grams of cooked meat per day. Water was given by stomach tube

		Amount of water given per day	Urine	
			Average amount per day	Average specific gravity
		cc.	cc.	
A. Female; weight 2600 grams	Control	100	103.9	1014.5
	Pituitary extract	100	114.2	1011.3
B. Female; weight 2750 grams	Control	100	104.1	1013.8
	Pituitary extract	100	107.1	1013.3
C. Male; weight 2900 grams	Control	100	101.8	1025.6
	Pituitary extract	100	103.0	1024.8
D. Female; weight 2750 grams	Control	50	68.0	1021.2
	Pituitary extract	50	62.3	1019.8
E. Female; weight 2700 grams	Control	50	64.1	1027.4
	Pituitary extract	50	57.1	1030.0
F. Male; weight 2800 grams	Control	200	175.2	1008.6
	Pituitary extract	200	165.4	1010.4
G. Male; weight 2860 grams	Control	100	95.0	1015.4
	Pituitary extract	100	117.6	1016.0
H. Female; weight 2680 grams	Control	20	41.6	1044.0
	Pituitary extract	20	33.6	1040.0
I. Female; weight 2710 grams	Control	20	46.8	1044.0
	Pituitary extract	20	40.8	1045.0
J. Male; weight 2890 grams	Control	20	30.6	1043.0
	Pituitary extract	20	26.1	1045.0

In attempting to inject doses larger than 2 cc. per day no satisfactory results were obtained. In two cases the output was reduced to one-half the normal. In three cases there was a very marked increase in daily output. The usual result, however, was that it was impossible to keep the injected animals from regurgitating the water introduced by stomach tube.

Rabbits gave practically the same results as cats so far as the effect of pituitary extracts upon daily output and specific gravity of the urine is concerned. This is shown in tables 3, 4 and 5.

The variation between the control and the injected animals was greater in the experiments on rabbits than in those on cats. This is accounted for in part by the shorter time covered by the rabbit experiments. It was not possible to keep the rabbits in good condition for

TABLE 2

Effect of large doses of pituitary extracts on urinary output in cat. Pituitary extract was given in two doses per day of 1 cc. each at six hour interval. Animal was given daily 100 grams of cooked meat and 150 cc. of water by stomach tube

	DAY	URINE	
		Cubic centimeters	Specific gravity
Control	1	183	1010
	2	163	1010
	3	155	1010
Injected	4	108	1009
	5	173	1014
	6	165	1014

longer periods of experimentation than those used. In order to avoid the loss of excessive amounts of water in the large amount of feces passed, it was found advisable to reduce the food supply to such an extent that a very small amount of material was normally passed from the intestine. The reduced food supply was also found to be necessary to avoid the development of diarrhea following the injections.

Another cause for the variation in the case of rabbits may be found in the tendency of the pituitary extract to increase defecation. This fact is referred to later in another connection.

The second part of our problem was to find out whether the subcutaneous injection of pituitary extract causes any variation in the rate of urinary excretion. Rabbits were found to be best suited for this line of work since by using males it was easy to collect the urine at regular

intervals by catheterization. Silk linen catheters no. 11 were found to be best adapted for this purpose.

It was found necessary to select rabbits that could be catheterized with ease since irritation of the urethra apparently exerted a reflex inhibition on the kidney. Rabbit 3 in table 4 illustrates this point.

Tables 4 and 5 show the effect of pituitary extracts on the rate of

TABLE 3

Summary of experiments on the influence of pituitary extracts on daily urinary output in rabbits. The averages for each animal are computed from three days of normal (control) and three days with daily injection of 0.5 cc. of pituitary extract. Each animal was given 50 grams of cabbage per day, 150 cc. of water was given daily to each animal

		URINE	
		Average amount per day	Average specific gravity
		cc.	
5. Male; weight 1677 grams	Control	139.6	1012.6
	Pituitary extract	181.3	1018.0
6. Male; weight 1774 grams	Control	139.3	1013.3
	Pituitary extract	181.6	1014.0
7. Male; weight 1810 grams	Control	201.6	1016.6
	Pituitary extract	191.3	1011.6
8. Male; weight 1751 grams	Control	192.3	1007.3
	Pituitary extract	212.0	1013.6
9. Male; weight 1698 grams	Control	180.0	1010.0
	Pituitary extract	257.0	1012.3
10. Male; weight 1830 grams	Control	227.6	1010.0
	Pituitary extract	187.6	1012.0

secretion, but this is even better shown in figure 1. It will be noted from the figure that the normal diuresis which follows the giving of 150 cc. of water to rabbits by stomach tube reaches its maximum in the second or third hour after giving the water. By the end of the seventh hour the diuresis has practically exhausted itself and the normal excretion of 2 to 3 cc. per hour follows. After the injection of pituitary extract the picture is quite different.

In this case the diuresis is held in check for seven or eight hours when it breaks through and may reach a level as high as that of normal secretion. As a rule the diuresis following the injection is prolonged for ten to twelve hours. The total output per day is found to be practically the same in both cases.

By referring to some of Motzfeldt's (15) figures it will be noted that they show an increase in urine output in the seventh and eighth hours,

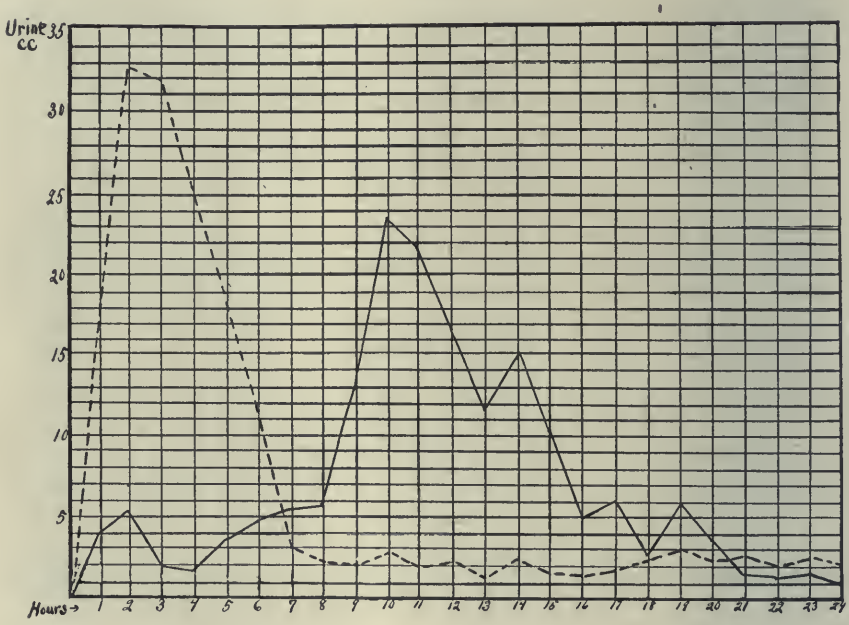

Fig. 1. Curves showing the effect of pituitary extract on urinary output. Each curve is plotted from averages from two animals (rabbits). At the beginning of the experiment each animal was given 150 cc. of water per os. The injected animals were each given 0.5 cc. of pituitary extract subcutaneously at the beginning of the experiment. The broken line represents the control animals. The continuous line represents the injected animals. Average 24 hours urinary output of control animals, 172.6 cc. Average 24 hours urinary output of injected animals, 170.5 cc.

that is, his observations were carried just to the beginning of the diuresis which has been delayed by the pituitary extract.

The third problem which concerned us was the cause of the delay in diuresis which we found followed the subcutaneous injections of pituitary extract. Several things which appeared in the course of the

experiments suggested to us that delayed absorption from the alimentary canal may be an important factor in causing this delayed diuresis. It has already been noted that in the case of injected cats there is a pronounced tendency to vomit. The vomiting may occur three-quarters of an hour after giving the water and the injection. Usually four- to five-sixths of the water was returned in the vomit, indicating that there

TABLE 4

Effect of pituitary extract on the urinary output in rabbits. Each animal was given 150 cc. of water by stomach tube at the beginning of the experiment. No food was given during the experiment. Rabbits 1, 2 and 3 were injected at the beginning of the experiment with 1 cc. of pituitary extract. The urine was drawn by catheterization

	(1) Male; weight 1720 grams pituitary extract urine	(2) Male; weight 1780 grams pituitary extract urine	(3) Male; weight 1675 grams pituitary extract urine	(4) Male; weight 1930 grams control urine
	cc.	cc	cc.	cc
6.00 a.m.				
6.30 a.m.	5.0	3.0	2.5	2.5
7.00 a.m.	20.0	4.0	4.0	5.0
7.30 a.m.	15.0	2.0	2.0	13.0
8.00 a.m.	4.0	1.5	2.0	13.0
8.30 a.m.	4.5	3.5	1.5	19.0
9.00 a.m.	0.5	5.0	1.0	18.0
9.30 a.m.	4.0	10.0	1.0	16.0
10.00 a.m.	9.0	5.0	1.5	0.5
10.30 a.m.	4.0	4.0	1.5	24.0
11.00 a.m.	0.5	2.0	2.0	7.0
11.30 a.m.	1.0	2.0	0.5	0.0
12.00 m.	3.5	2.5	1.5	5.5
Total first 6 hours.....	71.0	46.0	20.0	123.5
6.00 p.m.	56.5	57.5	11.5	18.5
6.00 a.m.	91.0	48.0	51.0	31.0
Total 24 hours.....	218.5	151.5	82.5	173.0

was a delay in passing the water from the stomach. The injection of large doses of the extract commonly caused diarrhea. The effect of the extract on the alimentary canal was much more pronounced in rabbits than in cats. Rabbits kept on a uniform diet of 50 grams per day of carrots or cabbage pass very small amounts of feces in the form of comparatively dry pellets. After injection with 0.5 cc. of pituitary extract there is a marked increase in the amount of feces which are of a

TABLE 5

Periodic variation in output of urine in male rabbits following injection of pituitary extracts. Each animal was given daily 50 grams of carrots and 150 cc. of water by stomach tube. The water and the pituitary extract were given at the beginning (6 a.m.) of the experiment. Injected animals received 0.5 cc. of pituitary extract subcutaneously

		DATE	TIME	URINE		Total amount urine per day
				Cubic centimeters	Specific gravity	
				cc		
11. Weight 1934 grams	Control	May 29–30	12 m.	101.0	1010	
			6 p.m.	0.0		
			6 a.m.	103.0	1006	204.0
	Pituitary extract	May 30–31	12.00 m.	34.0		
			6.00 p.m.	15.0		
			6.00 a.m.	95.0	1010	154.0
12. Weight 1677 grams	Control	May 29–30	12.00 m.	122.0	1005	
			6.00 p.m.	16.0		
			6.00 a.m.	30.0		154.0
	Pituitary extract	May 30–31	12.00 m.	18.5		
			6.00 p.m.	29.0		
			6.00 a.m.	134.0	1009	181.5
13. Weight 1740 grams	Control	May 29–30	12.00 m.	108.0		
			6.00 p.m.	17.0		
			6.00 a.m.	42.0		167.7
	Pituitary extract	May 30–31	12.00 m.	40.0	1004	
			6.00 p.m.	0.0		
			6.00 a.m.	45.0	1015	85.0
14. Weight 1734 grams	Control	May 29–30	12.00 m.	133.0	1004	
			6.00 p.m.	12.0		
			6.00 a.m.	38.0		183.0
	Pituitary extract	May 30–31	12.00 m.	37.0		
			6.00 p.m.	88.0	1002	
			6.00 a.m.	95.0	1010	220.0
15. Weight 2085 grams	Control	May 29–30	12.00 m.	158.0	1005	
			6.00 p.m.	17.0		
			6.00 a.m.	11.0		186.0
	Pituitary extract	May 30–31	12.00 m.	23.0		
			6.00 p.m.	30.0		
			6.00 a.m.	133.0	1007	186.0

TABLE 5—*Continued*

		DATE	TIME	URINE		Total amount urine per day
				Cubic centimeters	Specific gravity	
						cc.
16. Weight 2027 grams	Control	May 29–30	12.00 m.	145.0	1006	
			6.00 p.m.	27.0		
	Pituitary extract		6.00 a.m.	16.0		188.0
		May 30–31	12.00 m.	35.0		
			6.00 p.m.	43.0	1015	
			6.00 a.m.	87.0	1015	165.0
17. Weight 2285 grams	Control	May 29–30	12.00 m.	145.0	1008	
			6.00 p.m.	25.0		
	Pituitary extract		6.00 a.m.	5.0		175.0
		May 30–31	12.00 m.	43.0		
			6.00 p.m.	11.0		
			6.00 a.m.	133.0	1012	187.0
18. Weight 2640 grams	Control	May 29–30	12.00 m.	135.0	1008	
			6.00 p.m.	63.0		
	Pituitary extract		6.00 a.m.	35.0		234.0
		May 30–31	12.00 m.	42.0	1024	
			6.00 p.m.	2.0		
			6.00 a.m.	123.0	1012	167.0

semifluid consistency. This excessive amount of water feces accounts for the apparent decrease in the daily urinary output found in some cases after injection. An example of this is seen in rabbits 13 and 18 in table 5.

If rabbits are injected with doses of 1 cc. or more, large masses of semi-fluid feces are thrown off in five to ten minutes after giving the water and the injection. The large amount of water given was not the direct cause of the diarrhea since the controls passed small amounts of feces in the usual pellet form.

The effect of pituitary extracts on absorption was tested experimentally. The results indicate that there is a retarding of absorption after subcutaneous injection of the extract.

In order to avoid the possible effect of the anesthetic on the rate of absorption, a decerebrated dog was used in the following experiment.

When a diuresis was produced by the constant intravenous injection with the Woodyatt injection apparatus of 150 cc. of 0.9 sodium chloride

TABLE 6

Effect of pituitary extract on intestinal absorption in the cat. The animal was anesthetized lightly by giving urethane (2 grams per kilo) per os. The small intestine was exposed and washed, then ligated at either end

	AMOUNT OF WATER INJECTED INTO SMALL INTESTINE	WATER RECOVERED AFTER 1 HOUR	ABSORPTION
	cc.	cc.	cc.
Control animal	30	8	22
Injected animal 1 cc. pituitary extract, subcutaneously	30	27	3

solution per kilo per hour, the subcutaneous injection of pituitary extract had no effect on it (three experiments). On the other hand the injection of the extract caused a delay in the diuresis caused by giving 150 cc. of 0.9 salt solution by stomach tube.

The fact that the rate of diuresis caused by giving 0.9 NaCl solution intravenously is not affected by subcutaneous injection of pituitary extracts but is affected by such injections when the salt solution is introduced into the alimentary canal would indicate that the extract in some way causes delayed intestinal absorption.

TABLE 7

Effect of pituitary extract on intestinal absorption in the rabbit. Anesthetized and intestine prepared as in table 6

	AMOUNT OF WATER INJECTED INTO SMALL INTESTINE	WATER RECOVERED AFTER	ABSORPTION
	cc.	cc	cc.
Control animal	30	0	30
Injected animal 0.5 cc. pituitary extract subcutaneously	30	25	5

TABLE 8

Effect of pituitary extracts on intestinal absorption in a decerebrated dog. A 14 inch loop from the middle of the jejunum was used

	AMOUNT OF WATER INJECTED INTO INTESTINE	WATER RECOVERED AFTER 30 MINUTES	ABSORPTION
	cc.	cc.	cc.
Control period	75	42	32
Injected period	75	70	5

The fact that pituitary extract does not affect the diuresis following intravenous injection of normal salt solution would also indicate that the extract does not regulate the diuresis by an action on the salt content of the blood as has been recently advocated by Abrahamson and Climenko (20).

TABLE 9

Effect on the diuresis produced by giving 150 cc. of 0.9 NaCl solution per os in rabbits

		DATE	TIME	URINE		TOTAL AMOUNT URINE PER DAY
				Cubic centimeters	Specific gravity	
						cc
	Controls	July 25–26	12.00 m.	86	1013	
			6.00 p.m.	28		
			6.00 a.m.	42		156
Rabbit 19; male; weight 2100 grams	Controls	July 26–27	12.00 m.	122	1011	
			6.00 p.m.	51		
			6.00 a.m.	47	1019	220
	Pituitary extract 0.5 cc. per day	July 27–28	12.00 m.	82	1018	
			6.00 p.m.	94		
			6.00 a.m.	90		266
		July 28–29	12.00 m.	58		
			6.00 p.m.	55		
			6.00 a.m.	77	1013	190
	Controls	July 25–26	12.00 m.	182	1010	
			6.00 p.m.	58		
			6.00 a.m.	52	1024	292
Rabbit 20; male; weight 1550 grams	Controls	July 26–27	12.00 m.	114	1015	
			6.00 p.m.	70		
			6.00 a.m.	61	1025	245
	Pituitary extract 0.5 cc. per day	July 27–28	12.00 m.	97	1012	
			6.00 p.m.	63		
			6.00 a.m.	122	1022	282
		July 28–29	12.00 m.	90		
			6.00 p.m.	47		
			6.00 a.m.	73	1025	210

Previous workers have shown that subcutaneous injection of pituitary extracts does not affect the general blood pressure in anesthetized animals. We found that in decerebrated animals subcutaneous injection of the extract had no effect on blood pressure (two experiments). The effect of the extract on the rate of urinary excretion can not then be due to any general vasomotor change. It is possible, however, that

there may be a vasoconstriction in minute vessels of the intestinal wall thus causing a delayed absorption and incidentally a delay in the diuresis. This does not necessarily rule out the possibility that there may also be a simultaneous vasoconstriction in the kidney which is also a factor in retarding the diuresis.

CONCLUSIONS

1. Subcutaneous injections of pituitary extract do not alter quantitatively the daily output of urine in cats and rabbits, nor do they cause any marked variation in the specific gravity of the urine.

2. The subcutaneous injection of pituitary extracts causes a delay of seven to eight hours before the beginning of the diuresis which follows the ingestion of large amounts of water. This delay, however, does not cause any variation in the total amount of urine excreted in twenty-four hours.

3. The delay in diuresis which is produced by subcutaneous injection of pituitary extract is due in part at least to a delayed absorption from the alimentary canal.

4. The subcutaneous injection of pituitary extract has no influence on the diuresis induced by a continuous intravenous injection of isotonic salt solution.

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THE INITIAL AND PROGRESSIVE STAGES OF CIRCULATORY FAILURE IN ABDOMINAL SHOCK

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Received for publication January 28, 1918

INTRODUCTION

Although the circulatory failure in shock has been most extensively studied, it appears that the sequence of dynamic events, particularly in the earlier stages, has not been investigated with the degree of detail possible by optically recording manometers. Their use offers the advantages that, without resorting to extensive operative procedures, the pressure changes in the auricles, ventricles and arteries may be accurately recorded. With the knowledge gained from foregoing work (1) as to how individual factors of the circulation can modify the details of the optical pressure curves, it is possible by studying their modification during the progress of circulatory failure in shock to determine more minutely than in other ways the dynamic stages taking place in the circulation from time to time.

Since "exposure of the intestines" is generally considered the method "par excellence" for producing experimental shock and therefore the procedure most frequently employed, the study of the dynamic sequence in circulatory failure so produced is alone considered in this investigation.

EXPERIMENTAL PROCEDURE

The fundamental aim of this research was to analyze the consecutive changes evident in optical records of the right auricular, right ventricular and central arterial pressures. As it is undesirable, however, to take continuous photographic records of these pressure changes, the mean carotid pressure was constantly recorded on smoked paper as a rough index of the circulatory changes and the "effective venous pressure" was read at shortly spaced intervals from a differential water-manometer. Such a manometer is obtained when the fluid-filled limb

of an ordinary water-manometer is connected with a sound inserted via the jugular vein into the auricle and the air column of the other limb is connected with a trocar inserted into the thoracic cavity.[1] At stated intervals, marked on the smoked drum by a signal electrically connected to the photokymograph when the shutter opens, 50 cm. of pressure tracings were optically recorded.

After a period of preliminary observation under light ether anesthesia, abdominal shock was induced in the following way: A long median abdominal incision was first made through the skin and connective tissue. After studying the vascular reaction thus induced, the incision was extended through the muscles and peritoneum. Finally, the intestinal loops were removed and spread upon the abdomen and a stream of moist air allowed to blow over them during the reminder of the experiment. Experience showed that circulatory failure is more evenly produced without manipulation of the intestines and when this is indulged in it acts only to complicate the dynamic stages.

ANALYSIS OF RESULTS

The progress of the circulatory failure, as expressed by the changes in mean arterial pressure and effective venous pressure, by the changes in heart rate and respiration, are shown in a typical experiment plotted

[1] *Correction.* While casual consideration led to the impression that this type of manometer would offer a simple and direct method of reading the effective venous pressure in absolute terms, more careful reflection, together with subsequent experimental controls, shows that the effective venous pressure cannot be measured absolutely in this way. This is due, briefly, to the fact that the pleural cavity is virtual and not real; consequently the limited quantity of air in the connecting tubes suffers compression and rarefaction as the fluid level in the water manometer changes with the intra-auricular pressure. This makes the reading of the differential manometer considerably higher than the difference actually existing between the pressures in the pleural cavities and in the auricle. It has been found, however, that as long as intrapleural pressure variations do not change excessively, due to modified breathing, the figures so obtained follow the directional changes of effective venous pressure although they are not proportional to them. Inasmuch as these conditions obtain in all the experiments reported in this investigation, the values plotted, though neither absolute nor proportional as regards changes in effective venous pressure, are reliable as regards its directional tendency. This error is unfortunate in the fact that the absolute variations of effective venous pressure during circulatory failure are not available. Since the directional tendency is correctly indicated, however, it does not invalidate the essential conclusions in regard to the dynamics of the circulation in this form of circulatory failure here described.

in figure 1. The changes are in accord with those generally recognized as characteristic of circulatory failure in shock.

The onset of circulatory failure. A glance at the results presented in this chart shows that while the preliminary stage of the experiment involving the abdominal incision and removal of the intestines produces temporary reactions and often leaves the arterial and venous pressures somewhat low, the changes are not sufficient to be considered as even an initial stage of circulatory failure. After these operative stages have been completed, tracings of the auricular, ventricular and carotid pres-

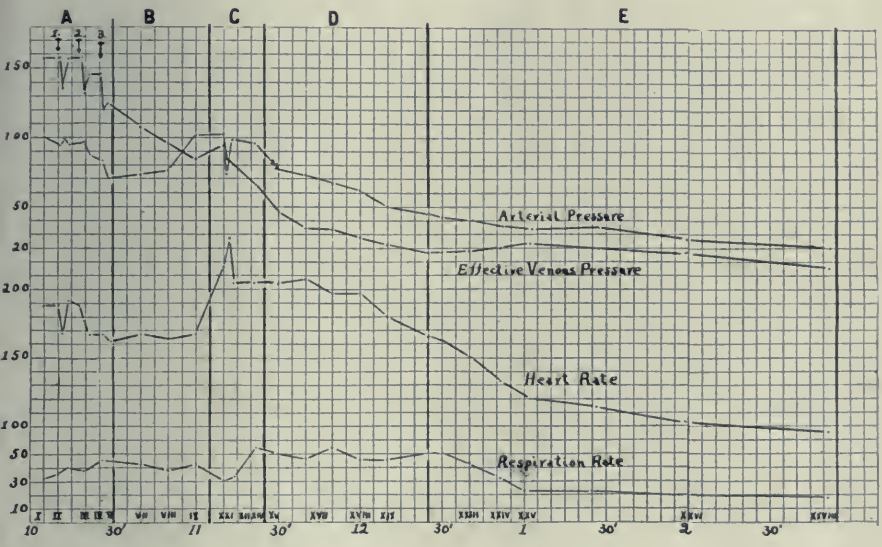

Fig. 1. Chart showing plotted data of circulatory stages in a case of abdominal shock (exp. C-157); *A*, operative stage; *B*, initial stage; *C*, effect of handling intestine; *D*, progressive stage; *E*, complete circulatory failure. Roman numerals in lower spaces refer approximately to times when optical records shown in figure 2 were recorded.

sures retain all the details that we regard as typical of normal dynamic conditions of the circulation (fig. 2, *I* to *IV*).

The reactions induced by the preliminary operative procedures are therefore physiological in nature and probably no greater than occur in many reflex effects on the circulation in everyday life. Furthermore, vigorous and repeated blows upon the abdomen with a wooden mallet were found to initiate no more than a temporary disturbance of the vascular system in every respect similar to that produced by opening

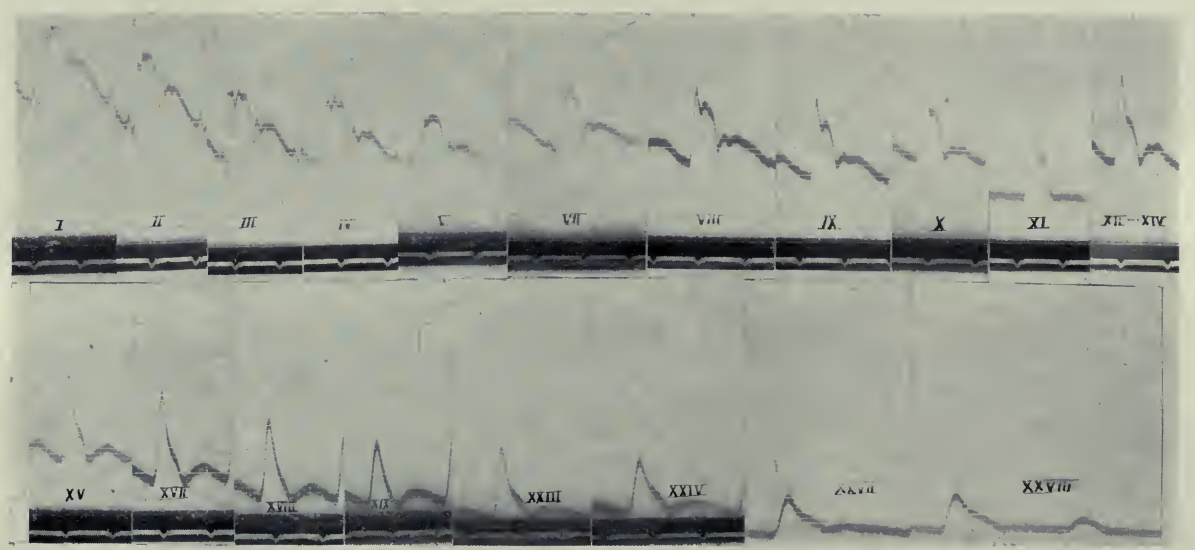

Fig. 2. (Three-eighths actual size). Segments of optical arterial tracings taken during progressive failure of the circulation in shock (exp. C-157). Numerals correspond to those in figure 1 so that relations to other events can be studied.

the abdomen and never followed by a condition that could by any stretch of the imagination be referred to as "circulatory failure." *Circulatory failure in abdominal shock, therefore, sets in only after the intestines have been exposed to the air for some time.* In the detailed analyses of records, however, we must consider carefully the possible bearings of these preliminary reactions upon the subsequent development of shock.

Effect of operative procedures on the circulation. Under light anesthesia the first incision through the skin and fascia of the abdominal wall produces a temporary cessation or diminution of the respirations, associated with a temporary fall of arterial pressure. The effective venous pressure is unaffected. From this reduced state of arterial pressure recovery is complete. Upon then extending the incision through the

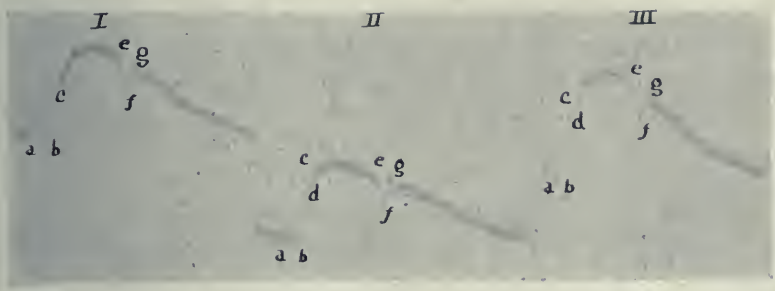

Fig. 3. (One-half actual size). Three segments of optical arterial tracings taken before (*I*), during (*II*) and after abdominal incision (*III*). (exp. C-152), showing the effect of cardiac inhibition on pressure variations: *a*, *b*, p eliminary vibrations; *b*, *c*, *d*, primary vibration; *d*, *e*, systolic summit; *e*, *f*, incisura; *f*, *g*, after-vibrations; *g*, *h*, diastolic period.

abdominal muscles and peritoneum the arterial pressure again falls in a similar manner but does not recover completely. The effective venous pressure is often lowered slightly (fig. 1).

An analysis of the kymograph records (fig. 1) corroborated by the more exact optical tracings of the carotid pressure (fig. 3) shows that the predominant cause of the blood pressure decline was a moderate reflex cardiac inhibition which was temporary after the skin incision but often became permanent after the peritoneal incision. The slight decrease in venous pressure was no greater than could be directly accounted for by the removal of the intra-abdominal pressure upon the abdominal veins and it is very doubtful whether the venous pressure

was sufficiently impaired to affect the systolic discharge appreciably. As a rule, this reflex cardiac inhibition left the arterial pressure at a level not far from but somewhat below normal, but in two instances it became so marked that the pressure fell very nearly to the critical level. It should be added that this reflex inhibition was obtained only when the heart rate was rapid on account of the anesthesia and was absent whenever the heart was already slow, due to high vagal tonus (e.g., after morphine). Nor was it present when the reflex arc was depressed either at the vagal terminals by atropine or centrally by deep anesthesia. It is therefore questionable whether this reflex inhibition comes into play at all in man when vagal control is already established. We may accordingly begin our analysis as to the real initiation of the circulatory failure in shock at this stage of the experiment.

Initial stages of circulatory failure in abdominal shock. Upon continued exposure of the intestines to the air, circulatory failure is initiated. As shown in the chart of figure 1, the first gross dynamic change occurred within the first half hour and consisted in a slight fall of mean arterial pressure. During this time the effective venous pressure remained unchanged or increased gradually if the cardiac rate remained unaltered (fig. 1). A careful study of the optical arterial tracings taken during the first half hour following exposure of the intestines, indicates clearly that this must be considered as the initial stage of circulatory failure even though the effective venous pressure does not alter or even increases and the mean pressure has fallen only to a small extent (Cf. note 2, page 493). These changes are well shown in segments *V* to *IX* of figure 2 which correspond to numerals indicated on the chart of figure 1. Owing to the larger amplitude of curves, they are shown even in better detail in the segments of another experiment reproduced in figure 4. In this experiment, twenty-four minutes after the intestines were removed the venous pressure was practically equal to that before removal and the arterial pressure had fallen only from 128 to 108, not an abnormal level of mean pressure. A comparison of the last three segments, taken at ten minute intervals, with the first segment obtained immediately after removal of the intestines, shows clearly that a definite reaction had been inaugurated in the vascular system. The preliminary vibration (*a* to *b*) has practically disappeared; the primary oscillation (*b*, *c*, *d*) is much larger and the systolic summit (*d* to *e*) gradually changes from an ascending to a horizontal and then to a descending plateau. The pressure at the beginning of diastole (*g*) is much lower and, in spite of the lower pressure level, the gradient of

the diastolic limb (*g*, *h*) is much steeper. *Such signs are indicative of a diminished distention of the large arterial trunks.* It is evident that the actual level of the mean pressure, as recorded by a damped mercury manometer, is no indicator of the important dynamic changes that have

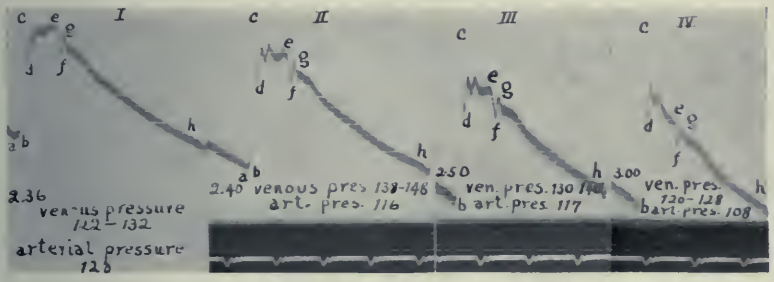

Fig. 4. (One-third actual size). Four segments of optical arterial tracings showing essential changes in arterial pressure variations in initial stage of circulatory failure when mean arterial pressure had fallen only slightly. Letters the same as before (exp. C-156): *I*, normal; *II*, after opening abdomen and intestinal exposure; *III*, ten minutes later; *IV*, twenty minutes later.

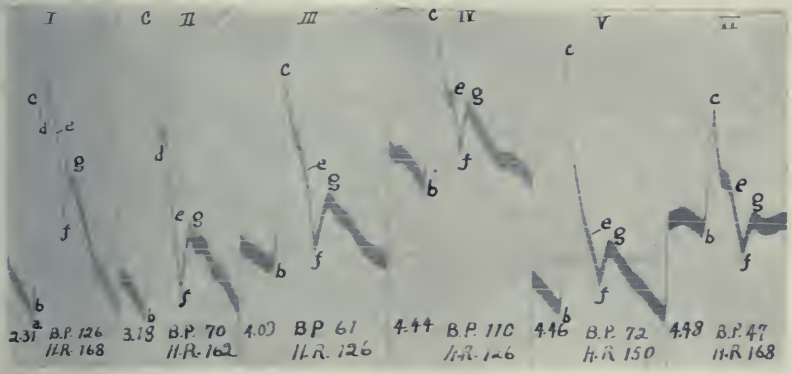

Fig. 5. (Two-fifths actual size). Segments of optical arterial tracings during initial and progressive stages of shock (exp. C-147). Letters same as before: *I*, after opening abdomen; *II*, during initial stage; *III*, during progressive stage; *IV*, after elevating animal board; *V*, immediately after 2.5 grains sodium nitrite; *VI*, two minutes later.

already taken place. Evidently, two factors tend to keep the mean pressure up in this case—the greater fling of the blood column, as indicated by the primary wave, and the abbreviation of diastole due to the cardiac acceleration.

Similar results are obtained in other conditions. Thus, when the aortic valves are suddenly rendered incompetent, the mean pressure sometimes undergoes no alteration or at most a very slight fall in spite of the most pronounced alterations indicated in the optical curves. When amyl nitrite is inhaled the mean carotid pressure may show no alteration or a rise, observations that have erroneously been attributed either to an irregular reaction of the animal by vasoconstriction or to the use of inferior drugs. Optical records show evidence of distinct vasodilation. In these cases also the mean pressure is prevented from falling by the same factors as we have in shock—the cardiac acceleration and the greater throw of the blood column in the less distended arteries.

Two factors may account for the diminished distention of the arterial trunks in the early phase of circulatory failure—a decreased total resistance and a reduced minute output of the left ventricle. In favor of the former are (1) the observation of the engorged intestinal vessels; (2) the more rapid decline of the diastolic limb of the pulse curve even

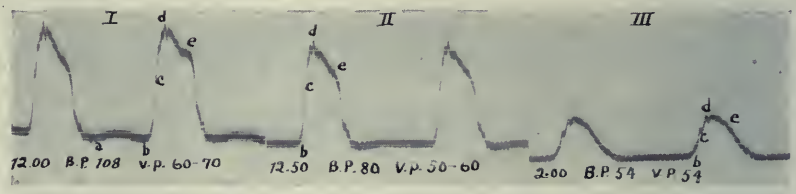

Fig. 6. (Two-fifths actual size). Segments of optical right intraventricular pressure curves in different stages of circulatory failure: *a*, *b*, auricular systole; *b*, *c*, isometric period; *c*, *e*, systolic ejection phase (exp. C-160). *I*, normal before operation on abdomen; *II*, after exposure and manipulation of intestines, initial stage; *III*, late portion of progressive phase.

though the pressure at the onset of diastole is much below normal; and (3) the fact that identical curves are obtained after the use of nitrites. Against the latter assumption are the high and often unaffected effective venous pressure and the accelerated heart rate which, on the basis of Henderson's work (2), may be anticipated to augment the minute output. Furthermore, the gradient of the ascending limb of the intraventricular pressure curve, which occurs during the isometric phase of cardiac contraction, remains unaltered even when venous pressure has actually become somewhat reduced. This is well shown in the first two segments of figure 6. In this experiment, fifty minutes after exposure of the intestine the right ventricular pressure curves, though smaller in amplitude, show no alteration of the gradient of the ascending limb (*b* to *c*) and the semilunar valves open (*c*) at the same level from the

base line. Only the slope of plateau (*d*, *e*) is modified. Yet at this time the effective venous pressure had fallen about 10 mm. of saline.

There is, therefore, no evidence that the diminished distention of the arterial trunks, which produces but a slight reduction of the mean pressure but pronounced changes in the optical tracings, is due to a decreased minute output of the heart, but distinctly favors the view that circulatory failure is initiated by reduced total resistance, presumably in the abdominal vessels.

The thought at once occurs to one: How is it possible, with a reduction of peripheral arterial resistance and the consequent accumulation of blood in the abdominal vessels, that the effective venous pressure can remain normal? Yet this is apparently the case, not only in the early stages of abdominal shock but in the most intense vasodilation it has been possible to produce by the use of nitrites. Augmented breathing and greater negative pressure variation within the thorax might be thought of, did this not happen even when respirations are entirely unchanged or are smaller and slower.

A record of the intra-auricular pressure in such experiments shows that the stability of the venous pressure is virtual and not real. It indicates, moreover, that an even finer conception of the relation between venous pressure and ventricular ejection is necessary than that stated by Henderson and Barringer (3). The differential water-manometer, as used, is a mean-pressure recording device capable of indicating only the respiratory variations with any degree of accuracy. The pressure in the larger veins and auricles, like the arterial pressure, is never constant but is always changing.[2]

The normal variations occurring in the auricle have been recorded by optical manometers and described by Piper (4), Garten and Weber (5) and the writer (6). They are also shown in segment *I*, figure 7. The pressure both rises and falls during auricular systole (*a*, *b*, *c*) and the fall is continued into that portion of auricular diastole comprised by the inter-systolic interval (*c*, *e*). With the onset of ventricular contraction (*d*) the pressure is abruptly elevated or lowered, differing

[2] The fact may be emphasized that mean pressure does not exist in any part of the vascular system. Theoretically it is a mathematical average of the individual pressure fluctuations. Practically, it is the pressure recorded by a very inefficient manometer which unfortunately rarely agrees with our theoretical figure. In the arteries it bears a variable relation to the systolic and diastolic pressures, depending on many circulatory factors which it is hoped some day to make the subject of a separate communication.

apparently in different animals. During the period of ventricular contraction (*d*, *e*) the pressure rises slowly and during the ventricular diastole it falls more or less until the next auricular systole. *It is the height of the effective pressure at the moment that the a–v valves open* (*e*) *which determines the diastolic ventricular filling; while the depth to which the pressure falls, in the common auriculo-ventricular cavities, just previous to ventricular systole* (*d*) *determines the volume and force of blood ejected by the ventricles, tersely expressed as the ventricular efficiency.* If we follow the changes in auricular pressure during the early stage of shock (segments *I* to *IV*, figure 7) it is found that exposure of the intestines alone reduces the return-flow of blood, as indicated by the

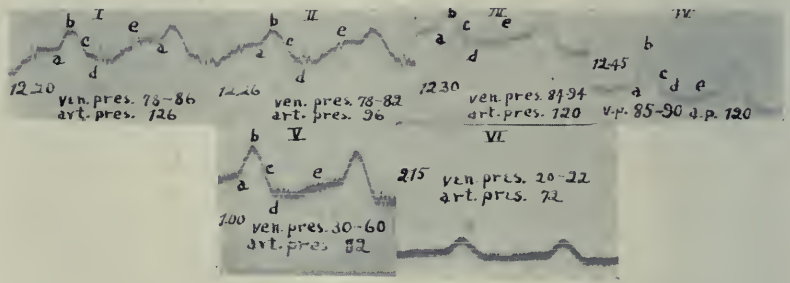

Fig. 7. (Two-fifths actual size). Segments of optical curves of right auricular pressure during different stages of circulatory failure (exp. C-158); *a*, *b*, *c*, auricular systole; *c*, *d*, intersystolic period; *d*, *e*, ventricular systole; *e*, *a*, ventricular diastose; *I*, normal; *II*, after skin incision; *III*, after intestinal exposure, initial stage; *IV–V*, progressive stages of shock; *VI*, complete circulatory failure.

slower rise of pressure from *d* to *e* (segment *III*). This is more than counteracted, however, by a greater pressure in some manner produced by auricular contraction (*a*, *b*) in consequence of which not only the general level of the curve is higher but the effective pressure and the initial pressure for ventricular contraction (*d*) is also higher. As long as the volume of blood returned to the auricles is not reduced too much these compensatory mechanisms are able to counteract the effects that would otherwise be produced by a deficient return of blood. By studying the finer details recorded by optical apparatus we can appreciate why the mean effective pressure can remain unaltered or even increase in spite of the fact that the return-flow of blood is partially reduced.

The stage of progressive circulatory failure. The stage of progressive circulatory failure extends from the initial stage to the time that the

mean pressure passes below 50 mm. of mercury. This figure has been arbitrarily selected by most experimenters as critical because it is difficult, by known methods, to restore the pressure to normal for any length of time when it has fallen below this level. The duration of this stage is variable, extending, in my experience, from thirty minutes to four hours. The general features are shown in the experiment plotted in figure 1. In this experiment the arterial pressure fell within one and one-half hours after intestinal exposure to 50 mm. This was accompanied by a rapidly progressing fall of the effective venous pressure. The heart accelerated. This is typical when vagus tonus is present at the beginning. The respirations increased in rate and amplitude.

The optical arterial pressure curves recorded during this stage become progressively smaller in amplitude and the features characteristic of a great depletion of the arterial trunks become more pronounced. This transformation is shown in segments *X* to *XIX*, figure 2, taken from the same experiment that is plotted in figure 1. They are also shown in the six segments of figure 5. If we compare segment *III* of this figure taken during the stage of progressive failure, with the normal, shown in segment *I*, we find that the complex central arterial pressure curve, normally present, is transformed to a curve with a very simple contour. The incisura (*e*, *f*) is less abrupt and is followed by a slow instead of a sharp rise (*f*, *g*) which results in the appearance of a dicrotic wave in the central pulse.

Recovery of the mean pressure, and in a certain measure of the typical form of the pressure tracing, was attained by increasing the venous pressure by tilting the animal board upward at an angle of approximately 30°. The changes are shown in figure 5, segment *IV*. When the pressure was thus restored, 2½ grains of sodium nitrite were given intravenously. Thereupon the mean pressure fell to 47 mm. mercury and the heart accelerated. The pulse contour during the early period of the pressure-fall is shown in figure 5, segment *V*. It is characterized by a rapid rise, mounting to a sharp peak; followed by a rapid systolic decline. The incisura (*e.f.*), which can scarcely be differentiated from this fall, is again followed by a dicrotic wave. During the depth of the mean arterial pressure fall the chief characteristics of the previous optical tracing are retained, except that the amplitude is smaller and the incisura deeper. The pressure line during the short diastole is practically horizontal (fig. 5, seg. *VI*).

The facts (1) that the mean pressure and the normal contour of the central pulse can in a measure at least be restored by increasing venous pressure, and (2) that the peripheral vessels are still capable of dilating in reaction to vasodilator drugs, indicate that the progressive deficiency in the arterial volume during this stage is predominantly due to low venous pressure. This is also corroborated by the changes in intra-auricular and intraventricular curves obtained during this stage of circulatory failure. As seen in figure 7, segments *V* and *VI*, the pressure within the auricle rises less and less during ventricular systole, finally remaining absolutely horizontal except when auricular contraction causes an elevation. In consequence, the initial pressure within the ventricle decreases and the intraventricular pressure curve, as shown in figure 6, segment *III*, shows a small gradual rise during the isometric period and a rounded contour during the ejection period, both characteristic of low initial pressure (1).

Stage of complete circulatory failure. When complete circulatory failure had been established the effective venous pressure was extremely low; the heart began to slow and thereby further reduced the minute output of the left ventricle. The impression is gained from these experiments that this is the final cause of circulatory failure in shock.

The progressive changes in the optical arterial tracings during this stage are shown in figure 2, segments *XIX*, *XXVIII*. The pulse form is always of a very simple type and resembles the peripheral pulse in normal animals. The rise is more gradual and the pressure falls markedly during the later systolic period. There is no sharp incisura and the pressure at the beginning of diastole is very low and decreases very little during the diastolic period. Evidently, the peripheral flow entirely ceases during diastole and is limited to the period of systole.

Effect of handling the intestines. The sequence of dynamic changes here discussed follows when handling of the intestines during the course of the experiment is reduced to a minimum. The act of manipulation was found to cause a variety of reactions in different animals. In some cases, as charted in figure 1, it caused a temporary reduction of arterial pressure accompanied by a decreased venous pressure. The optical arterial pulse shows an essential difference in that, during the diastolic period, the pressure line remained practically horizontal (fig. 2, cf. seg. *X* and *XI*). From this state the mean arterial pressure, as well as the contour of the pulse curve, recovered but the venous pressure often remained low. Apparently, the dynamic changes are associated with direct vascular effects produced by mechanical irritation. Hand-

ling was sometimes accompanied by reflex cardiac inhibition, in which case the mean arterial pressure fell markedly without any alteration of the effective venous pressure. Such effects were temporary, as a rule, and did not persist even when handling was long continued. In other cases intestinal manipulation caused an increase in rate and depth of respiration and a marked elevation of arterial pressure due to a removal of cardiac inhibition and consequent acceleration of the heart.

SUMMARY AND DISCUSSION OF RESULTS

The course of the circulatory failure in abdominal shock may be divided into three stages:

1. *The initial stage,* lasting about thirty minutes after intestinal exposure, during which effective venous pressure and cardiac discharge are apparently not appreciably reduced but the arterial pressure, as recorded precisely shows distinct alterations not adequately indicated by mean pressure manometers.

2. *The progressive stage,* lasting two to four hours, during which effective venous pressure falls progressively, cardiac efficiency is impaired and the arterial pressure falls toward a low level. The heart usually accelerates.

3. *Complete circulatory failure,* marked by a prolonged period during which effective venous pressure has reached its lowest level and the arterial pressure slowly falls further until death supervenes. During this stage cardiac slowing usually takes place.

A careful study of the optical tracings of arterial, intraventricular and auricular pressures, accompanied by constant readings of the mean arterial and effective venous pressures during these stages, corroborates the conclusion—in the ascendency at the present time—that *the decreased venous pressure and consequent reduction in minute output is the predominate factor in the pronounced fall of arterial pressure during the progressive stage of shock.* The dynamics of the circulation indicate clearly, however, that *a reduction in peripheral arterial resistance initiates the fall of arterial pressure and the diminished filling of the arterial trunks* before the effective venous pressure and cardiac discharge are reduced.

The rôle that a diminished arterial resistance plays in the circulatory failure is therefore directly established for the first time. Hitherto, evidence regarding the state of peripheral resistance in shock has necessarily been inferred from the state of the peripheral vessels.

Although vasodilation following vasomotor exhaustion has been suggested as a cause of circulatory failure in shock by Keen, Weir Mitchell and Morehouse (7) in 1864 and received experimental support from Fisher (8) in 1870 and from Crile (9) in 1899, the bulk of subsequent work has failed to confirm the hypothesis that peripheral resistance in shock is reduced. The experiments of Seelig and Lyon (10), of Seelig and Joseph (11), Morrison and Hooker (12), Muns (13), Guthrie (14) and others, in fact, indicate that the resistance in the peripheral vessels, on the contrary, is increased. The experiments of Bartlett (15) and Erlanger, Gesell, et al. (16), however point to a reduced vascular tone. In view of such results, the tendency of the present time is to favor the view that a reduction in peripheral resistance plays no part in the failure of the circulation in shock (cf. Henderson (17)).

With the direct demonstration in these experiments that a reduction of peripheral resistance occurs it is important to recall that the total resistance offered to the exit of blood from the arterial trunks is not entirely controlled *a*, by the state of arterial tone but may be modified; also *b*, by the caliber of the capillaries; *c*, by the "head on" pressure in the peripheral veins; *d*, by variations in extra vascular support; and *e*, by the viscosity of the blood. Furthermore, the total peripheral resistance is determined by the combined resistances in all the peripheral branches. It is therefore quite conceivable that the arterioles in the organs specifically subjected to trauma dilate and that a compensatory constriction occurs in other organs not so affected, in which case the total resistance to the aortic blood might still be reduced.

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THE MODE OF ACTION OF FOOD IN INCREASING OXIDATION

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Received for publication January 29, 1918

Lavoisier, shortly after his discovery that oxygen supported combustion, showed that food, work and cold increased oxidation in the body. Rubner (1) showed that of the foodstuffs, meat ingestion increased oxidation most, fat next and sugar least. Investigations have also been carried out to determine the stimulating effect of the different amino acids and of the sugars on metabolism, and it has been found that the different amino acids as well as the different sugars vary greatly in this respect. Lusk (2) found, for example, that the ingestion of glycocoll and alanin greatly increased heat production, leucin and tyrosin increased it very little while glutaminic acid was without effect. Several theories have been advanced in attempts to explain how food increases oxidation in the body. For discussion and literature on the subject consult Lusk, *The Science of Nutrition*, 1917; Dakin, *Oxidations and Reductions in the Animal Body*, 1912; and Kastle, *The Oxidases*, Bulletin 59, 1910, Hygienic Laboratory, Washington, D. C.

We (3) have shown that when oxidation is increased, as for example, by increasing the amount of work, by thyroid feeding, by fighting, in the excitement stage of anaesthesia there occurs a corresponding increase in catalase, and that when oxidation is decreased, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, in deep narcosis, in "surgical shock," or rendered defective as in pancreatic diabetes, there occurs a corresponding decrease in catalase. From these results it was concluded that it is probable that catalase, an enzyme found in the tissues and possessing the property of liberating oxygen from hydrogen peroxide, is involved in the oxidative processes, possibly in the manner suggested by Bach and Chodat. The object of the present investigation was to determine if the ingestion of food increases the catalase of the tissues and, if so, how this is brought about. Dogs were used in the investigation. The experiments were begun by

depriving the animals of food for twenty-four hours. At the end of this time, 300 cc. of a peptic digest were introduced into the stomach of each animal by means of a stomach tube, and 100 grams of finely ground lean steak were given also. The digest was made by adding 50 grams of a commercial preparation of pepsin to 75 grams of finely ground lean beef in 300 cc. of 0.5 per cent hydrochloric acid solution. The mixture was permitted to stand in a thermostat at 40°C. for twenty-four hours. Previous to the introduction of the digest into the stomach of the animals at least two determinations were made of the catalase of the blood taken from the external jugular vein. After the introduction of the digest the determinations of the catalase of the blood were also made at intervals of thirty minutes. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. After the volume of gas thus collected in ten minutes had been reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken at a fixed rate of one hundred and eighty double shakes per minute during the determinations.

In figure 1, curve 1 was constructed from data obtained from a dog previous to and after the administration of 300 cc. of a peptic digest. The figures along the abscissa indicate time in minutes while the figures along the ordinate represent the amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood. It will be seen that 0.5 cc. of the samples of blood taken previous to the introduction of the digest liberated 42 and 42 cc. of oxygen respectively; that thirty minutes after the digest was introduced, the blood liberated 48 cc. of oxygen; that sixty minutes later, it liberated 55 cc. of oxygen; after ninety minutes, 52 cc., and after one hundred and twenty minutes, 50 cc. It will be seen from this curve that the effect of the digest was to increase the catalase of the blood during the first hour by about 30 per cent, while during the second hour it was decreased. Curve 2 was constructed from data obtained in a similar manner as was that for curve 1, except this dog vomited the digest shortly after it was introduced. Curve 3 was obtained from a normal dog without the administration of anything. Curve 4 was obtained from a poorly fed dog. This dog was not at all particular about its food, eating everything that was given to him. Previous to and after the introduction of the digest, he gnawed on bones

continuously except at the times when the blood was taken from the external jugular. It will be seen that the catalase of the blood of this animal was increased by about 160 per cent one hour after the intro-

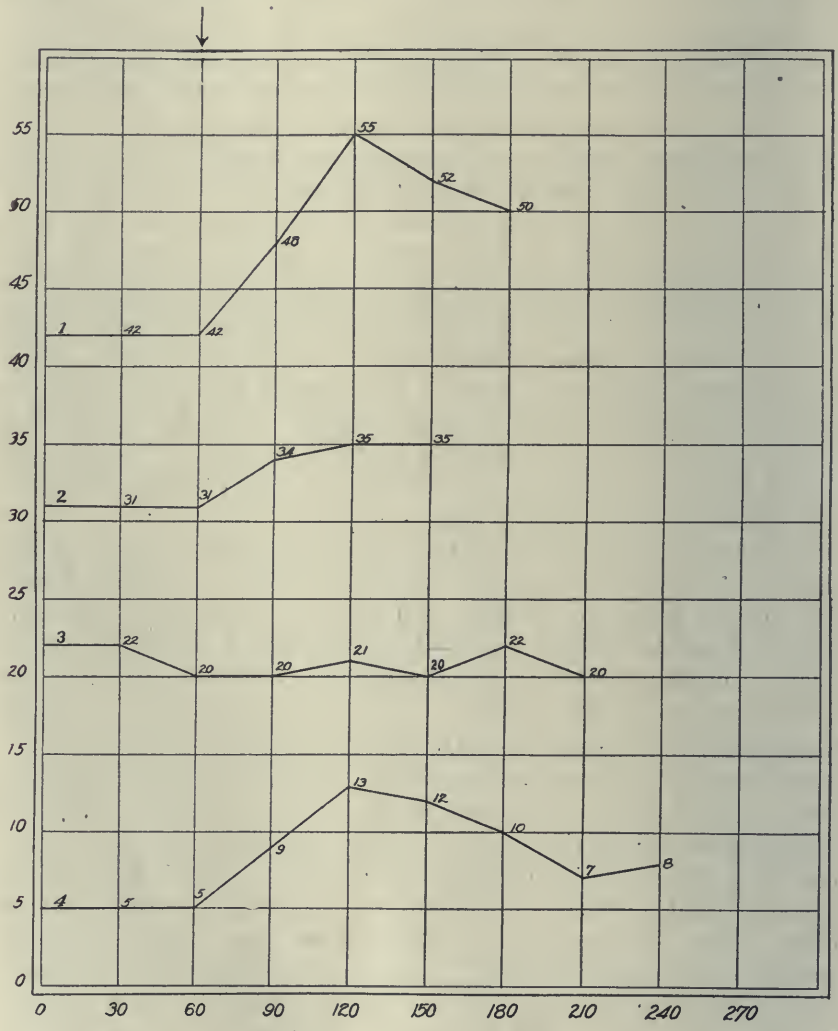

Fig. 1. Curves showing effect of food on the catalase content of the blood. The figures along the abscissa (0 to 270) indicate time in minutes, while the figures along the ordinate (0 to 55) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

duction of the digest, and that it had returned practically to the normal two and a half hours later. The low catalase of the blood of this dog is attributed to poor nutrition. These results show that food increases the catalase of the blood and hence of the tissues, parallel with the increase produced in oxidation.

The second part of this paper is concerned with determining how the ingestion of food produces an increase in catalase with resulting increase in oxidation. The digests used in the preceding experiments were tested for catalase and found to be negative, so the increase in the catalase of the blood could not have been due to catalase contained in the absorbed material. Furthermore, the contents of the stomach were also found to contain very little or no catalase, hence the increase in the catalase of the blood produced by the food must have been due to the stimulation of some organ or organs to an increased output of this enzyme. We had already found that the introduction of alcohol into the stomach of an animal greatly increased the catalase of the blood, and for this reason alcohol was the stimulant used in our attempts to determine what organ or organs are responsible for the output of catalase into the blood.

All the curves in figure 2 except curves 6 and 8 were constructed from data obtained from dogs previous to and after the administration of alcohol. Curve 1 was constructed from data obtained from three dogs whose livers had been cut out of the circulation by means of Eck fistulae and by the ligation of the hepatic arteries; curve 2, after the extirpation of the pancreas and spleen of two dogs; curve 3, from a dog into whose stomach 150 cc. of 45 per cent ethyl alcohol had been introduced through the walls of the stomach by means of a hypodermic needle after tying a ligature around the pyloric sphincter, thus preventing the passage of the alcohol into the intestines; curve 4, from a normal dog into whose stomach alcohol was introduced by means of a stomach tube; curve 5, after the extirpation of the pancreas; curve 6, from a normal animal without the administration of anything; curve 7, from two dogs into whose intestines 150 cc. of alcohol had been introduced through the walls of the intestines by means of a hypodermic needle after a ligature had been tied around the pyloric sphincter to prevent the passage of the alcohol into the stomach; curve 8, from a dog into whose jugular vein secretin, prepared according to the method of Starling, had been injected. In curve 1 it will be seen that after cutting the liver out of the circulation, the introduction of 150 cc. of 45 per cent ethyl alcohol into the stomachs of the animals increased the

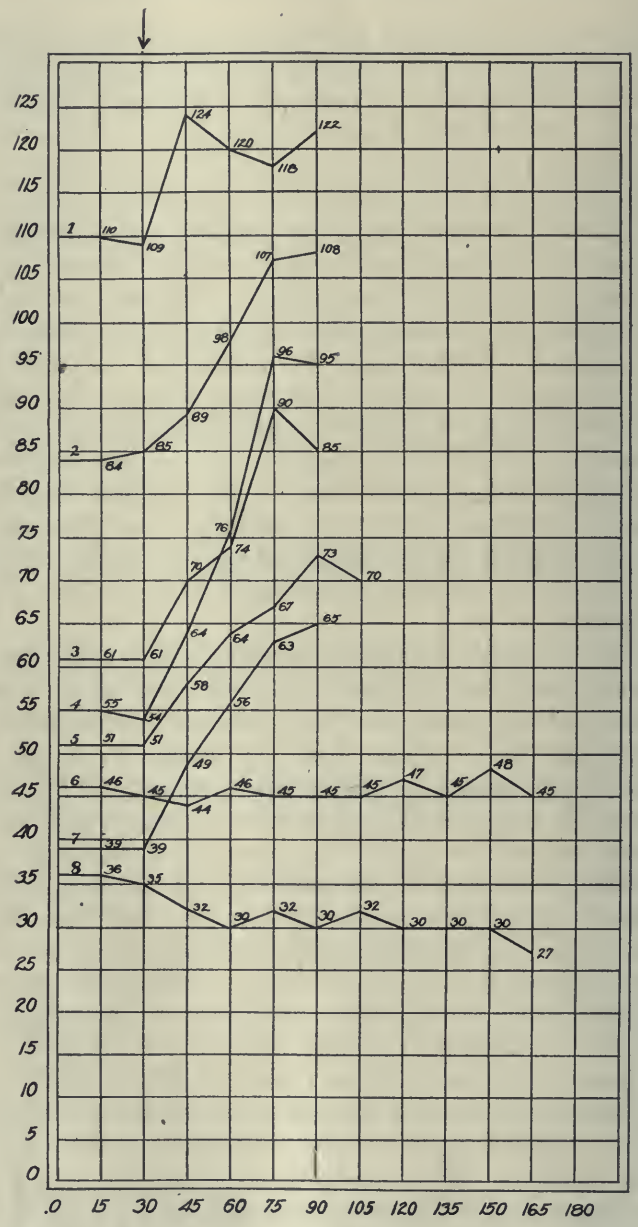

Fig. 2. Curves, except 6 and 8, showing effect of alcohol on the catalase content of the blood. The figures along the abscissa (0 to 180) indicate time in minutes, while the figures along the ordinate (0 to 125) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

catalase of the blood by about 13 per cent; while the introduction of a similar amount of alcohol into the stomach of a normal animal increased the catalase of the blood by about 80 per cent, as shown in curve 4, hence cutting the liver out of the circulation decreased the stimulating effect of alcohol by about 80 per cent. In curve 2 it will be seen that after the extirpation of the pancreas and spleen, the introduction of alcohol into the stomach increased the catalase of the blood by about 30 per cent, showing a decrease in the stimulating effect of alcohol by about 62 per cent from the normal. In curve 3 the introduction of alcohol into the stomach when the stomach was tied off increased the catalase of the blood by about 50 per cent; in curve 5 after the removal of the pancreas, alcohol increased the catalase by about 40 per cent; in curve 6 without the administration of anything, the catalase of the blood remained practically unchanged; in curve 7 after the administration of 150 cc. of alcohol into the intestines by means of a hypodermic needle when the pyloric sphincter was tied off, so that no alcohol could escape into the stomach, the catalase was increased by about 70 per cent; in curve 8 when secretin was injected into the external jugular vein of the dog, there was a small decrease in the catalase of the blood.

By comparing the data from which these different curves were constructed, it will be seen that the introduction of alcohol into the stomach of the normal animal greatly increased the catalase of the blood, while the introduction of a similar amount of alcohol into the stomach of animals whose livers had been shut off from the circulation, increased the catalase of the blood somewhat but not very extensively. This observation is interpreted to mean that the liver is one of the organs and probably the principal organ, stimulated by the alcohol to an increased output of catalase, thus producing the increase in the catalase of the blood. It will also be seen that the extirpation of the pancreas and spleen decreased the output of catalase into the blood after the introduction of alcohol into the stomach, showing that these organs, too, probably take part in the production of catalase after the administration of alcohol. That catalase is given off from the gastric and intestinal glands after the administration of alcohol is shown by the fact that the blood of the portal vein is the first to show an increase in catalase after the administration of alcohol.

From these observations it may be concluded that alcohol increases the catalase of the blood by stimulating the pancreas, the spleen, the gastric and intestinal glands and particularly the liver to an increased output of this enzyme. It is permissible, perhaps, to assume that food

like alcohol stimulates the production of catalase in the organs mentioned and in this way causes an increase in oxidations.

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THRESHOLD VALUES IN THE SPINAL FROG

I. Comparison of the Flexion Reflex and the Nerve-Muscle Response

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Received for publication January 19, 1918

The calibration of the inductorium by Martin (1) has made possible not only the accurate measure of the physiological intensity of induction shocks, but a statement of the value of these stimuli in units which can be used as standards of comparison or which can be duplicated in any laboratory. The completion of the calibration is so recent that only a small amount of data has been obtained regarding the irritability of various tissues. Martin (2) has applied his method chiefly to the determination of sensory thresholds under various conditions. Porter (3) has studied the variations in the irritability of the reflex arc in the spinal cat under normal conditions, in asphyxia and under the influence of strychnine. No observations taken by the Martin method have been reported on the thresholds of electrical stimulation for reflexes in cold-blooded animals. Such data would make possible a comparison of the irritability of various tissues in warm- and cold-blooded animals under similar conditions. It is of biological interest to know whether the threshold for the flexion reflex is of the same order of magnitude in the frog and in the cat.

The threshold values given in this paper are from a series of experiments which were preliminary to an investigation of the effect of changes of temperature on the synapse. They support the view that the synapse is a point of resistance in the reflex arc.

PROCEDURE

The preparation used was the spinal frog (Rana pipiens) made by destroying the brain in the ordinary manner. The shock of the operation disappeared entirely in about fifteen minutes, when the animal took the characteristic posture of a spinal frog and reflexes could be

easily elicited. During the period before recovery the frog was prepared for the measurement of the threshold of the flexion reflex in the left hind leg, and the right hind leg was prepared for the threshold of a nerve-muscle preparation giving extension of the foot. A section of the left sciatic nerve about 3 cm. long was freed from the surrounding tissue and a silk ligature placed just below the point of branching above the knee. Distal to the ligature the nerve was destroyed. The nerve could then be lifted over the point of a Lucas (4) fluid electrode. The right sciatic was exposed and a ligature placed high in the thigh. Central to that point the nerve was destroyed. This nerve could also be slipped into the groove of a Lucas electrode.

Before the measurement of the thresholds the frog was suspended by its jaws from a femur clamp attached to a stand. The points of the electrodes were slipped under the nerves, the electrodes themselves being supported by clamps from the same stand. The whole set-up could thus be moved without disturbing the adjustment of the electrodes. The frog and electrodes were placed in a glass jar of cold-blooded Ringer's solution or in 0.7 per cent sodium chloride at room temperature.

An example of an experiment in which both Z and β were determined is given below. In this case Z was obtained with three different amounts of secondary resistance for greater accuracy in calculating β.

Experiment 4. The frog was prepared as described above for the determination of the reflex threshold. The left sciatic nerve was placed in the slot of the electrode. The primary current was made and broken by means of a Martin key (5) which short-circuited the make shocks. The resistance in the primary circuit was adjusted so that 0.1 ampere passed through the primary coil. The secondary coil was pushed out to the end of the scale and while the current was repeatedly made and broken the secondary was gradually moved toward the primary. A point was reached where the slightest perceptible flexion of the left leg occurred in response to a break shock, which marked the threshold for the reflex. The secondary position was 21.5 cm. A calibration table of the values for $\frac{M}{L}$ for the inductorium used gave 78.7 for this position of the secondary. Since $Z = \frac{MI}{L}$, for this determination Z becomes 78.7×0.1, or 7.87. Then 10,000 ohms additional resistance were put in the secondary circuit by withdrawing plugs from a resistance box. Again the threshold was determined and the Z calculated was 19.7. With 20,000 ohms additional the third value of Z was 34.3.

The resistance of the nerve was determined by the Kohlrausch (6) method and found to be 2000 ohms. The secondary coil itself had a resistance of 850 ohms. With the values of Z and the resistances two values of β were calculated according

to the formula developed by Wilbur (7). The values obtained were 4.4 and 4.2. For this experiment $\beta = 4.3$, $Z = 7.9$, $\frac{\beta}{Z} = 0.55$.

PRELIMINARY EXPERIMENTS

Before making a series of threshold determinations of the ipsilateral flexion obtained on stimulating the central end of the sciatic nerve, it was necessary to determine with certainty that the response was a reflex. Lucas (8) has reported that with the fluid electrode a spread of current to tissue 5 mm. distant could only be obtained by increasing the strength of the current eighty times that of the threshold stimulus. In all of the experiments reported here the neighboring tissue, with the exception of the nerve itself, was from 5 mm. to 10 mm. distant from the slot in the electrode. Spread from threshold strengths would hardly be expected. The response obtained was always clear cut flexion. With direct stimulation by spread of current to nerve endings or muscle fibers an indefinite response would be expected since both flexors and extensors were equidistant from the point of the electrode. When the dorsal and ventral roots were cut or the cord pithed, the response obtained with increased strength of stimulus was always a combination of the contractions of both sets of muscles.

Direct evidence as to the nature of the response was obtained by determining the threshold of the response before and after cutting the spinal roots or pithing the cord, and also by comparing the results obtained with the thresholds of the nerve-muscle preparation in the opposite leg subjected to the same conditions. The results showed that in order to obtain a response after pithing the cord, the stimulus had to be increased from forty to one hundred times, while of course no increase was necessary to obtain the nerve-muscle response in the opposite leg. One seems justified in concluding that the clear cut flexion obtained at the lower value was a reflex. The procedure of destroying the cord at the end of the experiment was carried out as a matter of routine in each experiment. A few of the results are given in table 1.

RESULTS

The values reported in table 2, are the threshold stimuli in Z units and in β units for the flexion reflex and for the nerve-muscle extensor preparations in spinal frogs prepared as described above. The determinations have been made at least fifteen minutes after pithing and usually before an hour had elapsed.

The most important results are those of β for the reflex and for the nerve-muscle preparation. The thirty-four values of β for the reflex obtained from different animals average 6.9, and the thirty for the nerve-muscle preparation give an average of 4.7. The higher threshold for the reflex is in accordance with other wellknown characteristics of the reflex arc summarized by Sherrington (9). The average value of Z for the reflex is 11.7, while that for the nerve-muscle preparation is 4.7.

TABLE 1

Results of experiments to show that the response obtained from the stimulation of the central end of the sciatic is a reflex and is not due to current spread

DATE	NO.		REFLEX THRESHOLD Z UNITS	NERVE-MUSCLE THRESHOLD Z UNITS
11/30/15	4	Before pithing cord	9.4	
		After pithing cord	1176.0	
12/11/15	12	Before pithing cord	8.6	4.4
		After pithing cord	236.5	4.3
1/19/16	20	Before pithing cord	17.1	
		After pithing cord	1248.0	
12/ 4/16	28	Before pithing cord	34.3	6.2
		After pithing cord	266.4	5.9
12/ 5/16	29	Before pithing cord	10.5	3.4
		After pithing cord	675.0	3.5
12/19/16	39	Before pithing cord	7.7	7.2
		After pithing cord	618.0	9.7

The calculation of β in many experimental procedures may become too tedious and results stated in Z units may be sufficient for the purposes of the experiment. It is therefore of practical importance to know the ratio of β to Z for the tissue used, if the results are to be stated only in Z units. In the case of the reflex this value is found to average 0.59. For the nerve-muscle preparation it is 0.56. For the reflex the greatest deviation from the average ratio is 0.18 or 32.8 per cent. The average deviation is 0.09 or 16 per cent. In the case of the nerve-muscle preparation, the greatest deviation is 0.31 or 55 per cent. The average deviation is 0.07 or 13 per cent.

It will be noted in table 2 that a few of the values for Z and β deviate

TABLE 2

DATE 1915–1916	INTERVAL AFTER PITHING BRAIN	REFLEX FLEXION OF HIND LEG—SCIATIC			NERVE-MUSCLE EXTENSION OF FOOT—SCIATIC		
		Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$
	h. m.						
11/24/15*	1.00	2.2	1.6	0.72			
11/30/15	†	8.5	5.9	0.69			
12/ 2/15	0.15	5.8	3.1	0.44	3.5	1.5	0.25
12/ 5/15	0.30	11.0	5.0	0.45	5.5	3.4	0.62
12/ 5/15	0.10	14.6	10.6	0.73			
12/ 6/15	0.10	2.9	1.2	0.41	1.9	1.1	0.58
12/ 7/15	0.30	4.7	2.3	0.49	3.0	1.7	0.57
12/10/15	0.20	8.0	4.5	0.53	4.7	2.7	0.57
12/11/15	0.10	4.9	3.6	0.73	3.4	1.8	0.53
12/14/15	0.20	12.2	6.4	0.52	4.5	2.6	0.58
1/ 5/16‡	0.15	9.5	5.7	0.60			
1/ 5/16‡	0.25	7.5	4.1	0.55	2.3	1.5	0.65
1/ 5/16‡	0.15	8.6	4.2	0.49	2.2	1.2	0.56
1/ 6/16	0.15	27.0	17.8	0.66	4.2	2.5	0.59
1/17/16	0.54	7.2	3.5	0.48	2.0		
1/19/16	0.35	17.5			8.4		
1/19/16	0.32	9.2			4.4		
1/20/16	0.35	24.8			6.6		
1/20/16	0.20	8.3	4.6	0.55			
1/25/16	1.09	7.4					
1/26/16	0.35	37.0	26.4	0.70	4.9	2.2	0.45
1/26/16	0.20	44.4	27.0	0.61	11.2	7.3	0.65
1/26/16	0.15	20.4	11.6	0.57	5.3	3.4	0.64
12/ 4/16	0.20	25.9	12.9	0.50	6.6	4.0	0.61
12/ 6/16	0.20	10.5	5.8	0.55	3.0	1.0	0.33
12/ 6/16	0.15	7.2	3.5	0.49	3.4	2.1	0.62
12/ 7/16	0.22	9.0	5.2	0.57	7.8	4.7	0.60
12/ 7/16	1.10	15.8	9.4	0.59	9.9	5.2	0.53
12/ 8/16	0.47	6.1	3.1	0.51			
12/14/16	0.45	6.6	4.4	0.67	2.4	1.5	0.62
12/16/16	1.05	11.9	9.1	0.76	8.8	6.7	0.76
12/16/16	1.20	3.0	2.0	0.67			
12/19/16	0.55	5.4	2.4	0.44	5.1	3.0	0.59
12/19/16	0.15	4.4	3.3	0.75			
Average 1: All experiments		11.7	6.9	0.59	4.7	2.7	0.56
Average 2: Omitting all 100 per cent from Average 1		8.6	4.6	0.54	4.3	2.5	0.56

* Platinum points; electrodes in air.

† Average of five observations during one hour.

‡ Central end of IX root (abdomen open).

more than 100 per cent from the average. The frogs which gave these extremely high thresholds were always confined to shipments of frogs which had been delayed in transit from the west and had suffered exosure to cold. It may be pointed out, however, that the ratio of β to Z was very little altered in these cases. Here the greatest variation from the average is only 0.11 or about 19 per cent. It seems justifiable, therefore, to omit the values obtained from these frogs in making corrected averages.

The revised averages for Z and β for the flexion reflex are 8.6 and 4.7 respectively. For the nerve-muscle preparation, in which wide variations were very few, the corrected values of Z and β are 4.3 and 2.5. The corrections have altered only slightly the ratio of β to Z, which becomes 0.54 for the reflex and remains 0.56 for the nerve-muscle preparation. Martin (10) gives 8.1 as the average β for 18 observations on the frog's gastrocnemius stimulated directly with platinum points. He finds the ratio of β to Z to be 0.49, and the greatest deviation about 33 per cent. The average variation was 15 per cent. Porter (11) reports the average threshold stimulus for the flexion reflex in the spinal cat (seventeen determinations) to be 2.7 β units. An extensor nerve-muscle preparation at the wrist he found to have a threshold of 1.4 β units. The average Z units for threshold stimuli for the reflex (sixty-six determinations) was 5.2, and for the nerve-muscle preparation (fifty-two determinations) it was 2.3. He found the average ratio of β to Z to be 0.57, calculated from the combined results of reflex and nerve-muscle preparation. He does not state these values separately.

In comparing the results of Porter with those presented in this paper, one should consider that the thresholds on the spinal cat were taken at 38°C. while those on the spinal frog were taken at room temperature, from 15°C. to 20°C. That this difference of threshold might well be accounted for on a temperature basis alone will be shown in a subsequent paper.

It is interesting to compare the values reported here and those of Martin and Porter, with the thresholds for sympathetic responses in the cat reported by Mendenhall (12). He found the thresholds for contraction of the pupil through cervical sympathetic stimulation to be 5.7 Z units and 3.3 β units. The threshold for retraction of the nictitating membrane stimulated in the same manner was 6.3 Z units and 3.7 β units. For vasoconstriction in the nasal vessels, elicited through the cervical sympathetic, he found the threshold to be 7.9 Z units or 4.6 β units, and the ratio of β to Z in these observations was 0.58.

The values 5.7 Z, 6.3 Z, 7.9 Z obtained by Mendenhall for autonomic thresholds, 5.2 Z for reflex threshold in the cat reported by Porter, and 8.6 Z for reflex thresholds in the frog reported in this paper, are suggestive when compared with the thresholds for nerve-muscle preparations, as 2.3 Z by Porter for the cat, and 4.3 Z in this paper for the frog. It is usually accepted as a fact that each synapse increases the resistance to the passage of an impulse, although up to the present the only quantitative evidence has been that presented by Sherrington and Sowton (13) and Porter (14). In the autonomic and reflex paths, from which the above values were obtained, at least one synapse is known to exist.

The results of this investigation, showing a difference of 4.3 Z units, or 2.1 β units between reflex and nerve-muscle preparation, bear out Porter's observations which suggest strongly that a certain amount of resistance lies in the synapse.

SUMMARY

1. The fluid electrodes of Lucas have been used to determine the threshold stimuli for the flexion reflex and for a nerve-muscle preparation in the spinal frog. The values are reported in the Z and the β units of Martin.

2. The average threshold stimulus for twenty-five determinations of the flexion reflex in the spinal frog is 4.6 β units. For the nerve-muscle preparation the average threshold stimulus for twenty determinations is 2.5 β units.

3. The average threshold value in Z units for twenty-eight determinations of the flexion reflex is 8.6; and for twenty-three determinations of the nerve-muscle preparation it is 4.3.

4. The average ratio of β to Z for the reflex (thirty determinations) is 0.59. The average deviation is 0.09 or 16 per cent. The average ratio of β to Z for the nerve-muscle preparation (twenty-one determinations) is 0.56. The average deviation is 0.07 or 13 per cent.

5. The values of threshold stimuli for the flexion reflex and for the nerve-muscle preparation support the view that the synapse is a point of resistance in the conduction path of a reflex arc.

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THRESHOLD VALUES IN THE SPINAL FROG

II. Variations with Change of Temperature

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Received for publication January 19, 1918

The Martin method makes possible an exact study of the effects of various conditions on reflex thresholds. Since 1860, when Kunde (1) came to the conclusion that reflexes were depressed by cooling, the effects of changes of temperature on reflex irritability have been studied by many investigators. Statements which are quite conflicting are found in the literature. Recently (1916) Van Leeuwen and Van der Made (2) have reported that the optimum temperature for reflex irritability in the winter frog lies at about 5°C. and 2°C. higher for the summer frog. They find in some cases a second optimum around 20°C.

That there should be a disagreement about the effect of cold on reflexes might at first seem strange, when it is well known that cooling depresses the activity of protoplasm (3). But the explanation of the varied results will probably be found in the means used in determining the reflex irritability. The most common method has been that of Türch, in which the foot of the frog is dipped in acid and the time before its withdrawal taken as an index to the reflex activity. Since the introduction of induction coils into physiological work faradic stimuli have been applied directly to the skin of the leg or foot. In both of these procedures the sensory end-organs are involved and summation of stimuli is a chief factor. It is obvious that such methods cannot give so consistent results as one in which single break shocks of known intensity are applied directly to the afferent nerve and the threshold thus determined at various degrees of temperature.

But of perhaps greater physiological interest is the effect of change of temperature on the reflex threshold when it is compared with the threshold of a nerve-muscle preparation subjected to identical conditions. Sherrington (4) has summarized the characteristic differences between conduction in nerve trunks and in reflex arcs. Among other differences,

conduction in the reflex arc exhibits a greater variation in threshold value of a stimulus, a greater fatigability, much greater dependence on blood circulation and oxygen and a greater susceptibility to drugs. One might expect that cooling would also have a greater effect on reflex arc conduction, in which synapse and cell body are concerned, than in nerve trunk conduction.

Many observers working in this field have failed to distinguish between a change of temperature as a condition and a change of temperature as a stimulus. This is especially true of the early investigators. Heinzman (5), Goltz (6), Foster (7) and Sedgwick (8) were at one time concerned with the question of the effect of gradual application of heat on reflex excitability, as when a decapitated frog is placed in a water bath and heated.

Githens (9) has repeated and extended Kunde's experiments on the action of strychnine at various temperatures, finding that a given dose causes a longer period of spasms at 5°C. than at 31°C. He concludes that 5°C. is the optimum temperature for this drug. It seems curious, however, that this greater duration of activity could be due to the irritability of the cord determined by the low temperature. The possibility that the drug was eliminated more slowly at low temperatures was not considered.

The objects of this paper are to present the results of a series of experiments in which the effects of changes of temperature on reflex irritability and on nerve-muscle irritability are compared, and to consider these results in the light of the "all or nothing" principle.

PROCEDURE

A spinal frog was prepared in the ordinary manner and both sciatic nerves exposed sufficiently to slip easily into the groove of the Lucas fluid electrode (10). The left sciatic was used to evoke reflex flexion in the same leg, the nerve being destroyed at the knee. The right sciatic was destroyed high in the thigh and the lower leg used for nerve-muscle extension of the foot. The two responses used differed only in that the reflex arc contained afferent nerve fibers, cell body and synapse in the conducting path, in excess of the efferent fibers, motor endings and muscle fibers which were involved in both responses alike. Sensory end-organs were excluded by stimulating the afferent trunk directly. Temporal summation of inadequate stimuli was avoided by using single break shocks given at sufficiently long intervals. The

time resistance was taken at the end of the experiment by the Kohlrausch method with the electrodes in place.

The frog was suspended by its jaws from a femur clamp attached to a stand. The points of the two Lucas electrodes were slipped under the nerves, the electrodes being supported by clamps from the same stand. The whole set-up could be transferred from one jar of Ringer to another of different temperature without disturbing the adjustment of the electrodes. The actual temperature of the frog was not taken as a matter of routine. A few experiments showed that the temperature of the frog as measured by a thermometer inserted through the oesophagus into the stomach became the same as that of the surrounding fluid in seven or eight minutes. In all cases sufficient time was allowed after changing the temperature for constant threshold responses to be obtained.

In order to exclude the possibility that a rise of threshold as the experiment proceeded might obscure temperature effects, the temperature of the animals was changed, in different experiments and sometimes in the same experiment, in both directions, as from low temperature to high, from high to low and then to high temperature again. These procedures eliminated time as a factor in the results. When the experiment was started at a low temperature the pithed frog was kept packed in ice from half to three-quarters of an hour previous to the first determination. Although the threshold values in Z units are sufficient for these experiments, for completeness the data necessary for calculating β were sometimes obtained; i.e., three values of Z at different secondary resistances for greater accuracy, and the tissue resistances.

PRELIMINARY EXPERIMENTS

Before studying the effects of changed conditions on the responses involved it was necessary to find the variations which might be expected under the conditions of the experiment at a given temperature. Sedgwick (11) has suggested that a suspended frog loses its reflex irritability after thirty or forty minutes especially if warmed. Such an effect as this would obscure the effects of temperatures and tend to raise the reflex thresholds as the experiment proceeded. Several experiments were performed, therefore, to test the constancy of the reflex response.

In experiment 4 the fluid electrodes were used and the constancy of the reflex followed for fifty-three minutes, a much longer period than the duration of any of the subsequent experiments on the effects of tem-

perature changes. Recently Porter (12) has shown that the flexion reflex in the spinal cat remains comparatively constant for an hour or more. The accompanying protocol and figure 1 show that within an hour the reflex thresholds are fairly constant at a given temperature.

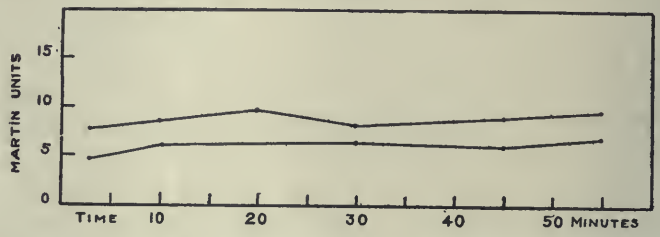

Fig. 1. Experiment 4, November 30, 1915. Variations in the threshold of the flexion reflex for a period of fifty-three minutes, plotted in Z and β units. Ordinate represents Martin units; abscissa, time in minutes.

Protocol of experiment 4, November 30, 1915

2.20 p.m. Brain pithed through foramen magnum.
2.25 p.m. Left sciatic nerve exposed.
2.30 p.m. Lucas electrodes applied to central end of sciatic.
2.40 p.m. Preparation immersed in cold-blooded Ringer solution at 20°C. Primary current 0.1 ampere.

TIME	TISSUE RESISTANCE AND COIL			10,000 OHMS ADDED			20,000 OHMS ADDED			0 OHMS
	Coil	$\frac{M}{L}$	Z	Coil	$\frac{M}{L}$	Z	Coil	$\frac{M}{L}$	Z	β
2.43	21.5	78.0	7.8	17.3	197	19.7	15.5	343	34.3	4.3
2.50	21.3	81.6	8.2	17.4	190	19.0	15.5	343	34.3	6.0
3.00	20.5	95.0	9.5							
3.10	21.4	80.4	8.0	17.1	209	20.0	15.7	319	31.9	6.4
3.25	21.0	86.0	8.6	17.0	216	21.6	15.6	331	33.1	6.1
3.40	20.4	97.0	9.7	16.9	222	22.2	15.5	343	34.3	7.2
Average...			8.6							6.0

The greatest deviation from the average for Z in experiment 4 was 1.1, or 12.8 per cent, while the average variation was only 0.6, or 7 per cent. For β the maximum variation from the mean was 1.7, or 28 per cent, while the average variation was 0.60, or 10 per cent. The ratio of β to Z was 0.69.

RESULTS

The figures presented in table 1 show clearly two important points. First, cooling increases the thresholds for both reflex and nerve-muscle response, while warming produces the reverse effect. Second, the reflex threshold is affected to a much greater degree than the nerve-muscle threshold by changes of temperature.

The first conclusion is to be expected from the general effect of lowering the temperature on biological processes, and the values obtained in these experiments without exception support this law. A few figures will serve to illustrate this point. In the experiment of De-

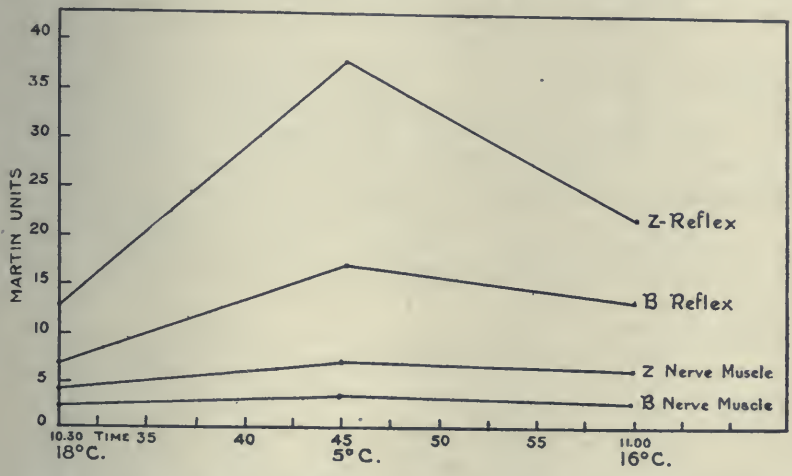

Fig. 2. Experiment 15, December 14, 1915. Reflex and nerve-muscle thresholds taken at 18°C., 5°C., and 16°C.

cember 11, 1915, a fall of temperature from 18°C. to 6°C. was accompanied by a rise of the reflex threshold from 4.9 Z to 10.7 Z, 3.5 β to 8.6 β, or a rise of about 130 per cent. The experiment of December 7, 1916, showed an increase of the reflex threshold from 10.5 Z to 24.7 Z or 5.2 β to 16.3 β caused by a fall of temperature from 20°C. to 6°C. The change here was about 170 per cent. The effect of a rise in temperature was shown strikingly in the experiment of January 17, 1916, when a change from 5°C. to 30°C. caused a lowering of the reflex threshold from 25.4 Z to 2.3 Z or 92 per cent. The nerve-muscle threshold in the same experiment fell from 3.0 Z to 1.5 Z or 50 per cent. A rise in the nerve-muscle threshold was shown in the experiment of December 14, 1916,

TABLE 1

Results of eleven experiments to compare the effect of change of temperature on reflex and nerve-muscle thresholds

DATE	TIME	TEMPERATURE	REFLEX				NERVE-MUSCLE			
			Z	Change	β	Change	Z	Change	β	Change
		deg. C.								
12/11/15	11.10	18	4.9		3.5		3.4		1.8	
	11.35	6	10.7	5.8	8.6	5.3	4.5	1.1	2.4	0.6
	12.00	16	8.6	2.1	6.9	1.7	4.4	0.1	2.9	0.5
12/14/15	10.30	18	12.0		6.4		4.4		2.5	
	10.45	5	38.0	26.0	17.1	10.7	7.2	2.8	3.6	1.1
	11.00	16	22.5	15.5	13.7	3.4	6.5	0.7	3.4	0.2
1/ 5/16	2.25	20	7.5		4.1		2.3		1.5	
	2.37	6	14.4	6.9	7.7	3.6	2.7	0.4	1.6	0.1
	2.55	19	10.3	4.1	5.0	2.7	3.1	0.4	1.9	0.3
1/17/16	3.35	5	25.4		18.0		3.0			
	3.44	16	7.8	17.6	3.8	14.2	2.0	1.0		
	3.49	16	7.2	0.6	3.5	0.3	1.8	0.2		
	3.57	30	2.3	4.9			1.5	0.3		
1/19/16	5.04	5	24.0				11.2			
	5.10	17	17.5	6.5			8.4	2.8		
1/19/16	2.25	5	24.0				9.9			
	2.35	15	15.6	8.4			5.9	4.0		
	2.45	24	9.2	6.4			4.4	1.5		
1/20/16	2.25	5	58.8				7.9			
	2.30	16	30.2	28.6			6.5	1.4		
	2.40	25	24.8	7.4			6.6	0.1		
	2.48	5	58.8	34.0			8.1	1.5		
	2.59	30	22.2	36.6			6.5	1.6		
1/25/16	4.15	16	17.1				16.6			
	4.32	8	20.4	3.3			17.1	0.5		
	4.39	32	7.4	13.0			14.4	2.7		
1/26/16	10.10	18	49.7				5.2			
	10.19	5	62.4	12.7			8.5	3.3		
	10.30	18	40.8	21.6			6.7	1.8		
	10.43	30	35.5	5.3			6.6	0.1		

TABLE 1—Continued

DATE	TIME	TEMPERATURE	REFLEX				NERVE MUSCLE			
			Z	Change	β	Change	Z	Change	β	Change
		deg. C.								
12/ 7/16	9.52	20	10.5		5.2		7.8		4.7	
	10.08	6	24.7	14.2	16.3	11.1	11.4	3.6	6.2	1.5
	10.22	20	18.5	6.2	9.8	6.5	10.9	0.5	6.5	0.3
12/ 7/16	2.44	4	18.5				10.5			
	2.53	18	12.5	6.0			9.9	0.6		
	3.07	7	26.9	14.4			11.2	1.3		
	3.18	18	15.3	11.6			10.7	0.5		

when a fall of temperature from 18°C. to 5°C. caused a change from 4.4 Z to 7.2 Z, about 60 per cent.

These figures and the others presented in table 1 seem to show that not only is the nerve-muscle irritability depressed on cooling, but, for the range of temperature between 30°C. and 5°C., cooling depresses reflex irritability.

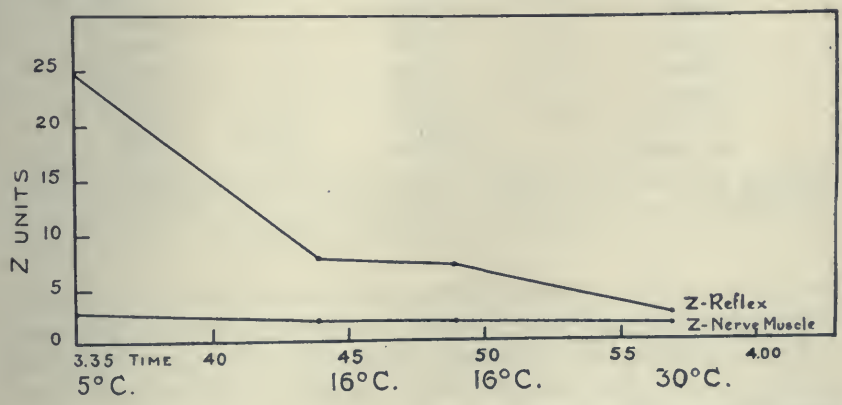

Fig. 3. Experiment 19, January 17, 1916. Reflex and nerve-muscle thresholds taken at 5°C., 16°C., 16°C. and 18°C. The frog was packed in ice for thirty-five minutes previous to the first determination.

The second conclusion, that the reflex irritability is more markedly affected by changes of temperature is in accord with other differences between the reflex arc and the nerve-muscle preparation. All of the determinations, with a single exception, in the eleven experiments gave

evidence for this point. In the experiment of December 11, 1915, for example, a fall of temperature of 12°C. caused an increase in the reflex threshold of 130 per cent, while the nerve-muscle threshold increased only about 32 per cent. In the experiment of January 17, 1916, a rise of temperature of 25°C. caused a fall of the reflex threshold of 92 per cent, and the nerve-muscle threshold was lowered but 50 per cent. An examination of table 1 will show other ratios of similar magnitude.

The figures in table 2 give the changes in Z units for a change of 1°C., both for the reflex thresholds and the nerve-muscle thresholds in each

TABLE 2

Change in Z units per 1°C. change of temperature

DATE	REFLEX	NERVE-MUSCLE
12/11/15	0.480	0.010
12/14/15	2.000	0.215
1/ 5/16	0.500	0.019
1/17/16	0.920	0.060
1/19/16	0.540	0.235
1/19/16	0.780	0.290
1/20/16	1.700	0.065
1/20/16	1.700	0.075
1/25/16	0.540	0.011
1/26/16	1.000	0.076
12/ 7/16	0.900	0.148
12/ 7/16	1.000	0.099
Average.....	1.005	0.109

experiment. The average change per degree Centigrade for the reflex threshold is 1.005 Z. For the nerve-muscle threshold this value is 0.109. The average deviation from the mean for the reflex is 0.31 or 31 per cent. For the nerve-muscle preparation the average variation is 0.074 or 68 per cent.

When the average change per degree for the reflex is compared with that for the nerve-muscle, the ratio is 9 to 1. In other words, for the range of temperature in these experiments the reflex threshold is affected nine times more than the nerve-muscle threshold by changes in temperature.

DISCUSSION

Little is known of the relative irritability of afferent and efferent fibers under various conditions nor is the phenomenon of conduction through the synapse well understood. If the reflex arc is more affected by changes of temperature than the efferent nerve fiber and muscle, as these experiments indicate, at what point in the reflex path does this greater effect take place? One must look to the afferent nerve, the synapse or the nerve-cell body, since the motor nerves and effector organs are common to both preparations.

There is no evidence that cold as a condition affects the irritability or conductivity of afferent fibers more than it does these phenomena

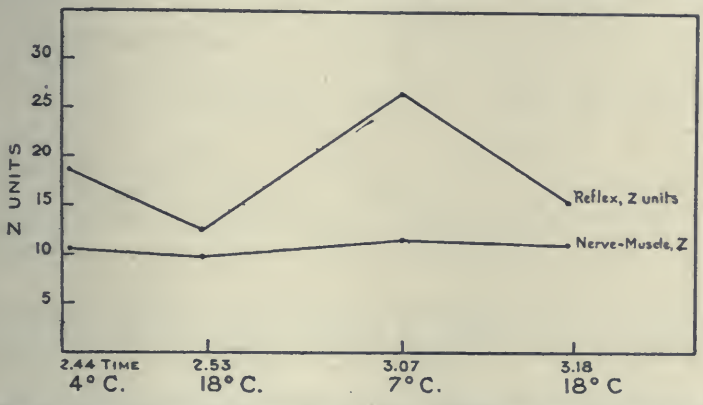

Fig. 4. Experiment 22, January 20, 1916. Reflex and nerve-muscle thresholds taken at 5°C., 16°C., 25°C., 5°C. and 30°C. The frog was packed in ice for fifteen minutes previous to the first determination.

in efferent fibers. Howell (13) states that motor fibers, however, are less easily stimulated by thermal applications than are sensory fibers, and suggests that this would seem to indicate some difference in structure or irritability between them. But until we have more direct experimental evidence that irritability and conductivity in afferent fibers are more susceptible to changes of temperature, it cannot be held that the afferent fibers would respond differently from the efferent fibers, either at low or high temperatures.

To the nerve-cell body is usually attributed the function of nutrition. Bethe (14) pictures the nerve fibrils passing directly through the synapse and cell body from one neurone to another. Whether such phenomena as "after-discharge," greater susceptibility to lack of oxygen, etc., are

due to the cell body, it is difficult to determine. Sherrington (15) rather favors the synapse as the point most easily affected by modifying conditions. The work of other investigators all indicates more or less directly that the characteristic features of reflex arc conduction are not referable to the nerve-cell bodies of the neurones. Bethe (16) in working on motor nerve-cells of Carcinus, and Steinach (17) in his studies on the physiology of the nerve-cells of the dorsal-root ganglia, suggest that the nerve-cell is little concerned with conduction. Langley (18) in showing that nicotine has little effect when applied to the dorsal-root ganglia, where no synapses are present, brings evidence to support the view that the synapse is the vital point in conduction through the reflex arc. It is probably the existence of the synapse

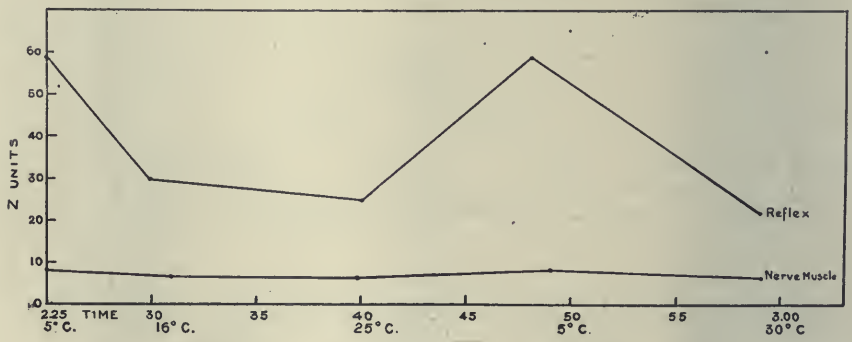

Fig. 5. Experiment 32, December 7, 1916. Reflex and nerve-muscle thresholds taken at 4°C., 18°C., 7°C., and 18°C. The frog was packed in ice for forty-five minutes previous to the first determination.

which makes the reflex arc so markedly susceptible to change of temperature.

How is a greater variation of threshold for the reflex arc than for the nerve-muscle preparation, when both are subjected to the same modifying conditions, to be reconciled with the "all or nothing" principle for nerve? In considering this question, it must be remembered that the threshold values obtained in the experiments reported here are an index of the number of nerve fibers stimulated to give a minimal response. A break shock of 2 Z, for example, stimulates all nerve fibers with thresholds lower than this value, and if 2 Z is the threshold value for the response, enough fibers must be stimulated to give the smallest discernible contraction.

For simplicity, let us suppose that the threshold of the nerve-muscle preparation N–M, figure 6, is 1 Z, while that for the reflex arc R is 2 Z. If these two preparations are cooled the threshold of N–M will be raised to 2 Z. If the "all or nothing" principle holds true for nerve, as Adrian's work seems to indicate, we may explain this rise in threshold by assuming that certain fibers are more susceptible to cooling than others and lose their function. But 2 Z will be strong enough to stimulate fibers of higher threshold that were not affected by 1 Z, and consequently enough functioning fibers are brought into activity at the higher threshold to give a minimal response. As far as the fibers themselves are concerned, we should expect that those of the reflex arc would be

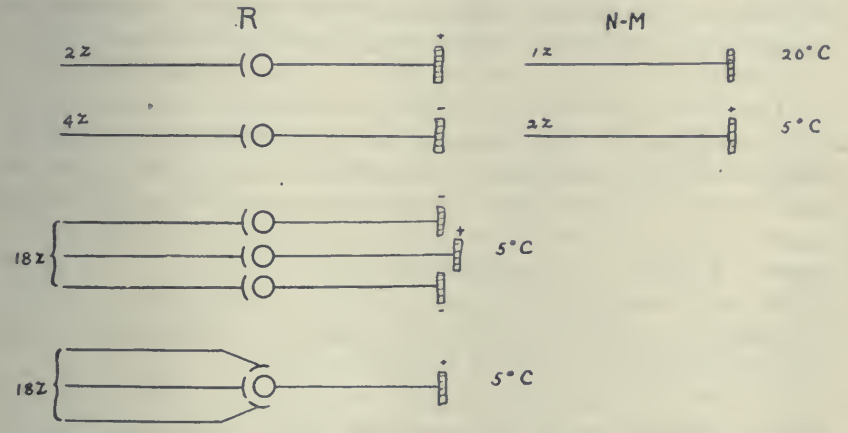

Fig. 6. In this diagram *R* represents the reflex arc with its synapse and nerve cell, *N-M* represents the nerve-muscle preparation. The + indicates a threshold response, the − indicates failure of the response at the stimulating values designated. Further explanation will be found in the text.

affected to the same degree by cooling, and a threshold of 2 Z would thus be raised to 4 Z. But a stimulating strength of 4 Z will not give a response. It is necessary to use a strength nine times as great as that for N–M under the new conditions, or 18 Z. It must be assumed that the synapses vary greatly, in themselves, in their susceptibility to cooling, so that although 2 Z at an ordinary temperature stimulated enough fibers with functioning synapses to give a minimal response, at a low temperature 18 Z must be used to stimulate enough fibers with synapses still functioning. There would be no necessity for any other view than that each nerve fiber, when stimulated by a shock sufficient to produce a local excitatory disturbance at the point of stimulation

great enough to discharge a propagated disturbance, responds with its maximum activity for the conditions.

Whether the actual propagated disturbance is smaller at low temperatures than at high is not known. Boruttau (19) has suggested that this is the case. Assuming this to be true, on cooling, enough afferent fibers carrying weakened impulses would have to converge on a single efferent fiber to produce a discharge through this path. In other words, there would be need for a sort of summation of stimuli through afferent fibers on a single efferent fiber. Very little is known about the passage of impulses from one neurone to another. The phenomenon of "after-discharge" suggests that some sort of summation may take place. And the greater number of afferent fibers than efferent fibers demands that more than one sensory fiber converge on a single motor neurone.

Adrian (20) has shown that anaesthetics affect the conduction of a nerve impulse by causing a progressive diminution in the intensity of the impulse until it finally ceases to exist. If cooling a nerve should cause a decrement in the size of the impulse as it passes away from its point of origin, this alone might account for the greater threshold necessary for the reflex response, because of the greater distance to the effector organs. A greater threshold stimulus would be necessary in order to affect a large enough group of fibers in which a certain proportion failed to conduct impulses to the muscle and in which enough fibers conducted the entire distance to produce a minimal response.

The results presented above may be interpreted according to the "all or nothing" principle by assuming that at low temperatures a greater threshold stimulus is necessary in order to include a sufficient number of functioning synapses, a certain number being entirely functionless at these temperatures. Or if it should be that the nerve impulse is much smaller at low temperatures, a greater number of fibers must be stimulated in order to conduct the weakened impulses to a smaller number of motor neurones, allowing summation and reinforcement to occur.

SUMMARY

1. The effects of changes of temperature on reflex arc and nerve-muscle preparation in the same animal are compared in spinal frogs. Changes of temperature were obtained, ranging from 4°C. to 30°C., by immersing the animal suspended by its jaws in normal saline or Ringer's solutions of various temperatures. The Lucas fluid electrodes were applied directly to the nerve-trunks.

2. Cooling depresses reflex irritability and nerve-muscle irritability, as indicated by a rise of the threshold strength of stimulus. Warming increases the irritability of both, as shown by a lowering of the threshold strength of stimulus.

3. Changes of temperature affect the reflex arc more than the nerve-muscle preparation. The average change per degree for the reflex is 1.01 Z units; and for the nerve-muscle, 0.11 Z units. The ratio is about 9 to 1.

4. The greater effect of cooling on the reflex arc is in accord with other differences between conduction in the reflex arc and conduction where no synapse is involved; and the results presented here suggest that the place of incidence of this greater effect is at the synapse.

5. The conclusions reached are reconciled with the "all or nothing" principle for nerve.

I wish to acknowledge my indebtedness to Dr. E. G. Martin, who gave me instruction in the use of his method, and to Dr. Alexander Forbes for his many suggestions.

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THE EFFECTS OF ADRENIN ON THE URINE FLOW OF ANESTHETIZED AND UNANESTHETIZED DOGS

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Received for publication January 14, 1918

The existing mass of data concerning the physiologic and pharmacologic action of adrenin on the various functions of the body is, with but few exceptions, complicated by the concomitant effects of general anesthesia. The data on the effects of the various anesthetics per se are lacking in many details. A possible synergic action of the drugs, which is entirely different from the action of either, may exist. Just what this action may be cannot safely be predicted. That anesthesia does affect the adrenin reaction has recently been shown in this laboratory by Berry (1), in his study of the effects of adrenin on the blood pressure reaction of the unanesthetized dog. The most striking effect observed was that ether anesthesia markedly depressed the blood pressure reaction to adrenin. The question then arises, Is this depression exerted on the vasomotor system as a whole or as is entirely possible, are the vasoconstrictor nerves selectively affected?

The extent of the data on the adrenin problem has reached such proportions that it would seem that the time is at hand for more intelligent generalization on the rôle played by the drug in the various normal and pathological activities of the body. Before such generalization can safely be attempted, however, the possible variable of anesthesia must be eliminated. The whole problem of the pharmacology of adrenin in the unanesthetized animal is therefore in need of study. Certain aspects of this problem are now under investigation in our laboratory. The first specific function to be considered is that of the effects of adrenin on the renal secretion.

The generally accepted action of adrenin on the secretion of urine is that of marked inhibition. The recorded literature on the subject, however, does not conclusively support this fact. Bardier and Fraenkel (2), who were the first to study the effects of adrenal extracts on the

urinary secretion, observed a marked reduction in the amount of urine passing from the ureters during an injection. With larger doses there was complete cessation of urine flow. These reactions were then followed by marked polyuria which lasted for some considerable time. Occasionally the injections were followed at once by polyuria. Their experiments were made on anesthetized dogs apparently under the influence of curare. Their extracts were made either from desiccated glands or from fresh glands macerated for twenty-four hours at body temperature. Judging from the effects on the arterial pressure, relatively large doses of the drug were employed. Whether the use of curare or the presence of protein decomposition products in their extracts played any part in their results was not determined. Schlayer (3) in his study of experimental nephritis observed that adrenin, when administered intravenously to animals containing much fluid, acts under certain conditions as a diuretic. In some unpublished studies on the effect of adrenin on the intestinal and renal secretion of the dog during hydraemic plethora, we failed to observe this effect. After repeated injections of varying dosages of adrenin the urinary output was decreased and was often one-fifth to one-tenth of that of the small intestine. Schlayer used large doses of adrenin without blood pressure control and the majority of his animals were renally abnormal. Biberfeld (4) reported that the subcutaneous injection of adrenin in doses of 1.5 to 2.5 mgm. per kilo, produces a marked diuresis in rabbits. Pollak (5) also observed diuresis to occur from adrenin injection. Sollman (6) observed that in the perfused kidney, when adrenin was added to the perfusate to make a dilution of 1-50,000, there was a marked decrease in the rate of urine flow. The concentration of adrenin used by this investigator, however, was such as to more nearly produce a toxicologic rather than the physiologic action of the drug. Meltzer and Auer (7) observed that adrenin restricts elimination and suggested that the substance probably interferes with the eliminating power of the kidney. Again, however, the effects of fairly large doses of the drug were studied whereas there is every probability that under physiologic conditions only minute quantities are ever present in the blood stream. Kleiner and Meltzer (8) later, in an extended comparison of the effects of subcutaneous and intramuscular injection of adrenin in rabbits, showed that the absorption of the drug must be at a very slow rate to produce diuresis. Diuresis occurred only with subcutaneous doses of 0.7 to 1.0 mgm. per kilo. Intramuscular doses of this size or larger doses subcutaneously were found to inhibit the flow of urine. Cow (9)

established the anatomical relationship of an anastomotic branch of the adrenal vein with the cortex of the kidney. From a series of perfusion studies he concludes that under certain conditions adrenin in appreciable amounts is poured directly into the kidneys from the suprarenal bodies of the intact animal, producing a diminution in the flow of urine.

Failing to find reported in the literature the effects of threshold dosages and of slow infusions of adrenin on the urine flow, which probably more nearly approach the normal discharge of the gland, it was necessary to study the effects of these dosages in the anesthetized dog for comparison with their effects in the unanesthetized animal.

METHOD

Medium sized dogs were used as experimental animals. Quiet, good-natured dogs were selected and great care used to avoid exciting them. After some petting, the dog was carefully and quietly laid on the operating board and strapped back down. In the early experiments one-eighth to one-fourth of a grain of morphine was administered but later with careful handling this was found to be unnecessary. One to two cubic centimeters of 2 per cent cocaine solution or an equal amount of 1 per cent quinine-urea bimuriate solution were injected intradermally over the femoral artery and vein just below Poupart's ligament. After waiting a few minutes for the anesthetic to take effect, arterial and venous cannulas were set. Next the skin and subcutaneous tissue over the lower median line of the abdomen were injected with the anesthetic. Again after waiting a few minutes an incision 5 or 6 cm. in length was made into the abdominal cavity, beginning at the upper border of the pubic bone and extending cephalad. The urinary bladder was then aspirated if necessary and drawn through this incision. The ureters were each isolated and cannulated close to the bladder and the viscus was replaced in the abdomen. The abdominal incision was then closed, leaving the cannulas protruding from the wound. All manipulations were made with as little trauma as possible.

The ureteral cannulas were led into a Y tube which extended over the edge of the table and the system was filled with water or salt solution. The blood pressure was recorded from the femoral artery with an undamped mercury manometer. The ureteral outflow was recorded in drops by means of a key and signal marker. Adrenin (Parke, Davis and Company's "Adrenalin") in varying doses was injected into the femoral vein, using a small amount of warmed 0.8 per cent sodium chloride solution to flush it in.

RESULTS

Rapid absorption of the cocaine solution was found often to make the animals very irritable. In one case the dog died in convulsions on the table. The observations made under cocaine anesthesia were therefore discarded. Quinine-urea bimuriate in 1 per cent solution was substituted. The perfect anesthesia resulting from its use was remarkable. The duration of the experiments was from three to six hours. Quite often the dogs would sleep throughout the greater part of this time.

For comparison, after a number of reactions had been recorded on the unanesthetized dog, ether was administered and the injections repeated. The effect of ether on the urine flow was very striking. As soon as the animal commenced to struggle against the anesthetic, the urine flow immediately ceased. The return was very slow requiring some fifteen to thirty minutes. In view of the following observed effects of adrenin on the urine flow, this might be interpreted as due to a discharge from the adrenal glands. It was not due to the struggling alone for in one dog that was sleeping the administration of ether occasioned a marked diminution in the urine flow without any struggling. The inhibitory influence of ether on the urine flow may well be due to adrenin for Elliott (10) has shown that ether produces discharge of the adrenal glands. On the other hand it must be recognized that this is only one of the various possible explanations. The recent work of Stewart and his collaborators (11) must make us cautious in ascribing to the adrenal glands the causation of reactions which can be explained on other grounds.

The effect on the urine flow of adrenin in all effective doses in both anesthetized and unanesthetized dogs was that of marked inhibition. In the unanesthetized animal the threshold for the reaction was found to be lower than after ether anesthetization. Figure 1 shows the results of an injection of 0.5 cc. of a 1-200,000 dilution of adrenin with practically a negative blood pressure reaction but with more than 50 per cent reduction in the urine output. This is the characteristic effect of injections of all dosages. The injection of larger doses produces a complete cessation of urine flow until ten or twenty seconds after the blood pressure has returned to normal. Figure 2 shows the effect of a pressor infusion. During the greater part of the blood pressure reaction there is practically a complete cessation of the urine flow. The normal outflow returns at about the same time the blood pressure

reaches normal. This type of reaction was observed with all strengths and rates of infusion, the degree of urinary inhibition varying directly with the concentration and rate of infusion.

The effects of infusions lasting fifteen to thirty minutes gave results which were somewhat striking. In dog no. 14 an infusion lasting thirty minutes and thirty seconds was started while the urine flow was approximately one drop in five seconds. During the first sixty-five seconds of the infusion, four drops of urine flowed. From then until the infusion was shut off, (twenty-nine minutes and twenty-five seconds,) but four drops flowed. Eight minutes after the infusion was shut off

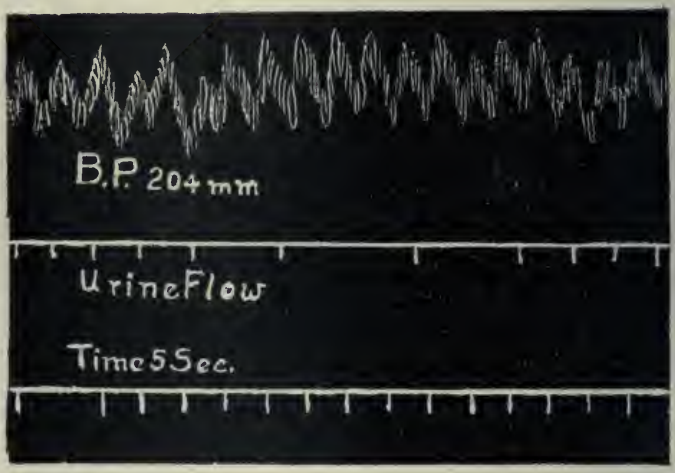

Fig. 1. Graph showing effects of threshold adrenin injection on urine flow and femoral blood pressure of an unanesthetized dog. Dose, 0.5 cc. of 1–200,000 dilution. Time, five seconds. No reduction. Dog weight, 14 kilos.

the urine returned to and remained normal. The quantity of the infusion was 30 cc. of a 1-50,000 solution of adrenin. There was a blood pressure rise of 20 mm. of mercury (from 120 mm. to 140 mm.). The most striking feature of such observations was that the urine flow should so soon return to normal after practically an anuria of so long a duration. It would seem to suggest that adrenin produces the marked inhibition on the urine flow in a way other than by the ischemia of renoconstriction. It is possible as Meltzer and Auer (7) have suggested that the substance in some way interferes with the eliminating power of the kidney and the action may be on the secreting cells directly. If this be true, the action is merely inhibitory for repeated injections

of the drug during an experiment did not permanently decrease the flow of urine.

Considerable attention was directed toward threshold dosages of the drug to detect the appearance of any diuretic effect but none was observed.

It was observed that when 10 to 20 cc. of the "flush in" solution were used with an adrenin injection, an "over-recovery" occurred in the urine flow. When smaller amounts of the "flush in" solution were used and with infusions when no "flush in" solution was used, this "over-recovery" was not observed and was due, therefore, either to the slight degree of hydraemic plethora induced by the salt solution or to a direct stimulation of the renal cells by the sodium chloride.

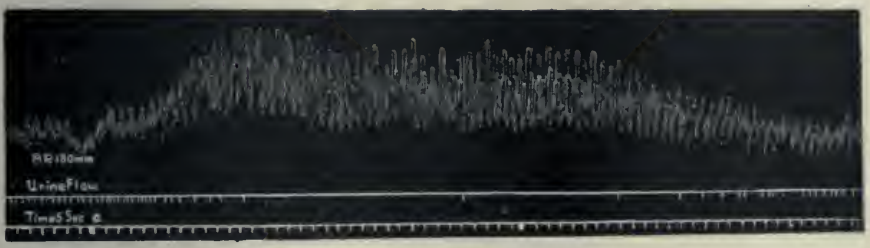

Fig. 2. Graph showing effects of adrenin infusion on urine flow and femoral blood pressure of unanesthetized dog. Dose, 20 cc. of adrenin 1-200,000 in one hundred and ninety seconds, from *a* to *b*. Time, five seconds. Reduced to one-third. Dog weight, 7 kilos.

SUMMARY AND CONCLUSIONS

1. Adrenin in all effective dosages administered intravenously inhibits the urine flow in both anesthetized and unanesthetized dogs.

2. The threshold of the reaction is slightly lower in unanesthetized than in anesthetized animals.

3. Small injections and infusions merely inhibit the flow of urine while larger doses produce a complete cessation of flow.

4. The inhibition usually persists until shortly after the blood pressure reaction is complete.

5. Diuresis succeeding the inhibition was not observed.

6. The rapid return of the flow to normal after prolonged infusions suggests that the drug exercises its inhibition on the kidney function in a way other than by the ischemia produced.

7. During the administration of ether the urine flow is completely checked and recovery under the anesthetic takes place slowly.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

Thirtieth Annual Meeting

Minneapolis, December 27 28, 1917; Rochester, December 29 1917

Some phases of industrial fatigue. Frederic S. Lee (for the Committee on Industrial Fatigue).[1]

The investigation of industrial fatigue was assigned to the author by the Committee on Physiology of the National Research Council. The Committee on Industrial Fatigue was thereupon organized under the Advisory Commission of the Council of National Defense and became associated with the U. S. Public Health Service, which appointed to positions on its staff several members of the Committee and appropriated a sum of money to defray the expenses of the work. Since July, 1917, the Committee has been engaged in an investigation of munit on factories from the physiological standpoint, with two aims in view: the more purely scientific one of learning how the human machine works in industry; and the practical aim of discovering the condi ions under which it can work most efficiently, and endeavoring to secure the establishment of these conditions in factories that are manufacturing war supplies. While visiting and observing the work in many places, two leading factories were selected for intensive study; a brass factory which was engaged largely in making fuses for explosive shells; and an automobile factory which was devoting a considerable part of its resources to government work. In the brass factory, the day shift works ten hours in two spells of equal length and has one hour's interval for luncheon; the night shift works twelve hours and has twenty minutes for luncheon immediately after midnight. In the automobile factory there is an eight hours' working period divided into two spells by a luncheon period of about one half hour. Especial attention has been given to studying quantitatively the output of the individual during each hour of the working period. More than thirty different operations, involving repetitive work, have been studied and from thirty to several hundred observations in each have been made.

The curve of output has been found to vary with the character of the work. Where close attention and exact muscular coördination are required, the average output curve of the individual during the first spell

[1] The Committee on Industrial Fatigue: Thomas Darlington, Chairman; Frederic S. Lee, Executive Secretary; Robert E. Chaddock, Raymond Dodge, David L. Edsall, P. Sargant Florence, Miss Josephine Goldmark, Ernest G. Martin, Ernest L. Scott and J. W. Scherescheswsky.

resembles that of a single excised muscle stimulated for a given period by a series of single induction shocks. In both there occur in succession a rise (treppe in the muscle, "practice effect" in the human being), a maximum, and a fall indicating fatigue. In operations that are markedly muscular, though here further observations are desired, there appears to be a steady fatigue fall from the first, with little or no practice effect, but often with a minor rise and fall ("spurt") just before the end of the spell. In monotonous operations involving frequent pauses, the curve after a slight practice effect becomes horizontal, with no fatigue fall. During the second spell, after rest and food, the curves are repeated as to their general form, but there appears at first a recovery of working power, if fatigue has resulted from the work of the first spell, and the final fatigue fall is more pronounced that at first. The operations that have been studied during the night shift of twelve hours reveal curves in general like those of attentive work, but during the last two hours output falls off greatly and during the final forty minutes often nothing whatever is produced.

Wherever the output curves show a pronounced fall as the spell proceeds, the introduction of rest periods is indicated, and the Committee has therefore recommended that in such cases each spell be broken by a ten minutes' resting interval. This has been done with certain groups of workers in the eight hour shift with the result, in especially fatiguing operations, of increasing the output by 6 to 8 per cent. The Committee further recommends that the twelve hour night shift be shortened by two hours, and this is about to be established experimentally with, it is hoped, no diminution and possibly an actual increase, of the total night's production.

The Committee finds very prevalent an output that is almost or quite uniform from day to day and week to week, even where piece-rate wages prevail. It is often far below individual capacity and perhaps represents a physiological defense of the individual against overdriving. How this "stereotyped" output may be eliminated and production be raised more nearly to physiological capacity is receiving the attention of the Committee.

The Martin spring balance muscle test, now used for the first time in industrial work, has shown that it is capable of revealing fatigue in the individual and also that each factory operation has its own standard of strength. The Committee recommends its use in factories in classifying workers and assigning them to their appropriate tasks and as a general test of the physical condition of workers from time to time.

The Committee has also made observations on certain chemical changes occurring in fatigue, on the rhythm of individual workers, on the course of accidents throughout the working spell, on the problem of labor turnover, and the occurrence of minor illnesses in workers, but their conclusions on these matters are not yet ready for publication. The work is still proceeding.

On Weichardt's so-called "fatigue toxin." FREDERIC S. LEE and B. ARONOVITCH.

When juice is pressed out of the muscles of fatigued animals and injected into other animals there result stupor and a temporary lowering of the body temperature. Such fatigue juice when administered to excised muscles causes a marked decrease in their working-power, as shown by a shortening of the working period and a decrease of the total work performed.

Juice from the muscles of non-fatigued animals exerts exactly the same action as fatigue juice, on both animals and excised muscles.

The experiments thus fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue. This conclusion is supported by an examination of Weichardt's writings, which shows that he uses the terms "toxin" and "antitoxin" vaguely and not in accord with the present use of serologists.

The quantitative measurement of general fatigue. A. H. RYAN (with the collaboration of J. H. Gordon).

The present experiments were begun in a search for further methods of measuring fatigue. The following tests were employed; visual acuity, as recently used by Stanley Kent; copying test, in which simple text was copied for a period of ten minutes, the characters written, and number of errors being computed; systolic and diastolic blood pressure; and the vascular skin reaction as described by Marey, consisting of the appearance of a white line on the skin, after the skin is gently stroked with a blunt instrument. The stroke was made on the flexor surface of the forearm, and sometimes on the chest and back, and the white streak which occurred was studied, in regard to latent period, time required for reaching the maximum intensity, time at which it began to spread and fade, and the time of complete disappearance. An instrument was devised by which the stroke could always be made with the same pressure, adapting the principle of Grützner's "myographion."

In the first series of experiments on a group of five hospital nurses, lasting three weeks, the observations were made in the morning, at noon, and in the evening, after meals. In regard to the visual acuity, the copying test, and the blood pressure, no definite relation could be established between work, rest in the afternoon, and loss of sleep, and the results of the tests. In regard to the vascular skin reaction it was found that the length of time between the making of the stroke, and the beginning of fading of the streak, fading time, was always decreased by work and activity, and increased by rest or sleep. The recovery at night was less if the night's rest was poor.

In several subjects climbing a hill for half an hour, there always resulted a definite decrease in the fading time of the reaction, which was recovered from, by reclining. Partial recovery from strenuous work was observed during the progress of lighter work.

Daily observations on the vascular skin reaction in one subject, for two weeks, at one and a half hour intervals during the day, showed a

continuous decrease in the fading time of the reaction during the day, in proportion to the severity of the work. Partial recovery occurred during lunch hour, and whenever the subject rested.

A short series of control experiments were made upon the effect of temperature; also upon subjects resting throughout the day. The influence of barometric pressure was ruled out.

Four of the nurses had "colds," and one had a recurrence of fever, following an old tuberculous peritonitis. These conditions were either preceded by, or occurred simultaneously with, a low level in the fading time of the reaction.

The following observations were made relative to the mechanisms concerned in the production of the streak. First, when a certain subject was "chilly," "goose-flesh" could be obtained wherever the stroke was made, and its appearance considerably preceded the appearance of the white streak, and disappeared sooner. Second, a white streak which has almost completely faded, may be revived by making a stroke in a neighboring skin area. Third, embarrassment may result in the appearance of a red streak preceding the white streak. This was first observed in a girl who had made a misstatement regarding a question in her history of the previous day.

Strength tests in industry. E. G. Martin.

Tests of muscular strength, according to a method previously reported, were made on operatives in munition factories. The strength is determined by measuring with a dynamometer the maximum resistance to a pull which overcomes the contraction of the muscle-group under examination. To make the test practicable for industrial conditions it was abbreviated by using only five pairs of muscle-groups (pectorals, flexors of forearm, flexors of wrist, adductors of thigh, abductors of thigh), and computing from these the entire bodily strength. Previous investigation had shown that this computation is accurate within an average range of 5 per cent.

The following results were obtained:

The normal non-athletic individual tends to show a strength whic is roughly twenty-five times his weight. The chief exceptions will be found among very light and very heavy individuals.

In industrial operations calling for the exercise of strength there is a pronounced tendency toward a standard strength for each job. In other words, operations in which a definite exertion is required tend to develop a degree of strength determined rather by the exertion than by the weight of the worker.

Comparisons of strength at the beginning and end of the work-shi t showed that in general there is in the easy jobs a tendency to make a better showing at the end than at the beginning. In the operations requiring moderate exertion the stronger workers do better at the end than at the beginning, but the weaker workers are likely not to do so well at the end as at the beginning. In the hard jobs all the workers tend to do less well at the end than at the beginning.

Comparisons of the daily strength records of workers with their daily production records show that on days in which good strength records are made production is likely also to be good, and on days in which poor strength records are made output is likely to be poor.

Nutritive factors in some animal tissues. LAFAYETTE B. MENDEL and THOMAS B. OSBORNE.

Rats fed on a diet in which skeletal bee muscle formed the sole source of protein and water-soluble vitamine grew for a few days and then declined. Addition of small amounts of yeasts to the food promoted rapid recovery and growth. Accordingly such muscle contains protein suitable for nutrition but is deficient in the water-soluble vitamine.

Beef thoroughly extracted with water and fed as the sole source of protein and water-soluble vitamine in an otherwise adequate food mixture failed to promote growth; animals thus fed declined immediately. The addition of small quantities of yeast, corn germs, wheat embryo, "protein-free milk" or dried liver to this diet checked the decline and induced rapid growth, showing that the protein of the extracted meat was adequate for growth, but that the water-soluble vitamine was lacking.

A sufficient quantity of the water extract of meat may furnish enough water-soluble vitamine to promote considerable growth.

Dried liver and dried heart suffice as the sole source of protein and water-soluble vitamine in an otherwise adequate dietary. Whether they can furnish sufficient fat-soluble vitamine is being determined.

Further observations on the production of lactic acid following alkaline injections. J. J. R. MACLEOD.

The immediate increase in the lactic acid content of blood produced by injections of sodium carbonate sufficient to lower C_H raised the question as to whether the function of acid production might be to neutralize the alkali. Production of lactic acid out of carbohydrate would then serve as an acid reserve of the acid base equilibrium of the body. If such should be the case, much of the acid would be excreted with the urine, and both in this secretion and in the blood no perceptible change in C_H of the blood would necessarily occur.

The following experments bearing on this aspect of the problem have been partially completed.

1. When 5 per cent sodium carbonate solution was injected intravenously in etherized rabbits at the rate of 1 cc. per minute, it gradually raised P_H of blood from 7.4 to 7.8 in about three hours, without having any marked effect on the respiratory rate. The urine was freely excreted, and the percentage of lactic acid rose from the normal of about 0.01 to over 0.30. A total of 7.5 grams Na_2CO_3 was injected, and a total of 0.654 gram lactic acid excreted. Between 5 and 6 had therefore been excreted as sodium lactate.

2. In a similar observation on dogs, the percentage of lactic acid in the urine rose in one case from 0.028 to 0.201, and in another from 0.045

to 0.495. In the former case 2 per cent Na_2CO_3 was injected (1 cc. per minute), and P_H of blood rose to 7.9 in four hours. In the latter case 1 per cent Na_2CO_3 was injected, the P_H rising to 7.6 in three hours. Diuresis was present in both cases.

3. An etherized dog was injected with 1 per cent Na_2CO_3 at the rate of 2.5 per minute, receiving in all 4 grams Na_2CO_3 in two and three-fourth hours. P_H of blood remained at 7.4. The CO_2 percentage rose only slightly and a total of 0.392 gram lactic acid was excreted by the urine. Between 5 and 6 per cent of the administered alkali was therefore excreted as lactate.

4. The daily urine was collected in a cat that was fed by stomach tube with a regular daily ration of minced liver and flour. After a preliminary period of eight days, sodium carbonate was added to the food for a further period of five days, this being followed by another normal period. During the alkali period, P_H of the urine rose from 6.1–6.3 to 8.2–8.3. The volume rose somewhat, and the relative percentage of ammonia-nitrogen fell from about 4 to less than 2. The total daily excretion of lactic acid during the normal periods averaged 5.5 mgm. (varying between 3.55 mgm. and 7.3 mgm.); during the alkali period the average was 10.5 mgm., being much greater (0.022 mgm.) on the first few days than subsequently. A similar result was obtained on a cat given bicarbonate in place of carbonate.

5. No difference was observed in the percentage of lactic acid in the daily urine of three rabbits according to whether they were starved for several days or fed with cabbage. The total excretion for twenty-four hours was greater in the case of the fed animals, but the very much greater water excretion of these as compared with the starved animals probably explains the difference.

6. No difference was observed in the percentage or in the total amount of lactic acid in the daily urine of rabbits fed liberally with cabbage with or without alkali.

7. Administration of sodium bicarbonate in therapeutic doses in man caused a distinct increase in the excretion of lactic acid which was most marked in the urine of the day following the administration.

(*a*) *The isolation and identification of the thyroid hormone;* (*b*) *The physiological action of the thyroid.* E. C. KENDALL.

The method for the isolation of the iodin-containing compound of the thyroid was briefly reviewed, and its empirical formula shown to be $C_{11}H_{10}O_3NI_3$, and its structural formula has been established as trihydro tri-iodo oxy-indol propionic acid. The oxy-indol group can change to the hydroxyl, and does change when the compound is dissolved in an alkaline solution.

Physiological testing has shown that this substance will relieve all symptoms of cretinism and myxedema to the same extent as desiccated thyroid. Furthermore, there is no relief of the conditions of cretinism and myxedema by all of the constituents of the thyroid other than this substance.

From a consideration of its structural formula, it is possible to relate its physiological activity to chemical reactions produced by the imino group of the indol nucleus and the carbonyl group. It has been shown that this substance will react in vitro with amino acids; that the carbonyl group condenses with the amino group of amino acids, and that the imino group is exceedingly reactive with the carboxy. It seems probable that the physiological activity of the substance is due to the action between itself and the amino acid. There may be three results of this action, (1) deaminization, (2) decarboxylation, and (3) both. To show the reaction of these two groups, the imino and carbonyl, derivatives of each were made. It was found that the animal organism could utilize the substance when the carbonyl group had reacted forming a nitrogen derivative, and this reaction presumably is involved in the deaminizing action of the substance in the body. However, replacing the hydrogen of the imino group with derivatives renders the substance inert within the animal organism. Hence, it is improbable that this action occurs in the functioning of the compound within the body. This reaction shows also that when the imino group can not function, the entire compound is rendered physiologically inert. Bearing in mind this physiological activity, the compound has been named "thyro-oxy-indol." This word, being too long to extend to every-day use, has been abbreviated to "thyroxin."

Evidence was submitted which supports the hypothesis that this substance is the only compound in the thyroid secretion which is essential for the production of thyroid activity in the body, and hence it may be considered the thyroid hormone.

The influence of music upon electrocardiograms and blood pressure. I. H. Hyde and W. Scalapino.

The object of the experiments of which only the beginning is here briefly summarized, is to ascertain the effect of different kinds of music upon the heart and blood pressure, in individuals who are known to have musical talent, and are fond of music, also in persons who are indifferent and have no fondness for music, in neurasthenics and in some animals.

The effects of the three following pieces of music were investigated, Tschaikowsky's death symphony, characterized by its tragic slow minor movements; Torcador's brilliant description of the bull fight, from Carmen, and the National Emblem, a stirring rhythmical march by Sousa. The experiments were conducted on three men who are fond of music. The data from subject "A" being more extensive and quite typical are here presented.

The cardiograms were obtained with an Einthoven string galvanometer, and the blood pressure with a Tycos and modified Erlanger sphygmomanometer. The records were taken before, during and after listening to the music.

A study of the cardiograms and tabulated results, revealed first, that the effects of the minor tones of symphony music, were a fall in

systolic, diastolic, and pulse pressure, and increase in the pulse rate, and also an increase in the E. M. F., or action current. The results were probably due to a reflex inhibitory action on the vagus nerve.

Second. Toreador's stirring song produced an increase in the systolic and pulse pressure, but a decrease in the diastolic pressure. The pulse was accelerated and the E. M. F. of the ventricular contraction was lessened. It may be, that this kind of music reflexly stimulates the accelerator or inhibits the vagus.

Third. The effect of Sousa's rhythmical march was increased systolic and pulse pressure, a decrease in the pulse rate but an increase in the action current. The effect seems to be due to vagus stimulation, and it is possible that this as well as other kinds of music may have a physiological influence on the system in other respects, and that by a careful selection of music from a definite source, prove an aid in the treatment of nervous disturbances.

A simple method for the resuscitation of the human heart. ARTHUR D. HIRSCHFELDER.

Observation of exposed dogs' and cats' hearts shows that death ensues in these ways—either the ventricles go into fibrillation, or they cease to contract and at once lose their irritability entirely, or they may cease to contract and yet for some time retain their ability to carry out a forcible contraction in response to mechanical stimulation. Such a stimulation may be furnished by directly slapping the ventricles with a blunt instrument or by suddenly pressing the ribs and chest wall down upon the heart. If this is done rhythmically at a rate of thirty to sixty times a minute the heart may respond to each stimulus, the circulation may be reëstablished, and spontaneous cardiac rhythm may then be resumed. The writer carried out this procedure successfully in one case of stoppage of the ventricles in a patient with Adam-Stokes' syndrome in which the stoppage of the ventricles was unusually prolonged. The convulsion and respiration had entirely ceased and the patient seemed beyond hope of recovery. The ribs and chest wall were then seized between the two hands, the right hand at the back, the left hand over the precordium, and the chest was forcibly compressed between them at a rate of thirty to forty times a minute, thus pressing ribs and chest wall suddenly down upon the ventricle. The heart responded at once to each stimulus with a contraction, circulation was rapidly resumed, the patient recovered and was living two years afterward. This manoeuver has been repeated as a routine upon the hearts of dogs and cats used in the laboratory experiments in which the hearts had been exposed by removal of part of the chest wall, and where the results could be watched with the eye. It is often successful in restoring the circulation. The rather slight stimulus offered when the chest wall is pushed suddenly down against the heart, without in the least subjecting the latter to compression, suffices as a mechanical stimulus to bring about contraction. This manoeuver is, therefore, a justifiable

procedure for the resuscitation of hearts which have stopped beating for a minute or more, and which are apparently beyond hope of the ordinary forms of stimulation. It is simpler and more readily applied than massage across the diaphragm or electric stimulation after the introduction of a needle into the heart.

Regulation of venous blood pressure. D. R. HOOKER.

The experiments were performed on dogs and deal especially with the reflex control of venous tone. The sigmoidal region of the large intestine was isolated as to its blood supply, double mass ligatures above and below the area interrupting collateral circulation and permitting elevation of the preparation above disturbing influences of neighboring viscera. The vascular bed was washed free of blood with warm Ringer's solution after which the artery was left freely open. It was assumed that arterial constriction would displace fluid backward rather than forward into the vein where the pressure was 10 to 15 cm. H_2O. The mesenteric vein was connected with a water manometer. A similar manometer was connected with the lumen of the isolated gut which had been previously filled with warm Ringer.

Stimulation of the nerve trunk running from the inferior mesenteric ganglion gave a rise of pressure in the vein amounting to 7.25 cm. H_2O in a number of cases. This reaction was readily and repeatedly obtained for periods of an hour or more. Stimulation of the saphenous nerve and asphyxiation gave rises of 3.5 cm. and 4.5 cm. respectively. The latter results were much less easily obtained, failing long before the peripheral mechanism was exhausted. Section of the peripheral nerve or destruction of the medulla destroyed the reflex. These venous pressure changes were independent of pressure changes in the lumen of the gut.

The evidence points to the existence of a central as well as a peripheral veno-pressor mechanism. The possible relationship of the mechanism to "shock" has not as yet been studied but the fact that the central mechanism loses function so easily suggests that it may be one of the early events contributing to the stasis of blood which is regarded as a cardinal symptom in the "shock" complex.

Blood pressure in sharks and the shock problem. E. P. LYON.

Blood pressure in sharks was studied on account of the peculiar circulatory arrangements. The blood pressure is about 35 to 40 mm. in the branchial arteries and 20 to 25 mm. in the systemic arteries. Under experimental conditions there is a slow fall of pressure through a long time, with corresponding gradual loss of reflexes and other activities. The equilibrium functions seem to be the first to disappear and the heart reflexes last, respiration being usually lost before the heart reflexes.

Many mechanisms which are effective in producing shock in dogs have little or no effect on sharks. These fish are remarkable in that stimulation of almost any part of the body causes inhibition of the

heart. There is an intimate relation between the respiratory and heart rates, a 1-1 rhythm being common, while at other times a 2-1, 3-1 or indefinite relation subsists. Artificial respiration (by a stream of water led into the mouth), produces variations of heart and respiratory movements. In a considerable number of animals change from light to dark lowers the blood pressure.

Observations in shock. C. C. GUTHRIE.

Dogs under ether anaesthesia were reduced to a state of shock chiefly by nerve stimulation alone, and combined with partial cerebral anaemia. Typically, the condition was associated with low blood pressure, decrease of respiratory movements, presence of eye reflex and absence of pronounced tendency to recover on discontinuing anaesthetic. Marked differences in resistance occurred in different animals as well as marked differences in the quantitative relations of the symptoms and in reflex functional response in shock. Also, marked quantitative variations occurred in chemical studies, such as the amount of oxygen retained and the amount of CO_2 eliminated by the lungs, the hydrogen ion concentration of the blood, and blood plasma bicarbonate.

Among the direct negative findings, as primarily causative of shock, were blood pressure; blood reaction, including reserve alkalinity concentration of chemical constituents or morphological elements, and viscosity of blood; blood volume; pooling of blood in vessels of alimentary canal and liver; temperature; cardiac weakness and cerebral embolism.

The amount of oxygen retained and CO_2 eliminated by the lungs was less in pronounced shock, but there is no reason for assigning to these conditions a causal relation to shock.

That acidosis was not a primary causative factor was shown by failure to produce shock by primary acidosis, as by injections of lactic acid; recovery from fatal lactic acid intoxication by injection of sodium bicarbonate, and absence of recovery from shock by such injection; and production of shock with alkaline reserve maintained by sodium bicarbonate injection.

Among phenomena observed possibly having causal relations to shock, were alterations in nervous activities, particularly fatigue of bulbar centers. Though presenting marked variations, results obtained in pronounced shock showed that both reflex vasomotor and respiratory response may be profoundly decreased. Interpretation of some of the reflex vasomotor phenomena is difficult.

Evidences point to decreased arterial and increased venous blood volume in shock, and derangement of the veno-motor mechanism may have an important causal relation to the condition.

Shock and its control. W. B. CANNON.

The observations were made at a casualty clearing station in France a few miles back from the front-line trenches. The main points may be summarized as follows:

1. There is a discrepancy between the red counts, haemoglobin and haematocrit readings of venous and capillary blood in shock cases, indicating a concentration of blood in capillaries. It seems probable that the "lost blood" of shock is in capillary areas.

2. The shock cases when received at the clearing station, have a diminished alkali reserve (an acidosis, in the Van Slyke sense).

3. A rough correspondence exists between the degree of acidosis and the degree of depression of the blood pressure.

4. Surgical operation in any case lowers the alkali reserve, and in these shock cases, with an acidosis already existent, operation may in a short time reduce the reserve to a serious degree.

5. In shock cases, surgical operation not only causes a sharp fall in an already low alkali reserve, but also a sharp fall in an already low arterial pressure. Thus two conditions unfavorable to recovery are made worse by the necessary operation.

6. By injection of sodium bicarbonate intravenously *at the start of operation* both of the unfavorable effects of operation are obviated—the pressure is higher at the end than at the start, and the alkali deficiency is abolished.

Observations on the volume flow of blood through the submaxillary gland. ROBERT GESELL.

The volume flow of blood through the submaxillary gland was studied under normal conditions, and under conditions of lowered blood pressure induced—(1) by hemorrhage, and (2) by tissue abuse.

Under normal conditions with a constant blood pressure, the basal flow of blood remained constant during periods of glandular rest.

On increased glandular activity, elicited by chorda stimulation, there was increased volume flow of blood which bore a linear relation to this increased activity.

Lowering of the blood pressure by hemorrhage reduced the ratio of secretory blood flow to secretion, and also reduced the basal volume flow of blood.

The initial fall in basal flow of blood was very much more rapid than the accompanying fall in blood pressure. This initial fall gave way to a fall in volume flow much slower than the accompanying fall in blood pressure. At a pressure of about 50 to 40 mm. Hg., the fall in basal flow of blood again became decidedly faster than the fall in blood pressure.

Lowering of the blood pressure by tissue abuse likewise lowered the ratio of secretory blood flow to secretion. In one experiment the secretion of one drop of saliva, at a blood pressure of 124 mm. Hg., called forth 13.9 extra drops of volume flow of blood through the gland. At a pressure of 37 mm. Hg. no extra flow of blood occurred. The basal volume flow of blood likewise fell; but more rapidly with the initial decrease in blood pressure than in the case of lowered pressure from hemorrhage. In one instance, the basal flow of blood at a pressure of 84 mm. Hg. was only 16 per cent of that obtaining at a pressure of 124 mm. Hg.

The curves of basal flow of blood were explained on the basis of the variations of three factors—viscosity of the blood, driving pressure, and caliber of the vessels.

The differences in the curves of basal flow obtained in the two types of experiments can probably be largely explained by the direction of change in viscosity of the blood.

In the case of hemorrhage, there is a decreased viscosity counteracting the effects of the accompanying fall in blood pressure; while with tissue abuse, there is an increased viscosity[1] augmenting the effects of decreasing blood pressure.

The experiments show the unfavorable conditions produced by lowered blood pressure for the maintenance of normal conditions, especially if any tissue is called upon for increased activity.

They show the gravity of even a small fall in blood pressure, for it is the initial fall in pressure which produces the greatest decrease in basal volume flow of blood.

Some reactions in the development of shock by diverse methods. Joseph Erlanger, Robert Gesell, H. S. Gasser and B. L. Elliott.

Shock supervenes in consequence of extensive tissue damage, not necessarily traumatic in origin. It appears after partial occlusion of the inferior vena cava, and of the descending aorta; after plugging the portal capillaries with lycopodium; and after large doses of 1-1000 adrenalin; in other words, after interfering for some time with the blood supply to a part, or the whole, of the body. At autopsy there are haemorrhages into many of the abdominal organs. The picture is similar when shock is produced by exposure of the intestines; and in addition a considerable quantity of plasma transudes from the serous surface. That there is a similar plasma transudation, but into the tissues, during the development of the other forms of shock, is indicated by the fact that in all there occurs a diminution in plasma volume; and in addition there is often a reduction in the volume of the blood as a whole.

The reaction of the vasoconstrictor center, followed by a modification of Bartlett's inflow method, depends largely upon the effect of the procedure by which shock is produced upon the cerebral circulation. The tone of the center at first is inversely as the cerebral arterial pressure, but long continued low pressure always leads eventually to loss in tone. It is possible to have shock with normal vasoconstriction.

The force of the heart beat seems to diminish somewhat as shock develops, but presumably only secondarily. Furthermore, late in shock, lowering the arterial pressure may paralyze the respiratory center without preliminary stimulation. The alkaline reserve declines as shock develops. The decline though may be extreme or not below the normal range.

[1] Gasser, Meek and Erlanger: Proceedings in this Journal.

The mechanism of shock, we conclude, is as follows: Extensive traumatization causes extensive local transudation of plasma, which, together with the primary haemorrhage, materially reduces the blood volume, and thus leads to general vasoconstriction which is enhanced reflexly by the pain. The blood stream is thus slowed to the point of damaging the cells, and of starting generalized transudation. The arterial pressure as a result eventually becomes so low, that the vasoconstrictor center suffers and the arterial pressure falls still further. At the same time, the alkaline reserve diminishes, possibly through the incomplete oxidation of metabolites. Thus a series of vicious cycles is started, the outcome of which is "Shock." Sometimes one, sometimes another of these three factors predominate.

At the front, fatigue entailed by transportation of the wounded, contributes toward the ultimate giving way of the vasoconstrictor center; it presumably works in the same way as does ether in laboratory experiments. If there is such a thing as traumatic shock in the absence of wound weeping and blood loss, we would suggest in explanation of it, that long continued pain stimulates the vasoconstrictor center, and so diminishes the blood supply to the tissues to the point where transudation begins, thus starting the vicious cycle just described. Naturally any other set of conditions tending to hold the arterial pressure low for some time, also would establish the same vicious cycles.

A method for the determination of blood volume. Walter J. Meek and Herbert S. Gasser.

A method for the determination of blood volume has been devised which is free from certain objections incident to the older methods. The general plan has been to inject into the blood stream some substance which was inert, which disappeared slowly, and which could be recovered quantitatively. A determination of the dilution of this substance in the blood would then afford data for the blood volume determination.

Acacia has been found to meet the above requirements. A given amount is added to blood in vitro for a standard or control. A given amount is then injected into the animal. Ten minutes later a sample of blood is drawn for determination. The amount of dilution indicates the blood volume. The acacia is determined as furfurol-phloroglucid, according to the method of Kröber.

The blood volume changes in shock and the modification of these by acacia. H. S. Gasser, W. J. Meek and J. Erlanger.

The volume of the blood was determined by the acacia method. The erythrocytes were also counted as an index to the plasma changes. The forms of shock studied were those produced by exposure of the intestines, injection of adrenalin, and temporary partial occlusion of the vena cava or the aorta. The findings, in all cases were essentially the same. There was a decrease of the plasma amounting to an

average of 22.2 per cent of the total blood volume. In the cases where the acacia was injected this decrease was strikingly less being on the average only 7.4 per cent of the normal.

Loss of plasma accounted for all the decrease in blood volume in one-third of the cases. Where the decrease in blood volume (acacia determination) was somewhat greater than that calculated from the red cell count the difference could be explained by haemorrhages into the tissues. These were a constant finding in the intestine, spleen and other organs. In a remaining group the blood volume decrease was much greater than that indicated by the red blood cell count. This difference could only be explained on the assumption of regions of stasis.

The filtration of the plasma might be due to decrease in the colloidal osmotic pressure of the plasma, rise of arterial blood pressure or increase in the permeability of the vessels. The first possibility may be at once discarded. The high blood pressure in adrenalin shock causes filtration, but the fluid returns when the pressure falls and polycythaemia only remains permanent when the decreased supply to the tissues has resulted in damage. When the cava is clamped the venous pressure is high but the arterial pressure is so low that there is little chance for filtration, other factors being equal. When the aorta is clamped neither the venous nor arterial pressures are high in the posterior part of the animal, but filtration is both a constant and marked phenomenon. One must turn therefore to decreased permeability of the vessels to explain the phenomena.

The acacia exerts its conserving influence on the plasma under conditions which are highly pathological, under conditions in which normal plasma leaves the vessels not only without a rise of arterial pressure but even when the pressure is low. Bayliss has suggested the use of acacia in the rendering of a solution isosmotic to plasma to aid its retention in the vessels when injected intravenously. In our experiments where normal plasma leaves the vessels acacia must have a further influence. In the amounts used the colloidal osmotic pressure of the plasma would be increased about 13.5 per cent. This would act in the direction of decreasing filtration and increasing absorption. Our blood counts, however, have given us no evidence that any appreciable expansion of the plasma volume from the tissues takes place in one hour. The possibility that acacia acts as a calcium salt in decreasing the permeability of the vessels is worthy of consideration.

The effects of injecting acacia. Walter J. Meek and Herbert S. Gasser.

In view of the use of acacia in perfusion solutions a series of experiments has been made to determine its effects on experimental animals. Injections of large amounts of 20 per cent acacia have practically no effect on blood pressure other than the mechanical one of increasing the blood volume. The heart rate is not influenced other than that due to the slightly raised blood pressure. After hemorrhage acacia seems

to maintain blood pressure better than salt solutions. Respiration is unaffected. The urinary secretion after acacia can be stimulated by sodium nitrate apparently as well as normally. An animal under ether may have acacia injected until the blood is a 10 per cent solution with no symptoms of disturbance. Intact animals have had their blood made up to 4 per cent acacia with no unfavorable symptoms. Acacia leaves the blood stream in the early stages at the rate not exceeding 10 per cent per hour. This rate soon slows and fairly large amounts of acacia may be found in the blood two days later. A pentose reaction may be obtained from the urine an hour after the injection of acacia.

Diet experiments bearing on carbohydrate luxus-consumption and wasteful eating. ADDISON GULICK.

The purpose of the tests was to determine whether excessive consumption of carbohydrate by a person of the characteristically lean physical type did not stimulate the organism to oxidize away a large part of the excess supply of fuel.

These experiments covered one and three-fourths years of more or less controlled eating with 330 days on strictly measured experimental diet. Diet in the strict periods was 90 to 675 grams per day of cereal foods (air dry) plus 3 pints milk, usually about 100 grams egg, and 0 to 50 grams butter.

At the start and finish, minimum food requirements were determined. Between these periods there was prolonged over feeding up to a maximum of 4100 calories and then a return to low weight by restricting the diet to about 1800 calories for six weeks. Activities were held as nearly uniform as possible.

Conclusions. 1. Minimum food requirements were somewhat greater than would be expected from the activities. Initial test: Slight weight loss during four weeks on 2750 calories, body weight about 62 kgm., sleep 8.2 to 8.5 hours per night, daily walking 5 miles or less. Final test: Weight constant twenty days on 3200 calories, body weight 61.5 kgm., average sleep 8.3 hours, daily walking 4.9 miles, bicycle 4.7 miles.

2. Under non-experimental conditions, the regulating influence of the sense of appetite and of satiety is an important factor in holding the weight at its rather constant level of 63 to 66 kgm. For upon eating persistently more than was relished (3600–4100 calories), the weight was gradually raised to 74.7 kgm. This conclusion probably has wide application, as the man experimented upon was believed, at the start, to show the very reverse condition.

3. If there is a luxus factor it is not overwhelmingly large. Metabolism was high in the periods of heavy feeding, but not high enough to give good proof of a true luxus oxidation, over and above the specific dynamic effect of the food, and the added fuel cost of activity of an organism enlarged by fattening.

4. If a luxus oxidation occurs at all, its effect is ended within 14 hours after taking food. Evidence is from the moderate or almost low post-

absorptive basal gas metabolism while on a 4000 calory diet. Gas exchange = 71.5 calories per hour, = 0.97 calories per kilogram, = 34.4 calories per square meter. (Determined by courtesy of the Carnegie Nutrition Laboratory in a Benedict bed respiration chamber.)

5. From an economic standpoint, the increased fuel expenditure found in these experiments with a high diet and moderate activities, was a pure waste.

Tests of methods of control of the clothes louse. Wm. Moore.

Sachets are not successful.

Talc 20 grams, creosote 1 cc., sulphur 0.5 gram is six times as effective a louse powder as NCI, causes less irritation to the skin and is dry, hence easier to apply.

Impregnation of the underwear is not possible, but a cheese cloth suit impregnated with a saturated solution of sulphur in creosote could be successfully worn outside of the underwear.

Chlorpicrin can be used as a fumigant, penetrating the clothing and killing the lice in all parts of the clothing in fifteen minutes and the eggs in thirty minutes. By increasing the heat in the fumigation chamber the time required to kill the eggs could be reduced.

The tension of the blood gases in the blood entering and leaving the lungs. R. G. Pearce and A. Nicholson.

We believe that our data indicate that so long as the minute volume of the circulation and the respiration increase directly with the metabolism, the difference between the tensions of the gases in the blood entering and leaving the lungs remains approximately constant. However, during acute exercise when the volume rate of the circulation fails to keep pace with the rate of increase in the circulation, the difference between the tension of the gases in the blood entering and leaving the lungs progressively increases, and the minute volume of the respiration is increased out of proportion to the degree of the oxygen intake or carbon dioxide output. The progressive increase in the difference in the tension of the gases entering and leaving the lungs is brought about by the hyperpnoea which reduces the tension of carbon dioxide in the alveolar air below that normally present or expected, and to the very rapid and disproportionate increase in the tension of the carbon dioxide in the blood entering the lungs.

The cause of the hyperpnoea in acute violent exercise is questionable. The failure to find a diminution in the alkali reserve of the body argues against the accumulation of organic acids from incomplete oxidation, and this together with the low tension of the carbon dioxide in the alveolar air speaks against the increase being due to stimulation of the center by increased acidity or carbon dioxide hormone. The possibility of oxygen want stimulating the center directly, or indirectly, through afferent respiratory fibers in muscle nerves is suggested by the high respiratory quotient found at higher levels of exercise. An inadequate minute volume of the blood flowing through the center during exercise

because of venous engorgement due to right heart insufficiency might explain the phenomenon.

The methods employed for the estimation of the tension of the respiratory gases in the blood entering and leaving the lungs at various levels of metabolism afford a means of judging the heart's reaction to and capacity for acute work.

Reflexes and clonus recorded graphically. R. Edwin Morris and L. G. Rowntree.

Hitherto the physician has been compelled to depend upon memory pictures when recalling reflexes and clonus. Obviously, this is unsatisfactory. It was with the idea of meeting a glaring need that an attempt has been made to devise methods of graphically recording these phenomena.[1]

Two great problems are encountered: (1) methods of record, and (2) interpretation of records. Our efforts up to date have centered on the former. In the beginning difficulty was experienced in securing consistent records, but, after a year's experience, various difficulties having been met one after another, we finally feel confident that consistent records of reflexes can be obtained. At present we are considering their interpretation and are meeting with some degree of success. However, as many of the records are relatively complex the question of interpretation will be deferred and will be the subject of further investigation and of future publication.

The electrocardiograph apparatus of the Taylor Cambridge type has been utilized, additions being made to meet our particular problems. The knee-kick and ankle clonus have been the special phenomena under investigation.

In securing records of knee-jerks, the patient is seated in a specially devised chair upon an electrode molded to the form of the gluteal region. The chair is sufficiently high from the ground to permit the feet to swing free. Mechanical leg attachments, in the form of laterally adjustable swing boards, are hinged to the front edge of the chair. These are adjusted to the patient's legs and secured to them by means of straps. Electrodes are attached, but only one at a time is connected with the string galvanometer. Preliminary study is made and the point yielding maximum resistance is marked. This constitutes the invariable point of contact in eliciting the reflexes. An insulated contact, and a small wire net attached by adjustable supports to the swing board, are placed over the area already marked. The hammer which produces an electric contact resulting in a fling in the signal magnet, is adjusted to the horizontal bar so that it strikes directly in the desired spot on either leg. Along the handle of the hammer there are five holes. The hammer is usually secured on the horizontal bar through hole (1), thereby fur-

[1] The credit for the mechanical devices utilized is due to Doctor Morris. After writing up our work—our attention was called to the work of Bornstein and Saenger (Deutsche Zt. f. Nervenheilkunde, 1914, Bd. 52), who also utilized the electrocardiograph in this connection.

nishing the lightest stroke possible with this apparatus. The second lighter horizontal set-bar is so placed that the hammer may be dropped from any desired angle. Ordinarily, however, 90 degrees is the one advised. In the other edge of the swing board are inserted three eyelets, to one of which is attached a hook which is secured to an inelastic cord which runs over an adjustable frictionless pulley to a hanging indicator suspended in front of the aperture in front of the lens of the electrocardiograph. The indicator produces a shadow parallel to that of the string galvanometer. Holding the indicator in position on the other side is a coiled steel spring, the tension of which is adjustable. So long as the indicator remains in a vertical position the tension on the string leading to the swing board is constant. A time-marker indicating one-tenth of a second has been used in most of the work.

In these records the upper tracing represents the time-marker, one tenth of a second being indicated. The second record represents the electrical response, the picture resulting from the shadow of the galvanometer string. The third record represents the mechanical response as pictured by the movement of the indicator; and the fourth depicts the instant of contact. In many of the records the deflection resulting from the introduction of a millivolt is shown just prior to the reflex. This indicates the tension of the string.

The cerebral center of mastication. F. R. Miller.

Ferrier, Mann and others observed that typical movements of mastication could be evoked in the rabbit by stimulation of the cerebral cortex a slight distance in front of the Sylvian fissure. The present research was undertaken with the idea of analysing more completely the cerebral mechanisms concerned in these movements.

By the procedure of dividing the lower jaw at the symphysis it was determined that stimulation of the cortical area of one side causes synchronous chewing movements executed by both halves of the jaw. It was also found possible to elicit similar bilateral chewing movements from the subcortical tracts as far back, approximately, as the commencement of the crus cerebri. Farther back than this only continuous jaw closure was obtained; this appeared to be mainly ipsilateral.

An endeavor was made to localise the subcortical centre of mastication. Réthi considered it to be within or below the thalamus and above the crus. A transverse section was made across both cerebral hemispheres at a distance of 16 to 17 mm. behind the anterior zygomatic angle. Stimulation of the appropriate point on the cross-section of each hemisphere yielded chewing and swallowing. A slice a few millimeters in thickness was next removed from the left hemisphere. Stimulation applied to the left section evoked now usually only continuous contraction of the jaw muscles apparently chiefly ipsilateral. Stimulation of the original point in the right section still elicited chewing and swallowing.

It is evident, therefore, that the masticatory centre is situated between the section of the right hemisphere, which passes through the corpora

mammillaria and the habenular nuclei, and the level of the section of the left hemisphere, which, in a number of experiments, passes through the medial geniculate bodies and the posterior commissure.

The point yielding mastication was localised on the more anterior cross-section by unipolar stimulation and was found to correspond with the medial portion of the pes pedunculi.

The relation of lesions of the optic thalamus of the pigeon to body temperature, nystagmus and spinal reflexes. FRED T. ROGERS.

As was long ago recognized by many early workers on the brain of the pigeon, the effects of decerebration vary according to whether or not the thalamus is also injured. Lesions of the thalamus are followed by a long continued flattening of the feathers against the body (Bechterew). Decerebration without thalamic lesion does not cause this change in the position of the feathers. Intra-peritoneal injection of pilocarpine in normal and in decerebrate pigeons causes a similar flattening of the feathers. The body temperature of decerebrate pigeons with thalamic lesions varies with the temperature of the cage. The temperature of decerebrate pigeons without thalamic lesions remains normal, but injection of pilocarpine in these birds is followed by changes in body temperature varying according to the temperature of the environment. Injection of pilocarpine sufficient to flatten the feathers of a normal pigeon produces very slight temperature changes (1 to 2°C.).

In decerebrate pigeons with *lesions* of the thalamus, the behavior varies with the body temperature. Depression of body temperature to 25° to 30° C. is followed by diminished reflex response to irritating vapors to the nostrils: to the puckering reflexes of the cloaca, and to the stimuli of hunger.

Complete decerebration leaving the thalamus intact does not lead to the disappearance of the nystagmus of head and eyes (Ewald). If the thalamus also be removed, whether or not the nystagmus disappears depends on the body temperature. If the temperature be lowered to about 30°C. the quick component disappears and the deviation (on rotation) persists. If lowered to 25°C. the deviation also disappears. If body temperature be brought back to 39°C. both deviation and quick component reappear. Involvement of the oculomotor nuclei causes disappearance of the eye nystagmus. In lowering the body temperature the head nystagmus persists after the eye nystagmus has ceased.

On the stimulation of the vagogastric medullary centers by drugs. FRED T. ROGERS.

In the turtle with the spinal cord sectioned at the level of the third cervical vertebra but the circulation through the head intact and the vagi normal, the injection of 0.5 cc. to 1 cc. of a 1:1000 solution of picrotoxin in Ringer's solution into the carotid artery leads to a powerful tetanic contraction of the stomach. This effect does not follow the injection if both vagi have previously been sectioned. Electric stimulation of the floor of the fourth ventricle causes a similar contraction.

This contraction is followed by a prolonged refractory stage of the gastric musculature to vagus stimulation.

In the dog, with splanchnic nerves previously sectioned, the injection of apomorphine in doses too small to cause vomiting leads to a marked diminution in the tonus of the stomach and to a cessation of peristalsis, so far as can be determined by the balloon method of recording gastric contractions. Vomiting caused by apomorphine is not preceded by any gastric contractions so far as this method will indicate. Picrotoxin caused the same changes in the gastric contractions as did apomorphine.

Comparison of the rhythm of the respiratory center and trapped wave in cassiopea. J. F. McClendon.

Mayer has shown that if a nerve impulse is started in a ring of nerve tissue of the jelly fish, cassiopea, it will pass round this ring at a uniform rate and can be tapped off at one point as a rhythmical impulse. The nerve tissue in the ring is in the form of a network with numerous synapses. I have shown that the rate of the nerve impulse per centimeter is not affected by stretching the ring and, therefore, the number of impulses per second that may be tapped off at one point in the ring may be varied by stretching the ring. The rate of the nerve impulse is affected, however, by additional stimuli applied to the ring or to nerve fibers coming into the ring. These additional stimuli retard the rate of the nerve impulse.

If we imagine the ring to be the respiratory center and that nerves are stimulated by the distention of the lungs and carry stimuli to the ring thus slowing the rate in the ring, the rate of breathing will be retarded. In this way we may make a model of the respiratory center out of very simple nerve tissue. Since the rate of the nerve impulse in the ring can be changed by altering several factors the rate of respiration would depend also on these.

Some points in the nervous regulation of respiration in the cat. C. C. Gault and F. H. Scott.

In these experiments respiration was recorded by recording the movement of the diaphragm by means of a spoon inserted between the liver and diaphragm (Rosenthal's method). Section of the vagi leads to the ordinary effects observed in other animals (prolonged inspiration and slow respiration). If in such an animal the cord be divided in the lower cervical region this type of respiration disappears and is replaced by one in which the ratio of expiration to inspiration is practically normal, although expiration and inspiration are prolonged. In a normal animal section of the cord leaving the vagi intact produces a respiration with prolonged expiratory phase. This same type of respiration is produced by section of the posterior roots of the thoracic nerves. It is evident that the impulses which come from the muscles and joints of the thoracic wall produce an opposite effect on the respiratory center from those coming from the lungs.

The effect of alterations of blood pressure on the blood of the rabbit. F. H. Scott.

Lamson reported that adrenalin did not produce a polycythemia in rabbits while it did in other animals. I find rabbits respond like other animals to alterations of blood pressure. A rise of pressure in the rabbit leads to an increase in the haemoglobin content of the blood and a decrease of pressure to a decrease of the haemoglobin content. Rabbits are extremely sensitive to the effects of small haemorrhage. The loss of a few cc. of blood is followed by a dilution of the blood. However, I believe Lamson's results are due to waiting till the blood pressure had returned to normal or sub-normal or to not getting an alteration in blood pressure.

Location of adrenalin vasodilator mechanisms. Frank A. Hartman.

Destruction of the brain does not interfere with the adrenalin vasodilator mechanism for the intestine. Pithing of the cord in the thoracic region often decreased the amount of intestinal dilatation from adrenalin. However pithing of the whole cord did not completely destroy this reaction of the intestine.

Loops of intestine perfused with Ringer's solution, but with intact nervous connections, dilated when adrenalin was injected into the external jugular, even though all splanchnic fibres were cut. Occasionally the dilatation was preceded by slight constriction. It seems from this that adrenalin produces dilatation of the intestine by stimulation of the sympathetic ganglia supplying them.

The adrenalin vasodilator mechanism for the hind limb must be below the thoracic cord because destruction of the whole central nervous system that far down does not prevent its action.

Adrenalin vasodilator mechanisms in the cat at different ages. Frank A. Hartman.

Adrenalin fails to produce a fall in blood pressure in kittens less than about eleven weeks old. The depressor effect at this age is small and may be inconstant. In fact, the increasing of the depressor effects from the slight fall succeeding a rise in younger animals to a marked almost pure fall in adults indicates a gradual development of the adrenalin vasodilator mechanism. This fall in blood pressure seems to be due to vasodilatation in skeletal muscle, for the two begin to appear simultaneously.

In three kittens from nine to eleven weeks of age both limb and intestinal adrenalin vasodilator mechanisms were sought. All three gave active limb dilatation, but no intestinal dilatation. It seems therefore that the mechanism for the intestine develops later. This supports the view that the two mechanisms are of different types.

Vasodilator nerves of the skin. H. Richardson and O. Wyatt.

Bayliss showed that stimulation of the posterior roots leads to a vasodilation of the vessels of the part. The work of Bruce rendered it very

probable that this was a case of axone reflex. Bruce used the inflammatory process as the basis for his work. We have followed the changes using a plethysmograph. If the paw of an animal be placed in a plethysmograph a vasodilation (increase volume of the leg) may be obtained by slow rhythmic stimulation of the peripheral ends of the cut cutaneous nerves outside the plethysmograph or by the same stimulus applied to the skin inside the plethysmograph. If cocaine be applied to the skin it is still possible to get a vasodilation from the nerve but none when the stimulus is applied to the skin.

A note on the mechanism of heart muscle contraction. MONTROSE T. BURROWS.

In this paper two facts were emphasized, the first is that tissue cells within a tissue culture cannot subsist upon the food material found within a medium of blood plasma. They grow also in a medium of salt solution. The cells that grow in a tissue culture are those at the periphery of the fragment. They obtain their nutriment from the cells that disintegrate in the center of the tissue fragment. The material use for food diffuses out over the surface of the medium. It is colloidal in nature and insoluble in the medium.

The second fact is that foetal heart muscle cells are essentially fluid in nature. The peculiar mechanical organizations essential for growth, migratory movement, rhythmical contraction or other forms of activity are differential surface tension phenomena established and controlled by the organization of the environment. By changing the mechanical organization of the environment one may change a contracting heart muscle cell to one which grows and divides by mitosis and is indistinguishable from a sarcoma cell. The growing and dividing cells are those cells which lie at the interval between the substances diffusing from the fragment over the surface of the medium and the medium itself. The contracting cells are elongated, cylindrical-shaped cells stretched through the liquid medium between a surface similar to that suitable for growth and the ends of tense bands of fibrin. The end of the cell in contact with the tense bands of fibrin is in metabolic equilibrium. Heart muscle cells completely embedded in a mass of fibrin show no metabolic activity in the presence of food and oxygen. They have been kept in this position in the incubator for six months without disintegrating. They grow again when removed to a suitable environment. The other end of the contracting cell is in contact with a surface similar to that upon which cells grow. The curve of growth of heart muscle cells has been studied and it has been found to follow the law of mass action and the cells come to an equilibrium before food and oxygen is exhausted. This inactivity or equilibrium is not disturbed by washing with serum but only when colloids (fibrin) or dead cells are added. It is assumed to be due, therefore, to the accumulation of waste product insoluble in serum but soluble in colloidal substances. This product in the presence of the surface food layer decreases the surface tension of the cell. With one end of the cell in equilibrium, the other yielding an

insoluble product it is evident that periodically this insoluble product may be broken up and an electric current pass (Bredig's phenomenon). It has been shown that oxygen is absorbed only during the relaxation period, tension is then developed. Lactic acid is liberated at contraction. The author has found that lactic acid causes an increase in surface tension.

Thus a theory for the mechanism of heart muscle contraction has been developed which explains the energy transformations and the physicochemical changes known to occur in heart muscle contraction.

The experiments were made with single and completely isolated rhythmically contracting heart muscle cells.[1]

Effects of external temperature and certain drugs on thyroid activity. C. A. Mills.

Effects of variations of external temperature on dogs, cats, guinea pigs, and rabbits were studied. It was found that animals kept at 30 to 37°C. for several days showed the following changes in almost every case: the colloid content of the vesicles was increased in amount, presented a uniform appearance, and stained rather intensely with eosin; the epithelial cells lining the vesicles were decreased in height, often entirely flattened, their cytoplasm and nuclei appearing rather compact and dense.

Animals subjected to low temperature, such as out-door winter temperature, for the same length of time exhibited a markedly different set of histological changes in the thyroid. The colloid decreased in amount, sometimes disappearing almost entirely, stained less intensely with eosin, and contained vacuoles of various sizes around the edge near the cells. These vacuoles, in some cases, entirely replaced the colloid, or left only shreds of it, looking as if the vacuoles were formed by the resorption of the colloid by the cells, leaving in its place a clear nonstaining fluid. The epithelial cells lining the vesicles became elongated to cuboidal or columnar types, both cytoplasm and nuclei apparently having enlarged and become less dense.

If we take as the index to the activity of the thyroid during the period of observation, the increase or decrease in the amount of stored colloid, its staining reaction, and the presence or absence in it of vacuoles, and also the character of the cells lining the vesicles, then the thyroid is shown to respond to temperature variations, other conditions being kept as near constant as possible. This same work is now being tried on opossums, using Bensley's stain to demonstrate the amount of true secretion antecedent, as he terms it in his work, found in the epithelial cells under these different conditions, in order to see if the two methods of gauging thyroid activity yield comparable results.

Certain drugs were also tried, and the histological changes in the thyroid noted. Morphine, injected into cats in amounts sufficient to produce hyperexcitability, caused changes in the thyroid closely resembling

[1] Burrows: Münchener Med. Woch., 1912.

those described above as resulting from low temperatures. On rabbits the effects were identical with those of high temperatures. Quinine had the same effects on the thyroid in rabbits as did morphine. Strychnine is now being tried to see if the activity of the gland is increased along with the hyperexcitability of the animal.

The influence of pituitary extracts on the daily output of urine. H. M. Rees.

The earlier work on the extract from the posterior lobe lists it as a diuretic, while recent investigators conclude that it is an antidiuretic.

It was our purpose in this investigation to find out: (1) whether the subcutaneous injection of pituitary extract will cause any quantitative variation in the daily output of urine; (2) whether such injection will in any way affect the quantity of urine excreted, and, if so, to find out if possible the factors involved.

Cats and rabbits were used as the experimental animals. The observations in each case were over three to ten days for the control and three to ten days for the injection.

The daily quantity and the specific gravity were the principal points noted. Variations in rate of output were also noted in several of the experiments.

It was found that pituitary extract does not, when injected subcutaneously, alter the amount of urine excreted per day in cats and rabbits, nor does it cause any marked variation in the specific gravity of the urine. There is, however, a very striking effect on the rate of excretion. Subcutaneous injection of pituitary extract causes a delay of seven to eight hours before the beginning of the diuresis which follows the injection of the large amounts (150–200 cc.) of water.

Evidence of toxic action of ovaries of gar. Charles W. Greene, Erwin E. Nelson and Edgar D. Baskett.

The reputed toxicity of gar ovaries was tested by feeding fresh ovaries to chickens, white rats, cats, and dogs. Chickens were fed from 5 to 75 grams each in amounts distributed over several feedings. These tests were run at intervals on individuals of pens under observation from thirty to fifty days. Control chickens were fed fresh gar meat and carp ovaries in addition to grain and kitchen scraps. This diet was also given to experimental individuals between tests. All ovaries and meats were fed fresh, generally within an hour after the fish had been killed. The symptoms produced were loss of tone and paralysis of the crop, loss of appetite, diarrhoea, loss of weight, muscular weakness, disturbance of the circulation as shown in the comb and wattles and depression of the central nervous system. If large amounts of the gar ovaries were fed the chickens became gradually weaker, dying after three or four days. If the ovary was withheld, in time the chickens slowly recovered. The ovaries are taken freely the first feeding, but never voluntarily the second time, hence forced feeding was used. Single feeds of 5 grams was the minimum toxic dose producing just perceptible symptoms.

White rats died after eating quantities as small as 5 grams of gar ovary. The toxic effects were general malaise, diarrhoea, marked diureses, loss of appetite, muscular weakness, and death. Autopsy showed stomach and intestines dilated by gas and usually with a severe congestion of the ileum.

Cats were given from 5 to 35 grams in a single feed. This was taken voluntarily the first time but never the second. Fresh ovaries and ovaries cooked by steam produced the same effects. In all tests the ovaries were vomited within two and a half hours, usually earlier. Occasionally there was a slight diarrhoea but no other symptoms were noted. The same effects followed when the ovaries were fed to dogs. Violent vomiting was exhibited. The last vomited material was partly digested Organ tests are under way and chemical separations likewise. The latter indicate that the toxic substance lies in the globulin fraction though we do not consider this point fully established.

Some electrical phenomena of the submaxillary gland. ROBERT GESELL.

The electrical variations of the submaxillary gland activated in various ways were graphically recorded. Both two gland leads and single gland leads were employed.

To interpret the deflections, blood pressure, secretion, and volume flow of blood were simultaneously recorded.

To maintain a constant condition of the animal the volume flow of blood was studied by an automatic and bloodless method especially devised for these experiments.

The electrical deflection obtained from prolonged chorda stimulation, with the usual lead, commonly shows four negative waves.

The deflection obtained by chorda stimulation, with the general condition of the animal remaining constant, is variable depending largely on four factors—duration of stimulation, strength of stimulation, duration of the period of rest, and the position of the electrodes on the gland.

By keeping all four of these factors constant, provided the period of rest is sufficient, superimpossible deflections can be obtained. This suggests the practicability of studying glandular processes by the electrical method, and permits the establishment of controls for studying the effects of introduction of other variables.

Results of some experiments indicate that contractility of the salivary ducts may account for the first negative wave. Other causes such as relaxation of the blood vessels were not ruled out, however.

The second and third negative waves have much in common. In most experiments the amplitude of these waves varied roughly with the rate of secretion. This correspondence may in part account for the dip in the deflection between the second and third waves elicited by prolonged chorda stimulation. Certain experiments, however, indicate that change in rate of secretion may not be the sole cause of this dip.

Frictional electricity as produced by flow of saliva or blood through the vessels is probably a minor factor in determining the deflection.

Obstruction of either the salivary duct or the carotid artery during glandular activity, however, markedly affects the electrical variation.

The effect of arterial obstruction is probably produced by the regulating effect of blood supply on glandular metabolism.

The fourth negative wave is not constant. When it does occur, it may last as long as twenty minutes, and may possibly represent the progress of recovery processes.

There seems to be a number of factors operating to produce the resultant deflections. Interpretations of the deflections are, at present, only tentative.

An automatic and bloodless method of recording the volume flow of blood. Robert Gesell.

The device used in this method consists of an electrical arrangement which permits the automatic filling and emptying of a segment of vein draining the tissue under study.

The apparatus described was devised primarily to record the volume flow of blood through the submaxillary gland, but it can be used for other tissues as well.

In measuring the volume flow of blood through the submaxillary gland, all the veins emptying into the external jugular vein, with the exception of those coming from the gland, are ligated. The jugular vein is then placed in a trough under an emptying plate and a cut off. The cut off is held down on the vein by the pull of a spring, preventing the flow of blood to the heart. In consequence, the blood accumulates in the vein below the emptying plate, raising this plate until an electrical contact is made, which simultaneously opens the cut off and presses down the emptying plate. The emptying of the vein breaks the contact, the cut off closes, and the process repeats.

The rate of filling and emptying varies directly with the volume flow of blood. This method can be made quantitative by calibration of the vein.

Vagotonic and sympathetic-atonic effects on gastric motility. T. L. Patterson.

The studies were made upon the bullfrog, Rana catesbiana, and the balloon method was used. All the animals were stomostomized[1] and normal records of the gastric hunger contractions with acid inhibtion obtained, 5 cc of 0.5 per cent hydrochloric acid being used in each case. They then underwent a second operation in which either both vagi or both splanchnics were sectioned and the above observations repeated. The vagi were cut in the region of the neck; the splanchnics in the region of the coeliac plexus after laparotomy.

Section of both vagi with the splanchnics intact leads to a sympathetic-atonic condition of the stomach with about the normal type of hunger contractions persisting, with the exception that, on the whole,

[1] Patterson: This Journal, 1916, xlii, 61.

they appear to be of a slower rate and slightly weaker, whereas the inhibition produced by the acid is quicker and more marked than in the normal animal. Section of both splanchnics with the vagi intact leads to a hypertonic stomach. The contractions are small tending to run into incomplete tetanus with an increased rate, while the acid inhibition is almost without effect there being only a very slight decrease in the height of the contractions.

Likewise, the same general influence which these two sets of nerves exert separately on the gastric apparatus may be shown when the splanchnitized stomach is superimposed upon the vagotomized stomach from two frogs of equal size. The latter or larger stomach represents the atonic and the former or smaller the hypertonic while the normal stomach takes an intermediate position between the two. It may be said, therefore, that the reciprocal or contrary innervation of Meltzer which may be termed antagonistic tonus, may be physiological as long as it serves the purposes of the organ in question in a beneficial manner. It is pathological as soon as the tonus of one or the other is so exaggerated that the common welfare of the organ is in danger and that is exactly what happens in the splanchnitized frog's stomach where the hypertonus of the vagus leads to a state of overexcitability, or to the Eppinger-Hess condition of vagotonia. Further investigations on this subject are in progress.

Studies on gastric secretion in man and dog: Gastric secretion and urine ammonia. A. C. Ivy.

This study consisted in the examination of the urine ammonia and gastric juice during (1) gastric stimulation followed by absorption in the intestine, (2) gastric stimulation without absorption in the intestine, (3) intravenous injection of water, (4) the absorption from the intestine of water, acid and alkali introduced by duodenal tube and duodenal fistula, and (5) during diuresis.

Gastric analyses were made every fifteen minutes. Urine was collected in fifteen or thirty minute intervals by catheter in dogs and voluntary micturition in man. Controls were made for one-half to one hour preceding the experiment to determine the continuous gastric secretion and urine ammonia. Conclusions are based on from three to ten trials of the same experiment in each individual. The work has been done on five normal men (the injections via duodenal tube were done only on one man), and has been repeated on female dogs with gastrostomy, duodenostomy and perineorrhaphy (perinaeum slit to expose urethral orifice). For the determination of urine ammonia the Folin macrochemical method and Folin-Nessler method, with the permutit modification[1] have been used.

It has been found that:

1. There is an increase in urine ammonia beginning one-half to one hour after the ingestion of a meal. This increase varies in the same individual and in different individuals on a constant diet.

[1] Folin and Bell: Journ. Biol. Chem., 1917, xxix, 329.

2. During gastric stimulation by food or water followed by absorption in the intestine there is an increase in urine ammonia.

3. During gastric stimulation by food or water *not* followed by absorption in the intestine no increase in urine ammonia results.

4. Intravenous injection of 200 cc. of water causes some gastric stimulation without an increase in urine ammonia or urine output.

5. (a) The absorption of water from the intestine causes some diuresis but no change in urine ammonia.

(b) The absorption of acid from the intestine causes some diuresis with an increase in urine ammonia.

(c) The absorption of alkali from the intestine causes diuresis with a decided decrease in urine ammonia.

6. Diuresis per se causes no change in urine ammonia.

So gastric secretion and urine ammonia are related in that the urine ammonia is increased by the absorption from the intestine of the acid product of the gastric secretion.

During the course of this study it has also been found that:

1. Copious water (300 cc. in Pavlov dogs and 500 cc. in man) with the meals causes an increase in the amount and in the free and total acidity of the gastric juice. There is no change in peptic activity.

2. The latent period of the gastric glands of man when stimulated by water is from five to seven minutes.

3. All stomachs are not stimulated by water, which seems to depend upon the rate of emptying the water, e.g., those stomachs that empty water slowly (less than 150 cc. in fifteen minutes when 400 cc. are drunk) respond much more than those that empty water fast.

The effect of water and sodium bicarbonate on gastric secretion. C. E. King and W. W. Hanford.

Dogs with the miniature stomach according to Pavlov were used. A uniform diet was maintained. The juice secreted after the introduction of distilled water was taken as the standard, and with it was compared the juice secreted after the administration of a 1 per cent solution of sodium bicarbonate. Experiments were carried out in which the water and alkali were introduced into the empty stomach, also with the meals and during the various stages of digestion. Dogs weighing about 15 kilos were used. The volume of liquid given each time was 400 cc. The following is a summary of the observations and conclusions:

1. Water excites the flow of gastric juice.

2. Water starved dogs secrete no appetite juice when shown water in the container from which they are accuston ed to drink, or when placed near running water.

3. The acidity of the gastric juice runs parallel with the rate of secretion until a maximum is reached. This maximum varies in different dogs but is near 0.5 per cent HCl.

4. Water given with meals or during digestion results during the following hour in an increase in the amount of juice secreted over that which would be secreted on the administration of either water or meat alone. This also holds true for 1 per cent sodium bicarbonate.

5. One per cent sodium bicarbonate excites the flow of gastric juice, being somewhat less effective than distilled water. The depression is not so marked as reported by Pavlov.

6. The acidity of alkali juice, when the alkali is introduced into the empty stomach, is on an average a little lower than that of water juice, but not materially different when given with meals or during digestion. This refers to total acidity. The combined acidity of alkali juice is a little higher than that of water juice.

7. Water and alkali juices possess approximately the same peptic activity, but both are much less active than meat juice.

8. No injurious effects were noted on the continued administration of 1 per cent sodium bicarbonate.

The effect of water on gastric secretion. Geo. F. Sutherland.

This is a study of the mechanism of the stimulating action of water on gastric secretion. Psychic secretion from the drinking of water was not tested, since its importance has already been shown by Carlson, Orr and Brinkman. Two other factors were studied, (a) dilution of the blood by intravenous infusion, and (b) more rapid absorption of secretagogues.

The intravenous infusion of 10 to 20 cc. of distilled water per kilo body weight in dogs with a Heidenhain or Pavlov pouch, or a simple gastric fistula, is stimulant to gastric secretion. Ringer's solution variously diluted, or a 5 per cent gelatin solution in Ringer's produced a similar response. Water introduced into the small intestine without the possibility of regurgitation into the stomach produces a slight though definite response. Introduction of water by stomach tube stimulates gastric secretion in man and dogs. Water introduced into the gastro-intestinal tract has a greater stimulating effect than by intravenous infusion probably because it increases the absorption of secretagogues.

A 0.23 per cent calcium chloride solution (16 cc. per kilo body weight by intravenous infusion) did not inhibit the secretion caused by gastric secretin.

There is a periodicity in the gastric secretion in starvation, periods of low activity alternating with periods of "spontaneous" increased activity.

In pups and kittens, the gastric glands are not able to secrete free HCl until about the time of birth. In guinea pigs, this power is developed earlier.

Further studies of the mechanism of the clinical measurement of blood pressure. Albert M. Bleile and Clyde Brooks.

The demonstration of the physics of the arm band indirect method of measurement of blood pressure by Brooks and Luckhardt[1] opened

[1] Brooks and Luckhardt: Demonstration before the American Physiological Society, December, 1 14, also, The chief mechanisms concerned in clinical methods of measu ing blood pressure, this Journal, 1916, xl, 49. .

and paved the way to a more complete understanding of the pulse sounds in the auditory method of clinical measurement of blood pressure.

The present work consists of a close observation of the pulse sounds in a large number of cases and an attempt to correlate these with what has just been discovered regarding the behavior of the blood vessel during the application of the arm band method.

The result indicates that the sounds heard are more varied and complicated than are generally recognized. There are certainly more than the four phases described by Korotkow. They seem too many and too complicated to describe here.

However it may be definitely stated that the "swish sounds" occur at such a time and in such a manner as to be in agreement with the hypothesis that they are due to the squirting of blood through the narrow orifice of the flattened but incompletely closed blood vessel.

A full report of these studies will be published shortly.

The length of the systole and the diastole of the human heart. Warren P. Lombard and Otis M. Cope.

There is great and immediate need of practical methods of determining the functional condition of the heart muscle, and of the irritability of the nervous mechanisms which regulate its action. Ordinary muscles when fatigued or degenerated show a longer latent period and a more prolonged contraction than normal, and presumably the same would be true of the heart muscle. Accurate measurement of a sufficiently long series of systoles and diastoles would give information not only concerning the heart muscle, but concerning the behavior of the respiratory and vaso-motor nervous mechanisms which influence the heart rate. There ought to be a table which would supply at a glance the average length of the systole and diastole of the normal heart, and the ordinary variations from the average, by every ordinary heart rate, the rate being estimated from the length of the individual cycles. The writers have begun the preparation of such a table. Although photographic records of the heart sounds would probably supply the most accurate measurements, the writers have thus far employed tambour records of the carotid pulse, because, if these proved sufficiently reliable, they would be of more practical use. The results of the measurement of the systoles and diastoles of 1600 heart cycles of twenty normal young men were shown in a chart. The chart gave for every heart rate from 60 to 120, as calculated from the cycles, the average length of the systoles and diastoles, the variations from these averages, and the number of cycles and of men supplying the data for each heart rate. Another chart illustrated the great variations in the lengths of the systoles and diastoles which may normally occur during one minute, as a result of inspiratory and vaso-motor effects.

A new criterion for the determination of the diastolic pressure in oscillatory blood pressure records. Bernard Fantus.

The shape of the oscillations may be of as great importance in determining the point of diastolic pressure in oscillatory blood pressure records

taken by the Erlanger method as the height of the oscillation. A compound lever tambour, such as the vertical membrane tambour of Zimmerman (Leipzig) or a 2.5 cm. tambour of Edward Meister (Johns Hopkins University, Baltimore), is more suitable for expressing the shape of the oscillations than the simple tambour commonly used. To bring out the true shape of the oscillations, it is also necessary that an adjustable leak be provided in the tambour space, as suggested by Dr. O. M. Cope of the University of Michigan, instead of the minute hole in the tambour provided by Erlanger. A rather soft thoroughly elastic rubber bulb and an arm bag with a stiff leather backing are also indispensable requisites.

When these conditions are met, it will be found that *the pulse oscillation that would make a triangle of greatest area* coincides with the fourth phase of Korotkoff. Under optimum conditions, a striking change appears in the succeeding oscillation, consisting of a lack of support in the downstroke of the pulse oscillation, causing a more definite expression of the dicrotic notch. This change is evidently due to the fact that the tambour lever flung up by the pulse wave is now no longer well supported during its descent, owing to the flaccid condition of the elastic system. Hence, the last well supported downstroke in the oscillation that would make a triangle of greatest area indicates the level of diastolic pressure. In experimental procedure a signal magnet marks the Korotkoff sounds, which were registered by an observer, without looking at the tracing that was being taken. The first sound of Korotkoff is indicated by one tick of the signal magnet, the fourth phase is signalled by two ticks, which are lettered "D." A monometer registers the pressure in the arm bag synchronously with the oscillatory record of an Erlanger bulb and tambour arrangement the tracing of which indicates the systolic criterion of Erlanger and the diastolic criterion here described. The carotid tambour record taken synchronously (breath being held) shows by the time relations that the marked notch following "D" in the Erlanger bulb and tambour tracing is the dicrotic notch.

The preanacrotic phenomenon and its relation to the arterial compression sounds of Korotkoff. A demonstration. JOSEPH ERLANGER.

Records obtained from the artery in situ where and while it is being decompressed[1] as in estimating the arterial pressure in man, show a series of small waves, the most prominent of which usually is negative, immediately ahead of the anacrotic limb of the pulse, but fully developed only in the more distal parts of the compressed length of artery. Korotkoff sounds first become audible in the artery beyond the compression simultaneously with the development of these waves. As decompression proceeds, these waves increase in amplitude and complexity, but disappear, usually quite abruptly, with the first pulse of the fourth sound-phase and when the artery first remains fully rounded but undistended at the end of diastole. During this first-to-fourth sound-phase

[1] Erlanger: This Journal, 1917, xlii, 588.

period the anacrotic limb of the pulse steepens as it proceeds along the compressed segment. These changes in pulse form we designate the preanacrotic phenomenon.

A similar phenomenon is seen in a model consisting mainly of a water-filled tube about one meter long, rolled out of thin rubber dam, through which artificial pulses are propagated. When this tube is partially filled, for instance so as to be half flat, the anacrotic limb of the pulse, as it proceeds, for a time steadily increases in steepness. About 7 to 12 cm. down the tube a preanacrotic negative wave begins to form. This wave deepens and shortens, and at about 15 cm. there appears in front of it a positive wave which also grows and eventually develops in front of itself another negative wave. At about 19 cm. the first negative and positive waves are found well up in the anacrotic limb of the pulse. This process then repeats itself over and over again some distance down the tube. The ascension of these *ripples* into the anacrotic limb is due to the faster propagation of the pulse proper. The same sequence can be traced in the records obtained from animals.

The position of ripple formation shifts downward as the tube fills, and at the degree of roundness (fullness) that is determined by a pressure of only 1-3 mm. Hg., ripple formation ceases. A lever resting across the tube is thrown violently from it, but only when the preanacrotic phenomenon is in evidence under it. A sharp snapping sound is produced by each pulse, but again only when and where the preanacrotic phenomenon is in evidence. Elsewhere and at other times if a sound is audible at all, it is dull in quality. Presumably, therefore, the sharp Korotkoff sounds are produced by the thrust of the steep anacrotic limb that develops in association with the preanacrotic phenomenon[1]. Sharp sounds are therefore heard in man during the stage of decompression included between the pulse following the first to penetrate the length of the compressed segment and the pulse following which the wall of the artery fails to be relaxed by the compression, that is, from just below systolic to just below diastolic arterial pressures.

Some uses of wire in the laboratory. Arthur D. Hirschfelder.

Easily adjustable semi-rigid holders for cannulas, glass tubes, funnels, etc., can be made by twisting soft iron stove-pipe wire a few turns around a ring stand then twisting tightly into a double strand from 6 to 10 inches and then twisting a few turns about the object to be grasped. The double strand of iron wire is just rigid enough to hold in place many of the ordinary objects used in experimentation and yet flexible enough to admit of adjustment in every direction.

Spring brass wire of various weights can be used to make convenient retractors for operations and dissections. The wire is first bent into a "U" of desired size ranging from 5 to 15 cm. and the hooks to grasp the tissue are then made at the end of each arm of the "U." These also are

[1] Erlanger: Proc. Wash. Univ. Med Soc.; Journ. Mo. State Med. Soc., June, 1917, 258.

"U" shaped with arms 0.5 to 2 cm. in length bent at right angles to the arms of the large "U," with the open portion of the "U" pointing outward, the connecting arm of the "U" facing inward. In this way the small "U's" grasp the tissue on each side and are pulled apart by the spring of the large "U." The small "U's" may be made most quickly by heating the ends of the large "U" to take out the temper before binding. A spherical drop of solder placed at the free end of each small "U" prevents the retractor from tearing the tissues. Retractors made in this way are very satisfactory.

A convenient lever for marking signals, recording contractions of muscles, ventricles, etc., can be made from spring brass wire by winding one end into a coil around the ring stand and leaving the other end to extend horizontally out from the ring stand for 15 or 20 cm. This horizontal arm forms the recording lever. A small ring twisted into the other free end of wire coil can be twisted into place directly below the horizontal lever arm and a thread can be run through this ring and attached to the lever arm. This thread may be pulled on directly or it may be brought out through a second loop of wire attached to the base of the ring stand. A pull upon the thread causes movement of the lever, which can be recorded on the drum.

Some simple valves for respiration apparatus. ARTHUR D. HIRSCHFELDER and EDGAR D. BROWN.

A valve for use in respiration apparatus is easily made by taking a $\frac{1}{2}$ or 1 ounce tin salve box, and cutting a hole in the top and one in the bottom of the box, and into the hole soldering a piece of brass tube. Care must be taken that the end of the tube does not protrude into the salve box. An elliptical piece of thick rubber dam, a little larger than the hole, is now placed so as to cover the hole and is held in place by a U-shaped bridle of spring brass wire whose ends are soldered down upon the inside of the salve box. For respiration experiments two such salve boxes are used—soldered upon each arm of a brass T-tube, the lid of one box on arm of the T, the body of the other box upon the other arm. In this way an inflow and an outflow valve are made. The apparatus is very easily constructed and the valves work well.

A simple stalagmometer. ARTHUR D. HIRSCHFELDER.

A piece of fine brass wire is inserted into the end of a piece of thick walled barometer tubing of 2 mm. lumen and 7 mm. outside diameter. The end of the tubing is closed by fusing in a small blast flame and the wire is then fused into the glass for a distance of about 15 mm. by gradually advancing the blast flame along the tube. It is then allowed to cool and a bulb to hold 2 to 3 cc. is blown into the tube 20 or 25 cm. away from the fused end. The fused end is now ground off square with a grindstone taking care to keep the grindstone moist. The coil of wire is now dissolved out. For this purpose a very fine capillary pipette is drawn out filled with nitric acid and introduced through the barometer tubing clear down into the fused portion, so that the acid comes into

contact with the upper end of the wire. The whole barometer tube is now placed in a test tube containing concentrated nitric acid 5 or 6 cm. deep so that the wire is acted on by nitric acid from within and without the barometer tubing. The test tube is now placed in a water bath and left there one or two days, after which the wire coil will be found to have been completely dissolved out and a fine capillary bore left in its place. If wire of no. 28 B. and S. gauge is used a stalagmometer which is very good for ordinary purposes may be prepared discharging 9 to 60 drops per minute. If wire such as is used for obturators of fine hypodermic syringe needles is used a very fine capillary is obtained delivering one drop in from twenty to thirty-six seconds. Any degree of frequency can thus be obtained.

An experiment for training students in the technique of intravenous and intraspinal injection. ARTHUR D. HIRSCHFELDER.

A human upper arm and forearm is obtained from the dissecting room, and a flap of skin dissected back for a distance of 10 cm. above and 10 cm. below the elbow on the pronator surface of the arm. An artificial vein is formed by taking a segment of rabbit's small intestine and connected by means of glass and rubber tube with a pressure bottle or funnel filled with water or colored fluid at an elevation of about 20 cm. above the table. The distal end of the intestine is closed off with a clamp. The filled segment of intestine is now introduced under the skin of the arm along the course of the median basilic vein and the skin flap is replaced and held in place with clamps. The distended segment of intestine presents under the skin in much the same appearance and position as the vein in man; and the student can now practice inserting a syringe needle into it, drawing up the "blood" and injecting any desired liquid into the "vein." The deeply seated veins of a fat individual can be simulated by interposing a thin layer of fat which can be kept in alcohol in a specimen jar. A sclerotic vein can be simulated by using a piece of white rubber tubing instead of the rabbit's intestine.

Practice in intraspinal injection may be afforded by two procedures. For preliminary practice, the spinal column of a human skeleton is articulated upon a bent brass rod. The segments may be held in place with modeler's clay or plasticin. Modeler's clay is now placed around one side of the vertebrae to simulate the tissues of the back. The student can now learn the sensation of introducing the needle between the bony structures of the vertebrae by introducing a stiff wire into the spinal canal. Having acquired the desired proficiency he can then practice upon the second model. This consists of the lumbar and sacral portions of a human trunk, screwed upright upon a piece of inch board which is just large enough so that, when in use it can be clamped to the table, and when not in use the whole specimen can be placed in a specimen jar filled with alcohol. Upon the left side of the specimen the muscles and fascia are dissected off along the spinal column so as to leave only the interspinous ligaments in place. The skin is left intact. A wedge of bone is sawed out along the anterior aspect of the

centra of the vertebrae, so as to expose the spinal canal. The student can now practice inserting the needle. He can observe upon the dissected left side exactly the bony and ligamentous structures through which his needle is passing and at the front of the vertebrae he can see exactly how and where his needle has entered the spinal canal and its exact relation to the spinal cord and the cauda equina.

The proportionate measurements of two hundred and fifty full term newborn infants. R. TAYLOR.

The results of the comparative measurements of 250 normal, full term, newborn infants show that from finger-tip to finger-tip is further than from crown to heel; that the occipital frontal circumference is barely greater than the sitting height, but decidedly out-measures the chest circumference; that the trunk length is greater than the arm, and the latter longer than the leg.

As regards individual variations, the spread of the arms is as long or longer in 81 boys and 82 girls, 65 per cent of the total. The head circumference is greater than the chest in 119 boys and 120 girls; the trunk length greater than the arm length in 119 boys and 123 girls, and greater than the leg length in 123 boys and 124 girls. The arm length exceeded the leg length in 111 boys and 107 girls. In 114 boys the mid point of the body lay at or above the navel and below in 11, the extreme figures being 32 mm. above and 10 mm. below. It was at or above the navel in 100 girls and below in 25. They showed greater variations, the extremes being 36 mm. above and 14 mm. below. The proportionate lengths of trunk, arms and legs, the proportionate chest and leg circumferences and the position of the center of the body relative to the navel are diametrically opposed in the newborn and the adult.

The rôle of the afferent impulses in the control of respiratory movements. HELEN C. COOMBS and F. H. PIKE.

To an earlier statement[1] and our statement of last year on section of the dorsal roots of the spinal nerves[2] we now wish to add certain other facts.

Cats were used in our experiments. Ether and tracheotomy were routine procedures. A control tracing of both costal and abdominal respiration was taken by means of Crile stethographs attached to Verdin tambours. The subsequent procedure was varied. Our findings are:

1. Section of the vagi alone produces a slow, deep type of respiration which has often been noted.

2. Section of the dorsal roots of the thoracic and cervical nerves results in a diminution or cessation of costal respiration. The effect of section of both thoracic and cervical nerves is a more marked diminution of costal respiration than after section of the thoracic roots alone. Abdominal respiration remains unchanged after section of the thoracic roots and there is no marked alteration in the respiratory rate.

[1] Stewart and Pike: This Journal, 1907, xix, 328; Stewart: Ibid., 1908, xx, 47.
[2] Pike and Coombs: This Journal, 1907, xlii, 395.

3. Section of the brain stem below the anterior corpora quadrigemina results in an abnormal form of respiration. It becomes labored, with respiratory gasps initiated by the diaphragm. This type of respiration resembles that which prevails after anaemia of the brain during the period of resuscitation before the afferent impulses become effective.[1]

4. Section of the vagi, followed by section of the dorsal roots produces, (1) a slowing of the respiratory rate, and (2) a diminution of costal respiration with dyspnea ensuing in from three to five minutes. This may be more or less severe, depending on the location and magnitude of the sections of the dorsal roots. Such respiration lasts for from fifteen minutes to an hour, gradually fading out with no terminal gasps. In its later stages it resembles respiration after section of the posterior corpora quadrigemina and vagi.

5. Section of the dorsal roots followed by section of the vagi produces (1) diminution of costal respiration, (2) a slowing of the respiratory rate with a return of costal respiration. This nearly normal type becomes dyspneic in the course of time, and gradually fades out, as when the procedure is reversed.

6. Section of the posterior corpora quadrigemina, followed by section of the vagi, results in dyspneic respiration which fades out much more rapidly than when the vagi are intact.

7. Section of the dorsal spinal roots after section of the corpora quadrigemina produces no more severe effect than section of the corpora quadrigemina alone. That the effects of transection below the posterior corpora quadrigemina are not due to trauma or shock from stimulation of efferent inhibitory fibers follows from the fact that essentially the same picture results from nerve section alone.

8. Section of the dorsal spinal roots followed by section of the phrenics results in (1) diminution of costal respiration and (2) return of costal respiration when the diaphragmatic is put out of commission. If the vagi are then sectioned there is a total failure of respiration coming on in a shorter time than when the phrenics are intact.

Parallel determination of amylase and dextrose-glycogen of the blood, liver and kidney after feeding. E. E. Brown and C. W. Greene.

Sets of animals have been prepared and fed meals consisting primarily of carbohydrates, carbohydrates and fat, carbohydrates and protein, and protein (lean meat).

The blood, liver, kidney and muscle of these animals have been examined for carbohydrates by the Lewis-Benedict method and for amylase by the Meyers-Killian method. The animals were killed at intervals during the digestion and absorption of the test meal. The blood and tissue curves in the sets of animals showed the following: The curves of variation in sugar content after any meal containing carbohydrate increases to the eighth to twelfth hour of absorption then gradually declines to the normal. This curve is closely followed by the curves of

[1] Stewart and Pike: This Journal, 1907, xix, 328.

variation in enzyme content though the enzyme does not vary to so great a percentage amount. A meal of pure protein gives only a slight rise in carbohydrate content of the tissues examined. The amylase content of the liver after protein is not increased more than usual but the blood and the musele both show a sharp increase. The liver shows the greatest increase in sugar during the cycle of carbohydrate absorption, it also exhibited the greatest augmentation of enzyme. The blood and the kidney present curves of slighter variation but of very constant character. No attempt has been made to determine the source of the enzyme observed.

Brain changes associated with pernicious anemia. HENRY W. WALTMANN.

While it has long been known that certain symptoms, such as numbness and prickling of the fingers and toes, ataxia, bladder disturbances, etc., referable to involvement of the central nervous system, may arise in the course of a pernicious anemia, it does not appear to be generally recognized, even yet, how constant these findings really are. Though Nonne, Minnich, and others who described the spinal cord changes present in this disease, could see no reason why these same alterations did not take place in the brain, it was not until 1913 and 1916 that Barrett described alterations in the brain analogous to the Lichtheim foci present in the cord. In addition to these foci, which are very characteristic, there is present in the brain also a diffuse parenchymatous degeneration of the medullary substance.

Many of these degenerative changes are intimately associated with the blood vessels, in the so-called peri-vascular space of which one often sees a hyalin-like material, probably containing a toxin, which extends into the areas occupied by the disintegrating white fibers. This would indicate that lymph stasis is an important factor in the mechanism of this destruction. The gray matter, particularly that of the convolutions, shows some pathological changes which also tend to support this view. Surrounding the pyramidal cells, one often notes a circular area of degeneration, the pyramids themselves showing all states of disintegration. It is possible that toxins present in Obersteiner's so-called pericellar space are responsible for this picture.

In conclusion, we might state that Lichtheim placques, analogous to those occurring in the spinal cord, are present in the cortex also, as Barrett reported.

Though only seven brains and cords were studied in this series, it would appear that these changes are present in the cortex in just as great a proportion of cases as appear in examinations of the cord, and that those who show a well marked subacute combined sclerosis of the cord are also the ones in whom these changes can be demonstrated in the cortex.

Relative to the mental disturbances associated with pernicious anemia it is generally recognized that well defined types of psychoses, such as manic-depressive insanity may occur in pernicious anemia patients which bear no relation whatever to this disease. The lesser disturb-

ances, such as irritability, somnolence, apathy, etc., may, however, be based, at least in part, upon the pathological lesions found in the cortex.

A study of the comparative anatomy of the biliary tract and the sphincter of Oddi with special reference to animals without a gallbladder. F. C. Mann.

The results of a previous research[1] performed at the suggestion of E. S. Judd, proved that definite changes were produced by removal of the gallbladder in dogs, cats and goats. These changes consisted in dilatation of all the extra-hepatic ducts, including the cystic duct when it was present. The results of other experiments seemed to show that this dilatation was due to the activity of the sphincter of Oddi.

The purpose of the present research was to determine, if possible, how animals without a gallbladder compensated for the lack of it. Several species, some with and others without, a gallbladder, were included in this study.

The following factors were investigated:

1. The diameter of the common duct.
2. The length of the common duct.
3. The point of entrance of the common duct into the duodenum.
4. Secretory pressure of the liver.
5. Tone of the sphincter of Oddi.
6. Histology of the sphincter of Oddi.
7. The walls of the common duct.

Of these the only points of difference noted so far were in the tone of the sphincter of Oddi and the thickness of the walls of the duct. In the animals without a gallbladder which have been investigated so far there does not seem to be very much tone in the sphincter of Oddi, and the walls of the duct seem thicker and stronger in these animals. The work is far from being completed.

Some further notes on the detoxification of potassium chloride in the guinea pig. S. Amberg and H. F. Helmholz.

After expressing the urine contained in the bladder guinea pigs of about 200 gram weight received intravenous injections of 2 and 3 cc. of a solution containing 5 per cent NaCl and 1.5 per cent KCl. Ten minutes after the injection only about 0.1 to 0.2 cc. of urine could be expressed from the bladder. The detoxifying action of the NaCl cannot be due to a hastening of the excretion of KCl. Previously reported experiments showed that injections of 5 cc. 5 per cent NaCl protected guinea pigs against 2 or 3 cc. 1.5 per cent KCl injected immediately afterwards, while 0.55 per cent NaCl did not protect. The possibility had to be considered that the injection of 5 per cent NaCl diluted the blood much more than 0.55 per cent NaCl. Haemoglobin determinations (Fleischl-Miescher) of the blood of guinea pigs were made before and after the injection of 5 cc. 5 per cent and 5 cc. 0.55 per cent NaCl. No marked differences were found in the effect of the 5 per cent

[1] Judd and Mann: Surg., Gynec. and Obst., 1917, xxiv, 43.

and the 0.55 per cent NaCl solution. The protective action of the 5 per cent NaCl solution therefore cannot be ascribed to a greater dilution of the blood. Guinea pigs of about 200 grams survive readily the intravenous injection of 2 cc. of a solution containing 50 per cent glucose and 1.5 per cent KCl. An intravenous injection of 2 cc. 50 per cent glucose leads to a more marked dilution of the blood than that of 5 cc. of the NaCl solutions. After such an injection the ear vessels are markedly dilated.

Development of certain types of malignant tumors of the thyroid. Louis B. Wilson.

The writer has in progress a pathological review of malignant tumors of the thyroid in man. These, while not of frequent occurrence are found to be much more frequent than is ordinarily supposed. They are often overlooked, especially in their early stages by clinicians, surgeons and pathologists, since many of the malignant types closely resemble both grossly and microscopically benign adenomas of "fetal" type. Both benign and malignant adenomas apparently have their origin in embryonic tissue which at first consists of relatively small cells with indistinct outlines and relatively large, round or slightly oval, densely staining nuclei. These cells are irregularly arranged and are separated only by scarcely discernible septa which consist essentially of thin-walled, flattened blood vessels. In the second stage the cells are arranged in cordon-like masses or long band-like platelets between which are the same "sinusoid" vessels. In the third stage the cordons or platelets are broken into roundish masses with the beginning formation of acini some of which may contain colloid. This stage is apparently the most critical. If the acini become well developed the tumor is apt to remain benign. If the acini are irregularly or imperfectly developed the tumor is apparently more apt to become malignant, if it is not already such.

A careful study of the histology of supposedly benign encapsulated thyroid tumors removed at operation in relation to these stages of development is important for the early diagnosis of malignant conditions.

Blood regeneration after simple anaemia. I. Curve of regeneration influenced by dietary factors. C. W. Hooper and G. H. Whipple.

In previous publications we have shown that the bile pigment secretion can be influenced at will by modification of the diet. We have shown that the curve of bile pigment secretion can be depressed below normal by a meat diet and can be raised much above normal by a diet rich in carbohydrates. A mixed diet in a healthy bile fistula dog is associated with a fairly constant mean bile pigment elimination. From the results of these experiments we have assumed that the liver may have a constructive ability in bile pigment formation which can be modified by diet, as well as the accepted eliminative function, which depends upon the destruction of red blood cells containing hemoglobin. Furthermore, we have considered the possibility that the liver may be concerned

in the formation of other body pigments than bilirubin; for example, hemoglobin.

At the present time we have very good evidence that blood regeneration, after simple anaemia of definite grade, can also be influenced at will by various dietary factors. The curve of blood regeneration on a meat diet is very rapid, a matter of days or a few weeks, while the curve of regeneration on a diet rich in carbohydrates is very slow, in some of the animals on a diet of bread and milk it has required months for complete blood regeneration.

Dogs of the bull mongrel type were used in all of our experiments. The following blood studies were carefully made a day before bleeding and at intervals varying from four days to a week after bleeding until complete blood regeneration had taken place. The hemoglobin was estimated according to the Sahli method. The blood was allowed to remain in contact with the $\frac{N}{10}$ hydrochloric acid exactly five minutes before diluting in order to insure accurate and comparable readings. The red blood cells and white blood cells were counted. The blood platelets were counted by the Ottenberg and Rosenthal method. Brilliant cresyl blue (dilution 1 to 300) was used for counting the reticulated red blood cells. Janus green (dilution 1 to 10,000) was employed to estimate the number of red blood cells containing mitochondria. Hematocrit readings of the percentage of blood corpuscles were made after centrifugation in graduated tubes for 3000 revolutions per minute for thirty minutes. The plasma volume was estimated by the introduction directly into the circulation of a non-toxic, slowly absorbable dye furnished by Dr. H. M. Evans. The dye was allowed to remain in the plasma long enough for thorough mixing and its concentration was determined colorimetrically by comparison with a suitable standard mixture of dye and plasma. The total blood volume was calculated from the plasma volume on the basis of the hematocrit readings. The principle of this method is similar to the Keith, Rowntree and Geraghty method for the determination of plasma and blood volume. The routine blood studies were always made before beginning an experiment. The animals were then placed on a bread and milk diet and on each of the following two days one-fourth of the calculated total amount of blood was aspirated from one of the jugular veins. The animals were then allowed to rest a day, and on the following day the routine blood studies were again made. Generally, the percentage of hemoglobin and the number of red blood cells were approximately one-half the normal. On this day the animals were placed upon the specified diets. Special care was taken in each case to give a diet containing a sufficient number of calories and amount of nitrogen to assure a positive nitrogen balance. The animals usually gained in weight.

The curve of blood regeneration on a diet consisting of lean scrap meat or beef heart was very rapid. Out of eleven dogs, ten showed complete blood regeneration in from two to four weeks. Usually the hemoglobin and red blood cells regenerated with equal rapidity. Several splenectomized animals made anaemic and placed on lean meat diets

regenerated as rapidly as normal animals. One bile fistula dog fed on a beef heart diet showed complete blood regeneration in three weeks.

In the next series of experiments we studied the curve of blood regeneration on diets rich in carbohydrates. The first group of dogs was fed a diet consisting of white bread and milk; the second group a diet of cracker meal, lard and butter; the third a diet of white bread, lard and butter. The curve of blood regeneration on each of these diets was very slow, requiring from four weeks to five months for complete blood regeneration.

In some of the animals, especially those on a bread and milk diet, the curve ascended slowly for a short initial period of a few weeks, when the animals lost weight, the hemoglobin fell and the anaemia progressed until they were placed on a meat or mixed diet. In some instances, the number of red blood cells increased very rapidly and within three or four weeks after the initial bleedings the total number of red cells was even greater than at the beginning of the experiment. However, the cells were small and fragmented. The percentage of red blood cells as indicated by the hematocrit readings was the same as on the days immediately following the initial bleedings. In other words, the increase in red cells was only relative, due to the development of small and fragmented forms.

Simple bile fistula dogs made anaemic and placed upon a bread and milk diet reacted the same as the normal dogs.

However, splenectomized dogs made anaemic and fed a bread and milk diet or a diet consisting of cracker meal, lard and butter, generally regenerated slowly for a period of from four to seven weeks when a sudden recidivation developed resulting in death within a very few days. In some cases the hemoglobin fell from 95 per cent to 24 per cent, the red blood cell count from six million to one million within a week without jaundice. Furthermore, there was always a leucocytosis and a diminution in the total blood volume. The anaemia was usually of secondary type in the animals that retained their appetites and usually of primary type in those that refused to eat. This latent anaemia period was always accompanied by an intense urobilinaemia without a noticeable rise in the bilirubin content of either the blood plasma or urine.

The curve of blood regeneration on a mixed diet consisting of meat, bread and milk, or meat, cracker meal, lard and butter was quite rapid, usually resulting in complete blood regeneration in from three to five weeks. Splenectomized dogs reacted on this diet as rapidly as the normal animals. One simple bile fistula dog made anaemic and fed a mixed diet recovered completely in three and a half weeks.

Iron in the form of Blaud's pills, administered daily during the anaemia period had no appreciable effect on the curve of blood regeneration in any of our experiments.

Blood regeneration after simple anaemia. II. Curve of regeneration influenced by starvation, sugar, amino acids and other factors. G. H. Whipple and C. W. Hooper.

These experiments were performed under conditions described in the preceding communication. The dogs were kept in metabolism cages, food and water being given by stomach tube every twenty-four hours after catheterization. The urine was analyzed for total nitrogen.

Starvation. A curve of blood regeneration may be formed by multiplying blood volume by the hemoglobin percentage. This curve of *pigment volume* will show a drop to about one-third normal after the unit bleeding. During the first week of repair there will be usually a small but definite rise in the curve—perhaps a rise of 10 per cent. The following weeks (3 to 4) will usually show a slow but steady rise of 5 to 20 per cent of the total pigment volume. This reaction may be observed in normal dogs, bile fistula dogs or splenectomized dogs, and we have no evidence of any marked differences in blood regeneration under these experimental conditions. It is interesting to note the same speed of blood pigment regeneration in a bile fistula dog with bile pigment excluded from the intestine, proving conclusively that the body does not rely on absorption of any pigment substance from the intestine (bile pigment, urobilin or urobilinogen).

Sugar feeding. The experiments are in every way similar to those just described but for a definite amount of cane sugar or glucose or a mixture of the two sugars (50 to 125 grams) dissolved in a uniform amount of water and given by stomach tube. The curve of pigment volume or blood pigment regeneration may be followed each week as before and will show some differences from the starvation curve. We note the same definite rise during the first few days or first week—a rise of 10 per cent of the original pigment volume, but this is usually followed by a fall of 2 to 5 per cent during the second week and minor fluctuations after this for the usual period of three to four weeks. In other words, after the initial rise in the first week, the dogs fed on sugar show no gain in blood pigment and often a slight loss in contrast to the starvation experiments which show a small but definite gain.

It is to be noted that the protein decomposition in the body as indicated by the urinary nitrogen elimination is much less in the dogs fed on sugar than in the starved dogs. Both groups of dogs are receiving no nitrogen by mouth and yet they are able to form hemoglobin and red corpuscles during this interval. How much new red blood cell construction is going on during these periods we cannot say, but surely there is some disintegration of red cells to be made up and the curve of pigment volume shows a definite rise, more pronounced in the starving dogs which of necessity are excreting more nitrogen and breaking down more body protein. We are forced to the conclusion that the body conserves certain substances from this body protein autolysis and uses them over again to construct red cells. We have evidence that a part of this conservation is due to liver activity. There is no evidence for increased protein katabolism.

The initial rise in the pigment curve during the first week is not easy to explain, but we have assumed that the body has stored in reserve certain necessary substances which are used in this emergency to form new red cells, but this reserve is rapidly depleted and we have experiments to show that the red cells formed in such emergencies may not measure up to the usual standard and may fail the body in service.

Gliadin feeding influences the curve of pigment regeneration no more than sugar feeding, and is associated with only minor fluctuations in the pigment volume. Four or five weeks of gliadin feeding may show the pigment volume at the same level as at the start.

Hemoglobin (dried washed red cells, 10 grams per day) added to a sugar diet will cause a definite rise in the curve of pigment regeneration, but the same amount of dried meat protein might give a similar reaction.

Gelatin feeding has a striking influence upon the curve of pigment regeneration and red cell formation. Gelatin added to a sugar diet will usually cause a sharp rise in the curve of pigment regeneration. This rise is not as marked as after meat feeding. It usually reaches its maximum after two to four weeks, and then may show a fall coincident with malnutrition which may develop after prolonged gelatin feeding. Gelatin combined with cracker meal and lard may show a complete regeneration to normal within three weeks. This may be followed by a fall below normal if the diet is continued several weeks.

Through the coöperation of Doctor Van Slyke, Doctor Rohde and Miss Foster we have been able to show that the monoamino acid fraction of gelatin hydrolysis can influence in a striking manner the curve of pigment regeneration—almost as efficiently as whole gelatin. However, the diamino acid fraction of gelatin has a similar effect, but little less marked. Histidine (1.5 gram per day) combined with a sugar diet has no notable influence on the curve of regeneration—perhaps a trivial increase. We can submit no evidence that any single amino acid or group of amino acids is the single essential factor in this reaction which determines the curve of pigment construction and regeneration of the red cells. It seems very probable that several diet factors are concerned—as well as body conservation of certain elements of protein katabolism. It is suggestive that a meat diet gives a maximum hemoglobin production and a minimum bile pigment secretion in the same bile fistula dog. Also, a bread and milk diet gives a minimum hemoglobin production and a maximum secretion of bile pigment in a bile fistula dog. It is possible that some substance in the meat enables the body to conserve or fix and reconstruct certain substances resulting from tissue katabolism. The amount of body protein autolysis or katabolism certainly is an important factor. There is a constant wastage of pigment substance (bile pigment) and a uniform construction of pigment substance in the body even during starvation. The products of protein katabolism in the body as well as dietary factors contribute to the steady construction of body pigment, especially hemoglobin.

Changes in reflex thresholds following shock from manipulation of the intestines. EUGENE L. PORTER.

Shock was produced in spinal cats by exposing and manipulating the intestines. In about 50 per cent of the animals the threshold of the flexion reflex was raised by this procedure in from one to five minutes. In the remaining animals no change could be detected. Commonly the new threshold was 30 per cent to 50 per cent higher than the original; the maximum observed was a 100 per cent increase. The high threshold was maintained as long as manipulation continued (ten to forty minutes). Upon returning the intestines to the abdominal cavity the threshold usually dropped, within about ten minutes, nearly or quite to its original level. In some cases it was possible to repeat the experiment on the same animal. There was no parallelism between the changes in reflex threshold and changes in blood pressure.

Note in regard to the amount of sugar normally in the blood of cats. ERNEST L. SCOTT.

In 1914 the author published a fairly extended account of the amount of sugar to be found in the blood of the domestic cat under ordinary laboratory conditions. At that time comparisons were based upon the average obtained from twenty-four cats which were prepared in a manner carefully described. Animals so prepared and killed by decapitation were found to vary but little from this average which was 0.069 gram of sugar per 100 grams of blood.

Since that time numerous determinations of glucose in the blood of cats which were prepared in a manner essentially similar to that adopted for the standard animals in the above series have been made.

In all, determinations upon something over one hundred such animals have now been made and while there is a slightly greater range of variation, the average for the longer series remains at the same place, 0.069.

On the comparative absorptive power for drugs of the bladder and urethra (male). DAVID I. MACHT.

In other communications dealing with the absorption of drugs from the conjunctiva[1] and from the vagina,[2] published elsewhere, the author called attention to the fact that apomorphin, by virtue of its being a centrally acting emetic, furnishes a convenient means of demonstrating absorption of drugs through unusual channels. If a 1 per cent solution of apomorphin hydrochloride is introduced into the bladder of a male dog through a hard catheter, the latter instrument being allowed to remain in place, the solution remains in the bladder and owing to the powerful spasmodic contraction of the urethral sphincter in the male dog, practically none of the drug gets into the urethra. Under these circumstances vomiting does not occur sooner than half an hour after the introduction of the poison and sometimes after the lapse of an hour

[1] Journ. Amer. Med. Assoc., 1917, lxviii, 1230.
[2] Journ. Pharm. Exper. Therap. (in press).

or more, and very often not at all unless the catheter be removed. If, on the other hand, the urethra of the same dog, on another day, be irrigated with the same solution or even weaker solutions of apomorphin, care being taken not to inject the drug into the bladder but to confine the irrigation only to the urethra and allow the fluid to run back, vomiting is produced in every case in from three to five minutes. Inasmuch as vomiting is produced in dogs almost as efficiently by means of morphin as with apomorphin, the same results can be obtained by using that alkaloid. Even strong solutions of morphin confined to the bladder produce either no vomiting at all or only after the lapse of a considerable period of time (half an hour to one hour). On the other hand, the introduction of a little morphin solution into the urethra is followed in the dog by vomiting in a few minutes. The remarkable difference in the absorptive power between the urethra and the bladder noted after morphin and apomorphin, holds good for a large number of other drugs and poisons. The author has studied in this connection the effect of various alkaloids, a number of antiseptics, some local anesthetics and a number of salts. The complete account of the investigation will be published in due time in the *Journal of Urology*. It may be stated in this place that an inquiry into the absorptive power of the ureters is also under investigation by the author.

A gradient of metabolism in the intestinal muscle. WALTER C. ALVAREZ.

There is a gradient in rhythmicity from the duodenum to the ileum, the rates, in the rabbit's intestine, varying from 15.3 per minute near the pylorus to 10.5 per minute near the cecum. There are also gradients of irritability and latent period. In the dog, the duodenal muscle responds to a strong stimulus after 0.13 seconds, the ileum after 0.22 seconds.

The best explanation for these differences is that there is an underlying gradient of metabolism. Child has shown that the regions with high rates of metabolism and faster oxidation suffer more from the action of weak solutions of KCN than do the regions with slower rates. Thus in the Ctenophore mnemiopsis there is a gradient of susceptibility to KCN in the conducting paths along the rows of swimming plates. The pace-maker region suffers so much more than the others do that the impulse may at times be even reversed.

I have caused five segments from different parts of the intestine to beat rhythmically in the same beaker of Locke's solution. The addition of 1 part of KCN to 1,300,000 parts of the solution caused a marked loss of tone and rhythmicity in the duodenum and jejunum, while the ileum and colon were much less affected. Some seventy-five drugs have been tried out, and several show similar differences in their action on different parts of the gut.

Simply shutting off the air bubbling through the solution has often been enough to show this graded effect down the intestine The duodenum generally suffered most; the jejunum came next, and the two segments of ileum and the colon suffered least.

These observations, so comparable with those of Child, furnish considerable proof of the presence of a metabolic gradient down the intestine. The practical aspect to the problem is the fact that the results obtained suggest strongly that some of the emetics and purgatives owe their effects to alterations in this gradient—alterations brought about by an unequal or dissimilar action of the drugs on the two ends of the tract.

Studies on cholesterol. V. The blood cholesterol in malignant disease and the effect of radium treatment on the blood cholesterol. GEORGINE LUDEN.

Between November, 1915 and December, 1917 a total of 1069 samples was tested for cholesterol. The above figure includes 1052 determinations of blood cholesterol on human blood, goat's blood, gopher's blood and dog's blood; 14 determinations on foodstuffs and 3 on human pus. Of 743 blood samples parallel determinations in triplicate were made with Bloor's original method (Bloor I, with sodium-ethylate) and its modification (Bloor II, without sodium-ethylate) making a total of 4658 determinations with Bloor's methods. The advantage of these parallel determinations by which the amount of cholesterol split-products present in the blood is revealed, was shown by tests made on pathologic human blood, including 70 miscellaneous conditions, 41 cases of pernicious anemia, 37 of exophthalmic goiter, 3 of myxedema tested 18 times at various intervals during the administration of the thyroid hormone (Kendall's thyroxin), 79 determinations on the writer's own blood as normal control, 9 cases of sarcoma, (16 determinations) and 92 determinations of the blood cholesterol in carcinoma before and after radium treatment including 20 weekly determinations on one patient.

The blood cholesterol values in carcinoma were found to be high in 43 per cent of all the patients and in 56 per cent of those that were to have radium treatment. In the latter 54 per cent had equal values with the Bloor I and Bloor II method. These equal values indicated some disturbance of cholesterol metabolism in carcinoma, since a difference between the values obtained by the two methods is always found normally and no equal values were found in 252 determinations on non-malignant cases. After radium treatment the equal values were found to disappear and the percentage of high values dropped to 10 per cent. In sarcoma the blood cholesterol values were found to be very much lower than in carcinoma, equal values were observed in two cases also and the effect of radium was similar to that observed in carcinoma.

In myxedema high cholesterol values (but not equal values) were found and the administration of the thyroid hormone brought the blood cholesterol back to normal at a rate parallel to the rise in basal metabolism which it induced.

These observations seem to warrant the following conclusions:

1. That the high blood cholesterol values found in carcinoma are

not due to cell destruction, since they are lowered by radium treatment although radium causes cell destruction, but that they are due to a disturbance of cholesterol metabolism.

2. That the disturbance of cholesterol metabolism may be but evidence of a low rate of basal metabolism, since the high cholesterol values in myxedema are reduced by the administration of the thyroid hormone by which the rate of basal metabolism is greatly increased.

3. That radium treatment, by lowering the blood cholesterol values, alters the chemical composition of the blood, a fact which has not hitherto been taken into account in the study of the effects of radium treatment.

4. That the administration of the thyroid hormone affects the blood cholesterol values in a manner similar to that of radium treatment and may therefore be expected to be equally beneficial to patients suffering from carcinoma. However, careful investigation (in progress by the writer) will be needed before definite conclusions can be drawn concerning the effect of thyroxin in carcinoma.

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