THE GASTRO-INTESTINAL TRACT
OF ELASMOBRANCH FISHES

THE MOTILITY OF THE GASTRO-INTESTINAL TRACT OF ELASMOBRANCH FISHES

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CHAPTER I

INTRODUCTION

The experimental work embodied in the following thesis has been carried out during the last four years and has been published from time to time in various journals, as each phase was brought to completion. These experiments were designed to investigate the reaction of the gastro-intestinal tract to temperature variations; to the action of drugs, such as, adrenaline, pilocarpine, acetylcholine, atropine and ergotoxine; and, finally, to sympathetic and parasympathetic nerve stimulation. For the last an anatomical, histological and physiological study of the vascular supply of the gastro-intestinal tract was necessary, because, as will be shown, the sympathetic nerves are distributed to the gut by means of periarterial nerve plexuses.

A careful search of the literature dealing with the above subjects uncovers very little, indeed. In regard to the effect of temperature variations upon the motility of the gastro-intestinal tract of elasmobranch fishes there is absolutely nothing. Little work of a systematic nature has been done on the whole group of cold blooded animals. Riddle (1909) carried out some investigations on the rate of digestion at different temperatures, which, however, unfortunately, did not reproduce natural conditions very accurately. He measured the stomach capacity of various fish, and constructed Mett's tubes to correspond. These tubes were filled with different food stuffs and placed in the stomachs of the fish under observation. On removing the tubes Riddle was able to measure the rate of digestion. He came to the conclusion that "within certain not very wide ranges of temperature the rule of van't Hoff applies to the digestive processes in living cold-blooded vertebrates (the average of eight valid temperature coefficients being 2.62)."

Turbin (1925) conducted some experiments, again on the rate of digestion taken as a whole, using Rana esculenta under almost normal conditions. Her results are of some value because of the similarity between the frog and the fishes, both being poikylothermal vertebrates. She found that both the motility of the gastro-intestinal tract and the rate of digestion were increased by a rise in temperature, and also that in the frog digestion stopped completely at and below 6°C. She quotes Feliche Supino, who found that the carp did not digest its food below a temperature of 7.0°C.

Kenyon (1925) worked out the temperature coefficients of digestion in representative species of fish, amphibia, reptiles, and mammals. He made extracts of the gastric and intestinal mucosae, and of the pancreas. He then measured the rate of action of their contained enzymes on various food stuffs at various temperatures. Kenyon concluded that peptic, tryptic and eriptic digestion were always more rapid at 37°C. than at room temperature.

The above researches have been dealt with at some length, because, while they are of little importance with regard to the gastro-intestinal motility of the elasmobranch, they do have a bearing upon certain experiments described in this thesis which were designed to show the effect of temperature upon the rate of digestion taken as a whole.

There is more literature, though still scanty, dealing with the effect of drugs and autonomic nerve stimulation upon the motility of the gastro-intestinal tract of elasmobranch fishes. Dreyer (1928-29) demonstrated the excitatory effect of adrenaline upon isolated strips taken from the ascending limb of the stomach and from the spiral valve of the skate. These strips were immersed in a suitable nutrient solution, namely, Mines', modified by adding 0.5 g. $Na\,H\,CO_3$ per litre of solution. On the other hand, he found that pilocarpine and atropine, even in massive doses, had no effect on either the stomach or the spiral valve. Lutz (1931) partially confirmed Dreyer's results. He found that adrenaline and extract of the chromophil tissue of the elasmobranch fish he was working on caused a marked increase of tone and sometimes of motility of all parts of the stomach, but that these substances caused a marked decrease of tone and inhibition of motility of the posterior end of the intestine and rectum. Stimulation of the posterior splanchnic nerves, however, gave an increase in the motility of these latter parts.

Bottazzi (1902) was the first to study the influence of vagal and sympathetic nerve stimulation on the motility of the gastro-intestinal tract of elasmobranch fishes. He found that both the vagus and sympathetic nerves supplied the stomach with motor nerves, and that no inhibitory effect could be detected when either of these nerves was stimulated. Moreover, stimulation of the sympathetic nerves after stimulation of the vagus always increased the contractions of the stomach. He obtained the same results with the spiral intestine. However, stimulation of the vagus or sympathetic nerves did not stimulate the rectum and colon, though excitation of the 45th and 48th segments of the spiral cord did stimulate those parts, which effect he thought took place through a sympathetic nerve outflow from this region of the cord.

Müller and Liljestrand (1918) repeated Bottazzi's work and came to the same conclusions. Lutz's work (1931) is in agreement. Babkin and MacKay-Sawyer (personal communication, 1931) also confirmed these findings. They found that by stimulating the coeliac axis contraction of the duodenum was produced, but this was not a very constant result. Stimulation of the superior mesenteric artery produced a strong ring of contraction at the lower end of the spiral intestine, which spread about one-third of the way up.

Drs. B.P. Babkin and D.J. Bowie, with the author, carried out a series of experiments investigating the structure and reaction of the arteries (and conus) in the elasmobranch genus Raja. The arteries chiefly studied were those supplying the intestine. For the sake of completeness these investigations are included here. Although the sympathetic innervation of the vascular system in elasmobranch fishes is not developed to the same degree as in mammals, as shown by Bottazzi (1902), Müller and

Liljestrand (1918), Isquierdo (1930), Lutz (1930a), it has been demonstrated (MacKay, 1931) that intravenous injection of a small dose of adrenaline produces in these animals a very marked and sustained rise of arterial blood pressure, lasting from one to two-and-a-half hours. Further, stimulation of the skin and viscera of the skate may produce sometimes a depressor reflex (Lyon, 1926; Lutz, 1930b), and sometimes a pressor reflex, during which the activity of the heart is not markedly changed (MacKay, 1931). It might be supposed that the sympathetic nervous system or chromophil bodies participate in this reflex. Since adrenaline produces only a moderate augmentation and acceleration of the heart contractions in elasmobranchs (Huntsman, 1931), the great and prolonged effect of adrenaline on the blood pressure is only to a certain degree attributable to increased activity of the heart. It would be natural to explain it by its action on the blood vessels, and to attribute its pressor effect chiefly to vasoconstriction of the arteries and arterioles.

However, there are certain facts which stand in the way of this explanation. Long ago Schoenlein (1895) doubted the existence of any innervation of the vascular system in elasmobranchs having relation to the heart but not to the blood vessels other than the vagus. His opinion was based on indirect proofs, such as the absence of Hering-Traube waves, the impossibility of changing the tonus of the blood vessels by stimulation of the central nervous system, e.g., through asphyxiation, and so on. Müller and Liljestrand (1918) have little to say concerning the innervation of the blood vessels except that all they have seen are mere streaks, staining with methylene blue, on the dorsal aorta. Like Bottazzi they did not find any sympathetic nerves to the heart. Wyman and Lutz (1932 a and b) observed a long-sustained pressor effect in Squalus acanthus after an intravenous injection of 2 c.c. of 1:500,000 solution of adrenaline. Since the percentage increase of diastolic pressure in the dorsal aorta was greater than that in the ventral aorta, they concluded that adrenaline produced vaso-constriction peripherally to the gill capillaries. They perfused the blood vessels of the spiral valve of Squalus acanthus with solutions of adrenaline and observed microscopically the minute vessels of the tail during the injection or direct application of adrenaline. No evidence of vaso-constriction was found in either case. Therefore, the region of adrenaline was not located.

There is very little evidence, with the exception of the work of Argaud (1908), dealing with the structure or innervation of the blood vessels in elasmobranchs. J.Z. Young (1933) demonstrated a fine network of nerve fibres, often containing nerve cells, around the muscular coats of the segmental arteries and dorsal aorta.

This work on the structure and function of the arteries fits in with, and leads up to a series of experiments carried out by the author, which were designed to study the effect of stimulation of sympathetic and parasympathetic nerves upon the gastro-intestinal tract of the skate. The method used was that of suspending in a suitable nutrient solution a strip of smooth muscle excised from the gastro-intestinal tract, in which the artery or the vagus nerve which supplied the part was preserved. This method was first elaborated by Rabinovitch (1928), using tissue taken from the oesophagus and stomach of the cat. On stimulation of the vagus he found that the make or break of a constant current had no stimulatory effect, but that a single induction shock or a faradic stimulation caused contraction in a minority of cases. McSwiney and Robson (1929b) repeated these experiments and found that stimulation of the vagus nerve with break shocks or Faradism would cause contraction. This effect was abolished by the use of atropine. Later, they extended these experiments, stimulating the periarterial sympathetic nerves (1929a, 1931), and found that on stimulation of the sympathetic nerve supplying strips of muscle from adjoining sections of the stomach a contraction or relaxation was obtained. If the muscle relaxed on stimulation of the nerve with a high faradic current, contraction was obtained on decreasing the strength or frequency of the current, but if contraction was obtained first alteration in the frequency or strength of the current did not affect the response. Ergotoxine abolished the motor effect.

Recently some very interesting work has been carried out by Babkin, Friedman and MacKay-Sawyer on the autonomic innervation of the stomach in the skate (in press). They placed two balloons in the stomach of a skate which had been immobilized by section of the spinal cord or by injections of Dial Ciba. These balloons were introduced through two small incisions in the stomach wall, one in the pyloric and one in the fundic portion. They were then connected with Marey's capsules so that the contractions might be recorded. The parasympathetic and sympathetic nerves supplying the stomach were stimulated by means of a Harvard induction coil. The above mentioned workers found that weak stimulation of various branches of the visceral vagus nerve caused only local movement of the stomach, whereas stronger stimulation of the vagus caused the whole organ to contract, the contractions beginning at the pylorus and running towards the oesophagus. Any effective stimulation of the anterior splanchnic nerves produced much more powerful contractions than vagus stimulation did; the contractions again running cephal-ward. They also found that the vagus and splanchnic nerves were not antagonistic in their action on the stomach, as they are in the mammal, but are synergetic. And finally, it was found by them that atropine injections stimulated the gastric contractions, and even in large doses did not inhibit the effects of sympathetic and parasympathetic nerve stimulation.

A study of function, as embodied in the elasmobranch fish, would seem to be of very great importance to anyone wishing to solve the complex physiology of the higher animals such as the mammal. For, in such a primitive vertebrate as the elasmobranch we can observe the function of a somatic and nervous organisation in an early stage of development, before refinements in structure and function have been added, as it were, to complicate the picture. The studies of Chevrel, 1887, Bottazzi, 1902, Müller and Liljestrand, 1918, Young, 1933, and, Babkin, Friedman and MacKay-Sawyer (in press) have clearly shown the simple structure of the autonomic nervous system of the elasmobranch as compared to that in the mammal. The experiments of the author, recorded below, and those of others, which have been discussed above, show that its function and its effect upon the gastro-intestinal tract of the skate are of a comparatively simple type. It was on this basis that the material for this thesis was compiled.

CHAPTER II

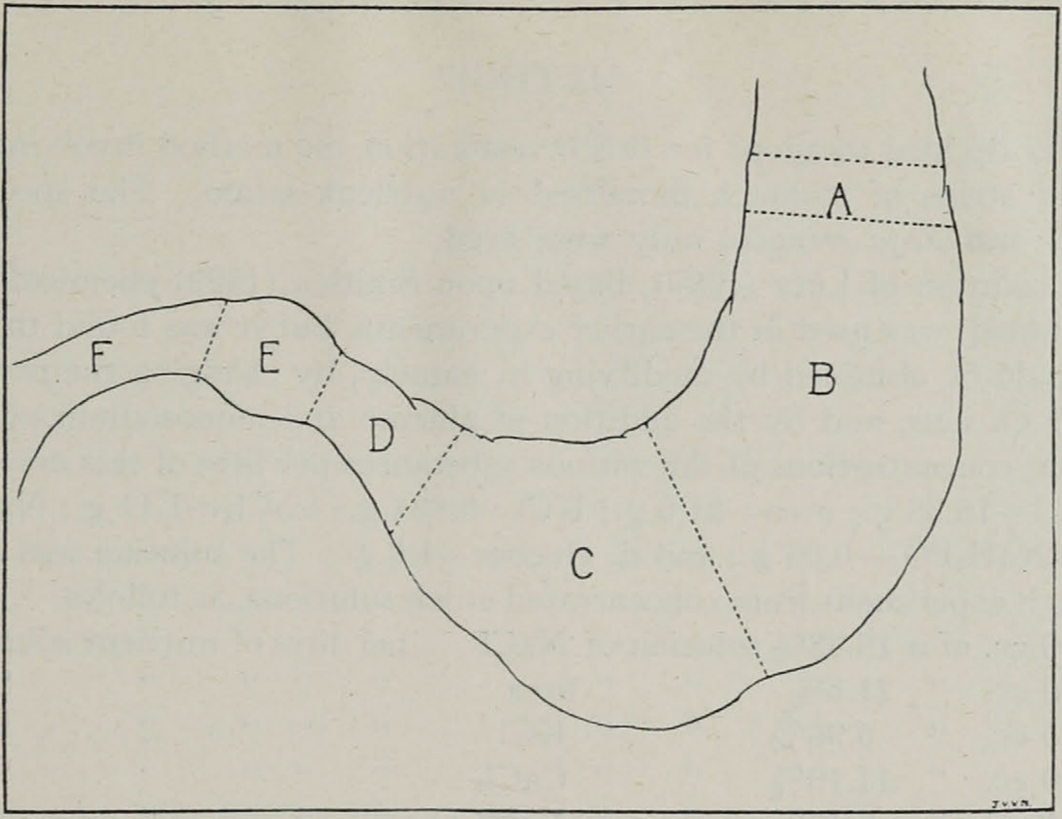
METHODS

The method adopted for the majority of the experiments was that of suspending strips of bowel in a suitable nutrient solution. The solution of Lutz (1930), based upon Smith's (1929) chemical analyses of skate blood, was used in the earlier experiments, but it was found that better results could be obtained by modifying it, namely, by changing the proportions of K and Ca ions, and by the addition of glucose and monosodium phosphate. The actual concentrations of the various substances per litre of this new solution were;-

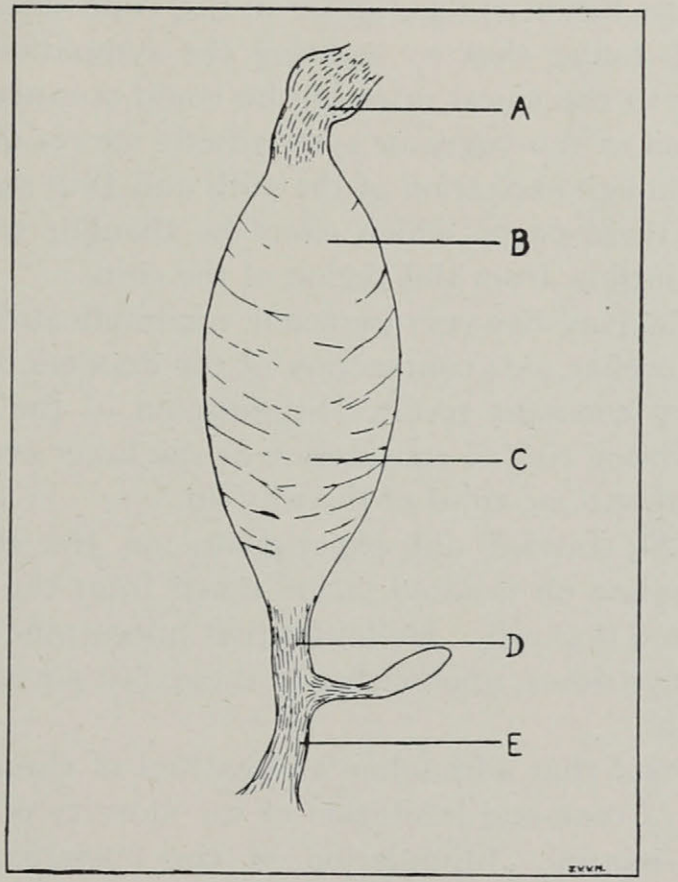
16.38 g. of NaCl
21.6 g. of urea
0.894 g. of KCl
1.11 g. of $CaCl_2$
0.378 g. of $NaHCO_3$
0.06 g. of NaH_2PO_4
1.0 g. of d. glucose

The solution was made up before each experiment from concentrated stock solutions, as follows:-

100 cc. of a 16.38% solution of NaCl per litre of nutrient solution
100 cc. " " 21.6% " " urea " " " " "
15 cc. " " 5.96% " " KCl " " " " "
10 cc. " " 11.10% " " $CaCl_2$ " " " " "
4.5 cc. " " 8.40% " " $NaHCO_3$ " " " " "
1.0 cc. " " 6.0 % " " NaH_2PO_4 " " " " "
10 cc. " " 10.0 % " " d.glucose" " " " "



~~Figure 1~~.—Diagram of the skate's stomach and upper part of the intestine. A—cardia; B—fundus; C—antrum; D—pyloric canal; E—pyloric sphincter; F—duodenum.



~~Figure 1~~.—Diagram of the spiral intestine. A, pyloric sphincter; B, duodenal or upper end of the spiral intestine; C, lower end of the spiral intestine; D, colon; E, rectum.

The resulting solution had a pH.7.8, which is only slightly more alkaline than the blood plasma of the skate. For instance, the pH. of the blood plasma of *Raja diaphanes* is 7.26, and that of *Raja stabuliforis* 7.21 to 7.63, according to the figures of Smith (1929).

The above stock solutions were kept on hand, except the glucose solution, which was made up each day.*

The procedure of obtaining tissue varied a little according to which part of the gastro-intestinal tract was to be investigated. In all experiments a skate that had been starved for several days was taken, and the spinal cord was divided in the region of the medulla oblongata. The abdomen was then quickly opened. In the investigations on the stomach, the whole organ was excised, washed inside and out with saline, and placed in ice-cold saline for several minutes. Then the muscular layer of the whole stomach was removed, without puncturing the mucosa. If this is done gently, the mucosa comes away quite easily with very little damage to the muscular tissue. It was found necessary to use the muscular layer alone, since foreign substances in the mucosa contaminated the preparation, and also autodigestion took place, inactivating the muscle within a few hours after setting up. Strips, 2 cm. x 0.5 cm., were then cut off this muscular layer that had been dissected away, in such a manner that the contractions of the circular layer would be most prominent in the re-

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* (Baker and Adamson's preparations of NaCl, $NaHCO_3$, $NaH_2PO_4 \cdot 4H_2O$ and d. glucose, and Merck and Company's preparations of $CaCl_2 \cdot 6H_2O$, KCl and urea were used).

cording.

Two such strips were then set up in the same bath containing 100 c.c. of saline at a temperature of about 12°C. This has the double advantage that one strip acts as a control against the other, and that it is possible to compare two different parts of the same stomach under exactly similar conditions. The lower end of each strip was fixed and its upper end attached to a light lever of the frontal drawing type (Evans, 1919), which (with a magnification of 8) drew a graphic record of the spontaneous contractions on an extremely slowly revolving drum. A thermometer reading to one-tenth of a degree centigrade was then placed in this bath, for the purpose of recording the temperature of the solution. The bath containing the preparations was then surrounded with another bath containing water, the temperature of which could be varied, and thus the temperature of the preparations in the inner bath could be controlled. Throughout the whole experiment the saline in the inner bath was aerated by water-washed air slowly bubbling from a jet with a small aperture.

This procedure of obtaining tissue was not applicable in the spiral valve, spiral intestine, colon and rectum as the muscular coats could not be separated from the mucosa without considerable injury. Consequently, a ligature was tied just above the upper end of the spiral intestine and another as far down the rectum as possible. The section of intestine marked off by these ligatures was then removed and placed in ice-cold nutrient saline in order to minimize the shock consequent on this procedure. Then strips were cut off carefully from the desired section of the intestine in a circular manner, in order that the contractions of the circular layer of muscle might be predominant in the recordings. The strips thus obtained were passed through several changes of ice-cold nutrient saline in order to wash them thoroughly. Two such strips were then placed in a bath containing nutrient saline at about 12°C. The rest of the procedure was the same as mentioned above for the stomach. By these means very constant results were obtained, the spontaneous contractions starting within an hour or two.

Photograph showing the general arrangement of the apparatus as used in the experiments on isolated strips taken from the gastro-intestinal tract.

In all the experiments carried out to determine the effect of temperature variations upon the motility of the gastro-intestinal tract a standard procedure was followed. As soon as the spontaneous contractions were established in the strips of stomach or intestine in the bath at 12°C., the temperature was slowly lowered to just below that point at which contractions could no longer be observed, and was then allowed to rise slowly during about seven hours to the upper limit at which contractions occurred. For these experiments the Raja diaphanes and Raja erinacea alone were used. In this way the effect of temperature upon the cardiac, fundic and antral regions of the greater and lesser curvatures and the pyloric sphincter of the stomach, upon the upper and lower ends of the spiral intestine and valve, colon and rectum was investigated.

Besides the above experiments, several others were carried out to determine the effect of temperature variations upon digestion as a whole. For these the skate was not used but a small fish belonging to the large group of Teleosts, the Fundulus heteroclitus. This fish was chosen for the purposes of this study for two main reasons; first, because of its great hardiness (it can be kept out of water for short intervals, and can be handled without ill effects); secondly, because its digestive processes are simpler than those of the higher vertebrates (Babkin and Bowie 1928). Fundulus has no stomach, and, therefore, is devoid of peptic glands; also, its digestion is entirely alkaline, and the complicating factor of acid control of the pyloric sphincter is absent. The fish were obtained from Birch cove, St. Andrews, N.B.

The lowest temperature at which any experiment was conducted was 5°C., at which point there is no digestion. The highest temperature was 29.5°C., at which point the fish were greatly weakened. One experiment was carried out at 35°C., but four out of the five fishes concerned died within fifty minutes. Consequently, it was not considered desirable to employ a temperature higher than 29.5°C.

In the case of temperatures between 15° and 17.5°C., no special means were used to maintain the water in which the fish were kept at a constant temperature. Each fish was placed in a hatchery jar filled with sea-water, and the temperature of the water, which depended on that of the surrounding air, was noted. The jars were then placed in a cool dark place, so as to avoid great variations of temperature during the course of the experiment.

For low temperatures, that is, between 5° and 14°C., the fish were placed in a hatchery jar which stood in a tin vessel filled with water, the temperature of which could be varied as desired. This, in turn, was placed within a wooden box lined with cork, and the whole within a second wooden box. By this arrangement the temperature of the fish could be maintained for twenty-fours hours within the range of one degree.

For high temperatures the hatchery jar containing the fish was placed in an incubator thermostatically controlled at the desired point.

In order that the water might be adequately ventilated, air was continually bubbled through it from two jets having small apertures. By these contrivances very constant temperatures could be obtained.

The actual procedure followed in each experiment was arrived at after a large number of preliminary tests, and was designed to reproduce as nearly as possible normal conditions. Fishes ranging in size from 7 to 10 centimetres were segregated in a perfectly clean aquarium. They were then starved for from three to five days. Twenty-four hours before feeding, the four or five fishes to be used were removed and each was placed in its own hatchery jar filled with clean water, the temperature of which was anywhere between 15° and 19°C. The jars were kept in the dark, for it was found that light excited the fish and they would not eat when desired. On the day fixed for the experiment about 140 mg. of the mantle of clams were added to each jar, an amount practically equal to the capacity of the duodenum. This substance was found, after many trials, to be the most suitable food. In almost every case the fish ate it almost at once. The time at which it did so was carefully noted. If the experiment were to be carried out at ordinary temperatures the fish was left where it was. If it were a low temperature experiment the fish was immediately transferred to a hatchery jar in which the water had previously been cooled to the desired point. If it were a very high temperature experiment the fish was transferred for a few minutes to water at a temperature half way between that at which the experiment was to be conducted and that at which it was fed. Then it was placed in the very warm water. In the case of an experiment at a moderately high temperature the fish was transferred immediately.

At certain intervals after feeding (2, 3, 4, or 5 hours, etc.) one fish was removed and its duodenal contents aspirated with a small pipette, the fish being kept in the air during the operation. The time of each aspiration was noted, as well as the presence or absence of clam and bile. The pH of the aspirate in each case was determined by the B. D. H. capillator method. If nothing, or only a small amount, could be aspirated, the duodenum was washed out with two or three drops of distilled water and the pH of the rinsing ascertained.

As has already been stated, digestion in Fundulus is carried out in an alkaline medium. During digestion the pH of the duodenal contents may be as high as 9.0, but in a starving fish average about 7.8, though the figure may sink as low as 7.4. Therefore, by taking these readings it is possible to tell when the duodenum is empty and when secretion of the digestive fluids subsides.

After each fish had had its duodenal contents aspirated, the experiment, so far as it was concerned, was finished. The fish was then placed in a perfectly clean aquarium and allowed to rest for three or four days before being used again.

The following are possible errors in the method employed which could affect the results.

1. Individual variations in the rate of digestion might be expected among the fish employed. These variations, such as they were, did not seem to have introduced any appreciable error.

2. Fishes that had been used for a number of experiments showed evidences of developing conditioned reflexes. For example, when the hand was passed over the aquarium, as was done when feeding them, the fish rose to the surface and opened their mouths. In the one or two instances in which this occurred the fish digested their food in a slightly shorter time, but not so markedly as to introduce any serious error. Such fish were not used again.

3. As has been stated above, in the case of high and low temperature experiments the fish were fed at about 17.0°C., and were then transferred immediately to warm or cold water, as the case might be. This procedure was found necessary as the feeding was hard to control unless carried out at temperatures between 13° and 19°C. However, it was found by experiment that the time necessary for the temperature of the fishes to rise or fall to that of the new environment caused only a negligible error. The body temperature of a fish (A) that had been kept in water at 14.7°C., for some time was obtained by passing a fine thermometer down the oesophagus into the duodenum, it proved to be 15.1°C. The fish was then transferred to water at 30.1°C. A second fish (B) which had been kept in water at 16.9°C., registered a body temperature of 17.0°C., and was then placed in water at 11.7°C. In each case the change of body temperature was taken at intervals of one minute. The results are given in the following table on page 18.

Time in minutes	Fish A (initial temp. 15.1°C.)	Fish B (initial temp. 17°C.)
1		
2	20.0°C.	15.1°C.
3	23.5	14.7
4	25.0	14.2
5	25.9	14.0
6	26.8	13.6
7	27.2	13.3
8	27.6	13.1
9	28.0	12.9
10	28.2	12.7
11	28.5	12.6
12	28.8	12.5
13	29.0	12.3
14	29.1	12.2
15	29.2	12.2
16	29.4	12.2
17	29.6	12.2
18	29.7	....
19	29.7	....
20	29.7	....

From this it can be seen that the temperature of the fish, under the given conditions, rises or falls rather markedly during the first five or six minutes, and practical equilibrium is reached in eighteen and fourteen minutes respectively.

4. In drawing conclusions from these experiments it was assumed that the body temperature of the fish was the same as that of the water. This is not quite the case. The above? table shows that there is a difference of 0.4° and 0.5°C. This error is so slight as to be negligible.

Finally to complete the picture of temperature relationships, several experiments were carried out to determine the survival time at different temperatures of the tissue removed from the gastro-intestinal tract of the skate. Again the species <u>Raja diaphanes</u> and <u>Raja erinacea</u> alone were used, and again the method of using isolated strips of gastric muscle in a suitable nutrient solution was employed.

Three series of experiments were carried out. In the first of these, several strips of muscle from the antral region of the stomach of a skate which had been starved for four or five days were placed in nutrient saline. In one case the tissue thus prepared was kept at 0°C.; in others at 5°C., 10°C., 15°C. and 20°C. respectively. Each day strips of muscle were removed and tested for spontaneous contractions at a temperature of 12°C.

In another series several fish which had been starved for four to five days were taken and immobilized by section of the spinal cord just below the medulla. One set of fish thus treated was kept at 5°C. and others at 10°C., 15°C. and 20°C. respectively. Each day one fish was removed and strips of gastric muscle from the region of the antrum were tested for spontaneous contractions at 12°C.

A final series of experiments exactly similar to the above was carried out, only fish freshly caught being used instead of fish that had been starved for four or five days.

The strips of tissue prepared according to the above three methods were placed in nutrient saline at about 12°C. and tested for the existence of spontaneous contractions, or other signs of viability, and their reaction to such drugs as adrenaline and pilocarpine. When such a strip gave no spontaneous contractions, tonal changes, nor reaction to drugs, it was adjudged dead. By these methods it was possible to estimate with some degree of accuracy the survival time of the tissue when kept under different temperature conditions.

The effect of certain drugs upon the cardia, pyloric sphincter, the fundic and antral regions of the greater and lesser curvatures of the stomach, upon the spiral valve, spiral intestine, colon and rectum, were also investigated. The isolated intestine preparation was used, the temperature of which was maintained between 12°C. and 13°C. Solutions of the drug to be used in distilled water were placed directly in the 100 c.c. of saline bathing the strip of tissue. In this way the effects of adrenaline, ergotoxine, pilocarpine, acetylcholine and atropine were determined. Fresh solutions, of 0.1 per cent concentration of atropine sulphate, pilocarpine nitrate, acetylcholine and ergotoxine phosphate in distilled water were used. A 1 in 1000 solution of adrenaline chloride (Parke, Davis and Co.) was used as a source of adrenaline. In the majority of cases 0.4 c.c. of each of these was used, except in the case of acetylcholine and ergotoxine where rather larger doses were used. In other words the final concentration in the bath of adrenaline, atropine and pilocarpine was usually 1:250,000, while that of acetylcholine and ergotoxine was rather greater. In the experiments dealing with all the drugs except ergotoxine, the species _Raja diaphanes_ and _Raja erinacea_ alone were used; _Raja maculata_ was used in case of the latter drug.*

The structure and reactions of the arteries of elasmobranch fishes were also investigated.** These experiments were performed on the ventral aorta, coeliac artery, superior mesenteric artery, anterior intestinal artery, as well as on the conus arteriosus. The species _Raja diaphanes_ and _Raja stabuliforis_ alone were used. Healthy specimens of these species, kept for several days in aquaria were immobilized by section of the spinal cord below the medulla. The blood vessels were excised, care being taken not to contaminate them with the mucous secretion of the skin, nor to distend them during the operation of removal. They were placed immediately in ice-cold nutrient saline (vide supra).

After one hour or more, according to the particular plan of each experiment, the blood vessels were prepared for ac-

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*(This was rendered necessary as the experiments dealing with the action of ergotoxine were carried out at Plymouth, England; whereas the other experiments were carried out at St. Andrews, N.B.).

**(This work was carried out by Drs. B.P. Babkin, D.J. Bowie and myself -- Dr. Bowie doing the histological part of the investigation).

tual experimentation. All loose tissue surrounding the vessel was removed. In order to investigate the reaction of the longitudinal layer of an artery to different stimuli, a piece of artery 8 to 16 cm. long was set up in a large glass test-tube containing 100 to 200 c.c. of the above mentioned saline at a temperature of 10° to 12°C. The lower end of the artery was fixed and its upper end was attached to the short arm of a very light lever.

The contractions of the circular layer of these arteries could not be investigated by the method usually employed for mammalian arteries, i.e., by suspending a single ring cut from the artery and registering its movements. The arteries even in <u>Raja stabuliforis</u> are smaller and their muscles weaker than in mammals. To obviate this difficulty a chain of 8 to 10 rings of artery, each about ½ cm. wide, tied together with fine silk thread, was set up in the bath in the same manner as the longitudinal pieces of artery mentioned above. This preparation gave quite a marked contraction. Experiments were also made on a single ring cut from the proximal or distal part of the conus arteriosus or ventral aorta. In order to load the arteries or the conus arteriosus riders of different weight were placed on the long arm of the lever at different distances from its fulcrum. The bath containing the preparation was placed in another bath containing water, the temperature of which was kept at a suitable level. The saline in the inner bath was continuously aerated by the bubbling through of water-washed air.

Finally, experiments were carried out to determine the effect of sympathetic and parasympathetic nerve stimulation upon the motility of the gastro-intestinal tract. It was decided to adopt the method of Rabinovitch (1928, vide supra), with certain modifications. The excised strips of intestine or stomach, obtained as described above, with the artery or nerve attached, were immersed in the nutrient solution mentioned above.

In the experiments dealing with the sympathetic innervation to these parts, the artery supplying the part in question was carefully dissected out for as much of its length as possible, one end being left attached to the strip of muscle. In the case of strips of muscle from the greater curvature of the stomach 3 or 4 cm. of the gastro-pancreatico-duodenal artery were thus left attached, while with strips from the lesser curvature the anterior gastric branch of the coeliac axis was left attached. Similarly, the superior mesenteric was left for strips from the spiral intestine and colon and the inferior mesenteric for strips from the rectum. For the purpose of stimulating the artery a Dubois-Reymond induction coil was used. A 2-volt accumulator with a make and break key were placed in the primary circuit and a short circuiting key in the secondary circuit. The secondary current was carried to the artery, which was held clear of the solution bathing the strip by means of conventional small electrodes. Thus the artery could be stimulated with a single or multiple break induction current or faradically.

The experiments on the effect of vagal stimulation were restricted to the cardiac and upper fundal regions of the stomach as both vagi nerves in the skate break up into small branches at the lower end of the oesophagus. But with some difficulty it was possible to dissect out 2 or 3 cm. of the nerve attached at its distal end to circular strips of muscle from the fundus and cardia. The nerve was stimulated in the same way as the artery.

The rest of the procedure of setting up the excised strips of muscle and recording their contractions was exactly similar to that employed in the above-mentioned experiments. The species *Raja maculata* alone was used, except in two or three cases, noted in the text, where *Raja naevus* was used.

Before closing this chapter it should be mentioned that the nomenclature of Daniel (1928) is followed throughout.

CHAPTER III

RESULTS

SECTION I

TEMPERATURE RELATIONSHIPS

The effect of temperature variations upon the motility of the cardiac, fundic and antral regions of the greater and lesser curvatures, the pyloric sphincter of the stomach, of the upper and lower ends of the spiral valve and spiral intestine, colon and rectum was determined.

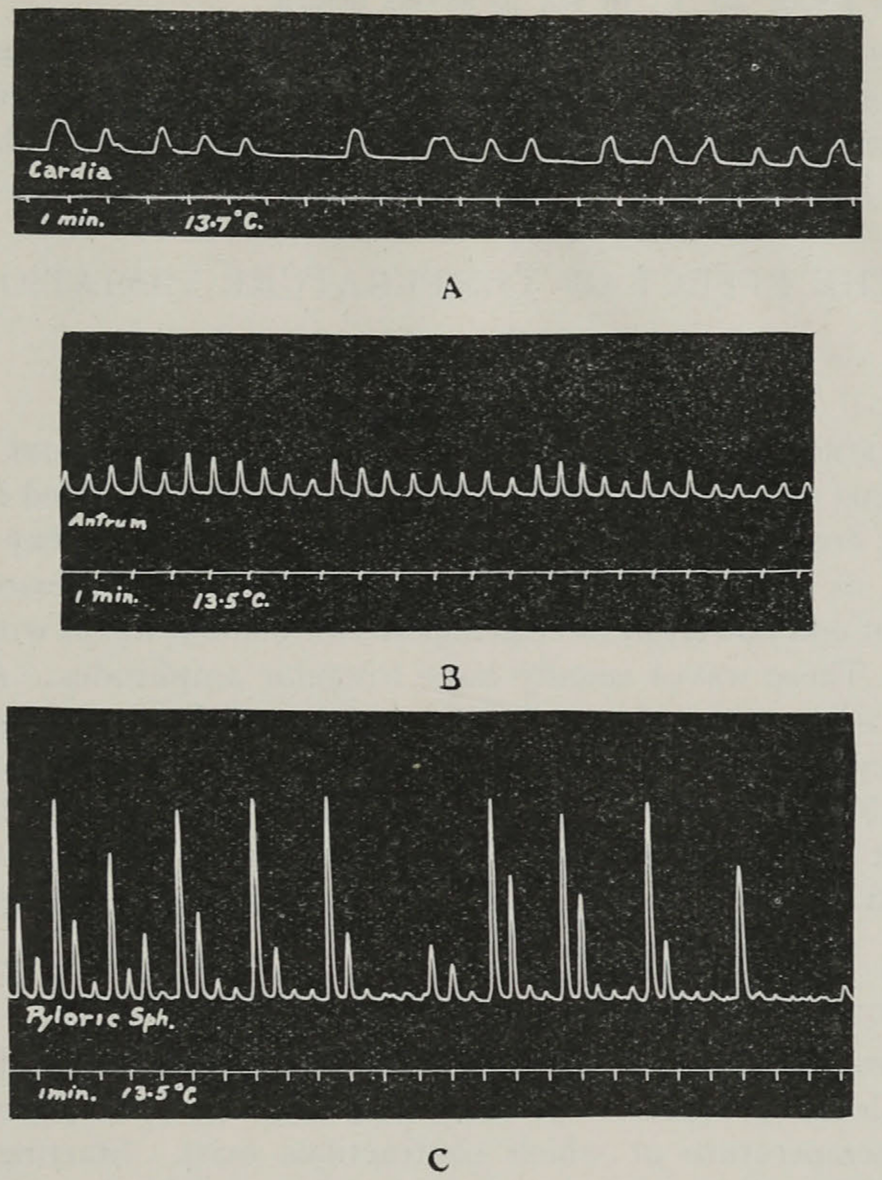
CARDIA

For the study of this part strips of muscle were taken from the area where the oesophagus and stomach meet. Contractions are obtained in the majority of cases, but are as a rule irregular, appearing in groups. They begin between 7° and 8°C. as mere waves, but as the temperature is increased they become more frequent and powerful, the tracings showing broad waves with rounded tops. These waves usually have irregular amplitudes. At 19.0°C. the contractions become irregular in shape, a number of them showing several peaks. Above this temperature they become weaker and very irregular and finally cease at about 24.5°C. As the temperature is raised there is very little change in tonus, except for an even and very gradual relaxation. The contractions are so irregular that an accurate count throughout the temperature range cannot be made.

THE BODY OF THE STOMACH

The characters of the contractions of the fundic and antral regions of the greater and lesser curvatures are essentially the same, except for the rate and the lowest temperature at which contractions exist. Starting at the lower temperature limit, the contractions begin as mere undulations which increase in rate and strength as the temperature is raised. Between the temperatures 8°C. and 14°C. the greatest amplitudes occur, with a maximum height of contraction at about 12°C. Above 14°C. the contractions gradually decrease in strength but increase in rate and maintain great regularity of rhythm. From 20°C. to about 24.5°C. they become slower, much weaker, more irregular and finally cease at about the latter temperature. This upper limit of temperature at which contractions cease is remarkably constant for all parts of the stomach, at about 24.5°C. If the temperature be allowed to fall from this upper temperature limit, contractions will be restored in the majority of instances. These contractions, as a rule, are slow and irregular in rhythm and amplitude. Within a short time they usually become weaker and finally cease altogether. The lower temperature limit varies for different parts of the stomach; for the fundus it is 3.0° to 5.5°C., and for the antrum 1.5° to 4.0°C.

Often between 8°C. and 15°C. a definite rhythmic variation is seen in the height of the contractions, but never is there any such variation in the base line. There is merely a slow and gradual relaxation as the temperature is raised. It is doubtful whether this should be considered as a tonic change; rather it should be considered as a gradual stretching.



~~FIGURE 2~~.—A—record showing the normal contractions of cardia at 12.7° C.; B—record showing the normal contractions of the antral region of the greater curvature at 13.5° C. The antral region of the lesser curvature and the fundic region of both curvatures have the same type of contraction; C—record showing the normal contractions of the pyloric sphincter at 13.5° C.

PYLORIC SPHINCTER

The contractions here are very similar in character to those of the body. The contractions start between 1.5°C. and 3.0°C. and cease at about 24.5°C. They differ from those of the body in that they are faster, that weakness first appears at about 16° to 17°C., and that there is very little periodic variation in the amplitudes between 8°C. and 14°C., but rather a gradual and irregular increase in height, which reaches a maximum at about 12°C. and then gradually decreases.

By the method of setting up the preparations, where two strips from different parts of the stomach are immersed in the same bath, it was possible to compare the rates of contraction of various parts of the same stomach under exactly the same conditions. The results of five experiments are reproduced in the table on page 29. From these it can be seen that the pyloric sphincter contracts the fastest; then in order of decreasing rate the lesser curvature region of the antrum and fundus, the fundic region of the greater curvature, and the central region of the greater curvature. The figures denoting the rate of contraction of the cardia in the table on page 29 should not be taken as strictly accurate; they merely indicate the slow rate of contraction of this area.

SPIRAL INTESTINE

Into the upper end of the spiral intestine the pancreatic and common bile ducts empty; hence this part is often referred to as the duodenum. Experiments were carried out to show the effect of temperature variations on circular strips cut from this part and from the lower end.

DUODENUM

Contractions start at about 3°C. as mere faint, irregular undulations. As the temperature is raised, these become more regular and of greater amplitude, which characteristics are most evident at 10°C. Above this temperature the contractions decrease gradually in amplitude. At 17°C. the rhythm and amplitude become irregular and contractions cease at about 24°C.

The rate of contraction of different parts of the skate's stomach at different temperatures.

Exp.22			Exp. 13			Exp. 8			Exp. 19			Exp. 15		
Temp. in °C.	Rate/ min.in pyloric sph.	Rate/ min.in fundus L.C.	Temp. in °C.	Rate/ min.in fundus G.C.	Rate/ min.in antrum G.C.	Temp. in °C.	Rate/ min.in fundus G.C.	Rate/ min.in fundus L.C.	Temp. in °C.	Rate/ min.in fundus L.C.	Rate/ min.in antrum L.C.	Temp. in °C.	Rate/ min.in pyloric sph.	Rate/ min.in cardia
2.0	irreg.	-	1.4	-	0.70	3.0	0.46	0.54	4.1	-	irreg.	3.2	0.80	-
3.6	0.67	0.62	2.4	-	0.71	4.0	0.50	0.56	5.5	0.64	0.62	4.4	0.82	-
5.0	0.70	0.64	3.3	-	0.83	6.5	0.64	irreg.	7.0	0.80	0.80	5.6	0.90	-
6.5	0.80	0.71	5.1	irreg.	0.83	7.7	0.67	0.75	8.5	0.83	0.85	6.8	1.25	-
8.1	0.89	0.81	6.1	1.00	1.00	8.7	0.71	0.78	9.5	0.93	0.92	8.1	1.50	-
9.6	1.17	0.92	7.3	1.16	1.14	9.5	0.78	0.80	11.0	1.00	1.00	9.4	1.71	irreg.
11.0	1.44	1.13	8.5	1.25	1.25	10.7	0.80	0.86	12.5	1.21	1.20	10.6	1.82	irreg.
12.5	1.58	1.29	9.3	1.33	1.25	11.5	0.82	0.93	14.0	1.25	1.25	11.7	1.67	0.70
14.0	1.63	1.38	11.0	1.40	1.29	12.5	0.95	1.00	15.5	1.38	1.36	13.0	1.89	0.88
15.5	1.71	irreg.	12.0	1.44	1.44	13.5	1.14	1.00	17.0	1.50	irreg.	14.5	2.00	irreg.
17.0	1.60	1.43	13.5	1.65	1.50	14.7	irreg.	1.14	18.5	1.55	1.57	15.8	1.64	irreg.
18.5	1.44	1.50	14.9	1.83	1.63	15.5	1.11	1.25	20.0	1.36	1.38	17.0	1.50	0.72
20.0	1.50	1.60	16.5	2.00	1.75	16.5	1.22	1.36	21.5	1.40	irreg.	18.3	1.50	0.70
21.5	irreg.	irreg.	18.0	2.13	1.83	17.5	1.29	1.30	22.1	1.25	irreg.	20.0	1.35	0.62
23.0	irreg.	irreg.	19.7	2.17	1.91	18.5	1.33	1.50	23.5	irreg.	irreg.	20.4	irreg.	0.64
23.9	-	irreg.	21.0	2.22	1.90	19.5	1.50	1.60	24.5	-	-	22.1	irreg.	0.60
24.1	-	-	22.5	2.50	irreg.	20.7	1.40	1.833				23.9	-	irreg.
			23.9	2.55	-	21.9	1.43	2.00				24.2	-	-
			25.0	-	-	22.5	irreg.	irreg.						
						23.5	irreg.	irreg.						
						24.3	-	-						

Note; G.C. means Greater Curvature
L.C. means Lesser Curvature

LOWER END

The above characteristics are true of this part also. The table on page 31 shows the relation of temperature to rate.

SPIRAL VALVE

Experiments were carried out showing the effect of temperature variations on the contractions of circularly cut strips taken from the upper or duodenal end and the lower end of the spiral valve.

Contractions start at about 3.0°C. as undulations of regular low amplitude and regular rhythm. The amplitude gradually increases and reaches a maximum at about 12°C. and then gradually decreases until contractions cease at about 24°C. Throughout the temperature range a remarkable regularity of rhythm is preserved. The table on page 31 shows the relation of rate to temperature.

COLON AND RECTUM

These two sections of the intestine are dealt with together, since they are so similar in many respects. They both are remarkably distensile -- when contracted the outer diameter is about equal to that of a pencil, when dilated it is about equal to that of a walnut. Further, they both are very sensitive to mechanical stimulation such as pinching or stretching, reacting to such stimuli by contracting powerfully. At no time on opening a fish were peristaltic contractions observed; both rectum and colon were entirely dilated or contracted.

Circularly excised strips of colon and rectum do not show rhythmic contractions but merely spontaneous spasmodic contrac-

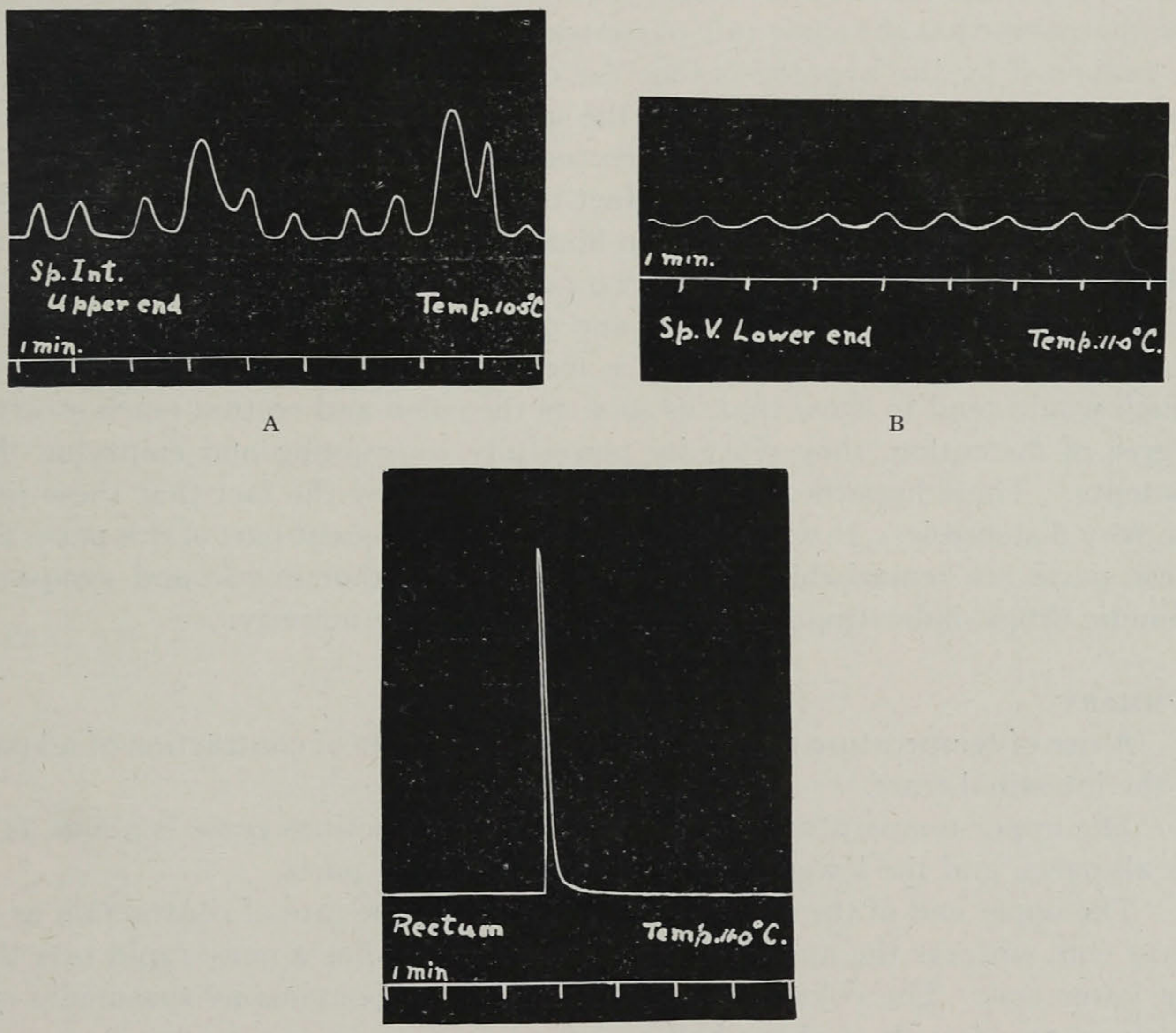
Relation of rate contraction of spiral intestine to temperature in Raja

Temperature in °C.	Rate/min. Duodenal end	Rate/min. Lower end
3.0	irreg.	irreg.
4.2	0.6	0.6
6.0	1.0	1.0
7.5	1.16	1.11
9.0	1.48	1.45
10.5	1.41	1.43
12.0	1.29	1.29
13.5	1.15	1.12
15.0	irreg.	irreg.
17.0	"	"
19.0	"	"
21.0	"	"
23.0	cease	cease

Relation of rate of contraction of spiral valve to temperature in Raja.

Temperature in °C.	Rate/min. Duodenal end	Rate/min. Lower end
3.0	0.40	irreg.
5.0	0.50	0.40
7.0	0.71	0.50
9.0	0.78	0.57
11.0	1.09	0.67
13.0	1.30	0.75
15.0	1.40	1.00
17.0	1.40	1.00
19.0	1.30	0.88
21.0	1.20	0.80
23.0	irreg.	irreg.
24.0	cease	cease

tions which at 10°C. recurred about every half hour. The interval between these contractions varies greatly from fish to fish, but in general it becomes somewhat shortened on a rise in temperature. These contractions start at about 3.0°C. as round-topped waves of great amplitude and of about two minutes' duration. As the temperature is raised the amplitude increases and the duration of the contraction becomes much shorter; at 10°C. it is about one-third of a minute. Above 12°C. the amplitude decreases and contractions cease at about 24°C.



A

B

C

~~FIGURE 2~~.—A, normal contractions of upper end of spiral intestine at 10.5°C.; B, normal contractions of lower end of spiral valve at 11.0°C.; C, normal contractions of rectum at 11.0°C.

Thus it can be seen that throughout the gastro-intestinal tract of the skate a rise of temperature within certain limits causes an increase of the spontaneous rhythm.

A series of experiments was carried out on Fundulus heteroclitus to determine the effect of temperature variations upon the rate of digestion taken as a whole. These experiments, strictly speaking, have no place in a paper dealing with an elasmobranch fish like the skate. However, they are included here since it is believed they will round out somewhat the picture of temperature relations. The following may be taken as a typical experiment.

Exp. XLI. August 1, 1930.

Four fishes were used; temperature during feeding, 18.1°C.; temperature after feeding and throughout the course of the experiment, 22.5°C.

Time	Fish	Contents of stomach	pH
9.27 to 9.33 a.m.	A B C D	Each fed with about 140 mg. of clam	Clam had a pH of 6.5
3.00 p.m.	A	Nothing was brought up, but on washing with distilled water, a minute amount of clam and bile was obtained. (Interval, 5½ hrs.)	8.0
3.20 p.m.	B	About 1½ drops of bile and no clam were obtained; on washing with distilled water nothing more was removed. (Interval, 6 hrs.)	8.8
4.00 p.m.	C	Nothing was obtained. On washing out with distilled water only a very small amount of bile was removed. (Interval, 6½ hrs.)	8.6
4.30 p.m.	D	Nothing was obtained either before or after washing out. (Interval, 7 hrs.)	7.6

The time required for the completion of duodenal digestion was here estimated to be $6\frac{3}{4}$ hours.

On following the same procedure some thirty relationships were established. See table on page 35.

These results have been plotted according to the formula of Arrhenius. Thus, log K x 10^2 against $\frac{I}{T^o}$, where K is the rate and T is the absolute temperature. K has been multiplied by 10^2 in order to obtain a positive logarithm. The table on page 35 shows that with a rise of temperature the time of digestion is decreased.

There are at least three reasons why the rate of digestion should be increased by elevation of temperature.

1. With a rise in temperature there is probably an increased secretion of the digestive fluids. It is difficult to prove this in small fish, but it is logical to suppose it to be the case, since it is known that a rise in temperature accelerates a large number of physiological processes.

2. A rise in temperature increases the action of enzymes, as has been shown by several workers. To verify this, special experiments were carried out on the activity of lipase, obtained from the duodenum of Funduli, at 28.0° and 15.8°C., respectively.

A cream substrate was made up as follows. Some fresh cream was boiled and then diluted with an equal quantity of distilled water. Two samples of 10 cc. each were then taken and three drops of toluene were added to each. In each sample, also, were placed seven drops of the pooled intestinal contents from

Rate of digestion in *Fundulus heteroclitus* under different temperature conditions.

No. of exp.	Temp. in deg. C.	Time in hrs.	Rate (K)	Log. K x 10^2	$\frac{I}{T^o}$
37	29.5	3	0.3333	1.5228	0.003306
38	29.5	3	0.3333	1.5228	0.003306
55	28.0	3½	0.2857	1.4559	0.003322
57	28.0	3¼	0.3077	1.4881	0.003322
49	27.0	4	0.2500	1.3979	0.003333
59	26.0	4½	0.2222	1.3468	0.003341
35	24.5	5¼	0.1905	1.2799	0.003361
36	24.5	5¼	0.1905	1.2799	0.003361
60	23.4	5¾	0.1731	1.2382	0.003374
40	22.5	6¾	0.1481	1.1706	0.003384
41	22.5	6½	0.1540	1.1875	0.003384
46	21.5	6¾	0.1481	1.1706	0.003396
61	21.0	6½	0.1540	1.1875	0.003402
62	20.0	7	0.1428	1.1547	0.003413
65	19.1	7¼	0.1379	1.1396	0.003423
32	18.7	7	0.1428	1.1547	0.003428
30	18.5	7¼	0.1379	1.1396	0.003431
31	17.4	7½	0.1333	1.1249	0.003444
34	16.5	8¼	0.1212	1.0835	0.003454
19	15.5	8½	0.1176	1.0705	0.003467
24	15.3	8¾	0.1143	1.0580	0.003469
22	15.1	8½	0.1176	1.0705	0.003471
66	14.0	9	0.1111	1.0457	0.003484
17	13.7	9¼	0.1081	1.0338	0.003488
48	12.8	10	0.1000	1.0000	0.003499
51	12.1	10¼	0.0976	0.9939	0.003508
42	9.8	12+	......	......	0.003536
43	9.8	15½	0.0645	0.8096	0.003536
50	9.0	15½+	......	......	....
45	5.8	26¾+	......	......	....

several Funduli that had been fed with clam about one and one-half hours previously. One preparation was maintained at a constant temperature of 28.0° and the other at 15.8°C. At intervals of one hour one cubic centimetre was removed from each and titrated with 0.2 normal sodium hydroxide solution, using phenolsulphonphthalein as an indicator. Control experiments were also carried out.

Hours	Temp. 28.0°C. Amount of one-fifth normal NaOH required in c.c.	Temp. 15.8°C. Amount of one-fifth normal NaOH required in c.c.
1	0.15	0.15
2	0.50	0.25
3	0.55	0.35
4	0.55	0.35
5	0.55	0.40
6	0.60	0.35
7	0.65	0.50
8	0.60	0.50
Total amt. of alkali used in 8 hours.	4.15	2.85

From this it is clear that the action of lipase is more rapid at the higher temperature.

The control test was carried out by taking 1 c.c. of pure substrate and titrating with 0.2 normal NaOH. By subtracting the amount of sodium hydroxide solution used to neutralize the control from that used in the primary experiment the amount of 0.2 normal NaOH required to neutralize the fatty acids could be determined. The figures given in the table on page 36 were obtained in this way.

3. A rise of temperature causes increased motility of the intestinal tract while a fall has the opposite effect. A few experiments were performed touching this point. One of them may be quoted here.

The duodenum of a Fundulus that had been starved for five days was excised and suspended vertically in a beaker filled with Tyrode's solution. The lower end was fixed and the upper end was attached to a very light lever arranged so as to register any contractions on a kymograph. The preparation proved to be a very powerful one. Three kinds of movements could be distinguished -- contractions of the longitudinal and of the circular layers of muscle, and peristaltic waves. The preparation was raised in temperature slowly from 18° to 29°C., during which time the contractions became so irregular that it was not possible to count them throughout the range. In general, there were about 3.3 contractions per minute at 18° and about 4.0 at 29°C. The preparation was then slowly cooled to 5°C., over a period of 3 hours and 26 minutes. At about 23°C. variations in tonus appeared, on which were superimposed the contractions. Tonus variations disappeared between 15° and 16°C., when the rate of the contractions was about 1.7 per minute. With further cooling of the preparation the contractions became gradually less, until at 5°C. they ceased entirely.

The data reported above show that in a poikylothermal animal, such as a fish, the temperature of the medium surrounding it influences greatly its digestive processes. Employing Fundulus heteroclitus, coincident with a rise in temperature of the water in which it was kept was an acceleration of the digestive processes in the first part of the intestinal tract, whereas with a fall there was a retardation.

Finally, the survival time of tissue, taken from the stomach of the skate, at different temperatures was determined.

SERIES I

The following results were obtained, when strips of stomach musculature were excised and kept in nutrient saline at various temperatures.

1. For the first four days after excision regular contractions were observed in the strips taken from the preparation kept at 0°C. On the fifth day contractions were definite but slow. On the sixth day they were irregular in rate and amplitude. This irregularity and weakness increased each day. Finally, on the fourteenth day after excision of the muscle no contraction whatever could be obtained. The addition of 0.4 cc. of adrenaline stimulated the tissue from the antral region, which had been kept at 0°C. for three or four days, although this dose inhibited the contractions of fresh tissue from this region. On other parts of the stomach adrenaline also had a stimulating action, though each day the effect became weaker and finally on the

fourteenth day no effect could be obtained.

2. Tissue that had been kept at 5°C. first showed weakness on the third day, after which the contractions became weaker and more irregular, ceasing on the sixth day after excision of the tissue. Adrenaline had a stimulating action each day up to the sixth day.

3. Tissue that had been kept at 10°C. showed extreme weakness on the second day, and on the third day no contraction could be obtained.

4. Tissue that had been kept at 15°C. showed slight weakness after twenty-four hours, and reacted weakly to adrenaline. No contractions were obtained after forty-eight hours.

5. Tissue that had been kept at 20°C. showed extreme weakness after twenty-four hours, and would not react to adrenaline; twelve hours later no contractions could be obtained.

It was found that the saline solution in which the preparations were kept would be clear and odourless until the day before which the contractions ceased, when it would quickly become cloudy and smell strongly of ammonia within a few hours.

SERIES II AND III

The results of these two series of experiments are so similar that they may be dealt with together.

1. Fish kept at 20°C. After twenty-four hours the fish were found to have a faint ammoniacal odour. On opening the abdomen the stomach and intestines were found to be relaxed and soft. Strips removed from the antrum of the stomach gave small irregular weak contractions, but reacted to adrenaline and pilo-

carpine. Twelve hours later the fish were found to have a stronger odour and the flesh was softer. The intestines were soft and flaccid and strips removed from the antrum of the stomack gave no contractions and did not react to adrenaline and pilocarpine.

2. Fish kept at 15°C. After twenty-four hours the fish seemed to be in good condition; the flesh was firm and had no bad odour. Similarly the intestines seemed in good condition. Strips removed from the antrum of the stomach gave fairly strong, somewhat irregular contractions and reacted to adrenaline and pilocarpine. Twelve hours later the fish gave a slight ammoniacal odour, the flesh was less firm and the intestines in less good condition. Contractions of strips from the antrum gave very poor, irregular contractions and reacted poorly to adrenaline and pilocarpine. Twelve hours later the fish gave off a strong odour, the flesh was soft and the intestines were in poor condition. No spontaneous contractions could be obtained from strips taken from the antrum of the stomach and there was no reaction to drugs.

3. Fish kept at 10°C. After twenty-four hours the fish were in good condition. The flesh was firm and gave no bad odour. The intestines were also in good condition. Strips taken from the antrum gave strong, regular contractions and reacted powerfully to adrenaline and pilocarpine. Fish removed twenty-four hours later were in poor condition, the flesh was soft and had a strong odour of ammonia. The intestines were also in poor condition, being soft and flaccid. Strips removed from

the antrum gave very poor spontaneous contractions and reacted very poorly to adrenaline and pilocarpine. This tissue seemed to have nearly reached the end of its vitality. This was proved by the fact that strips taken from a fish six hours later did not react to these drugs.

4. Fish kept at 5°C. After twenty-four hours the fish were found to be in excellent condition. The flesh was firm and had no bad odour. The intestines were also in excellent condition, being firm and odourless. Strips removed from the antrum of the stomach gave good regular spontaneous contractions and reacted powerfully to adrenaline and pilocarpine. Similarly, fish removed forty-eight, seventy-two, and ninety-six hours after killing were found to be in good condition, and strips removed from the antrum gave good regular spontaneous contractions and reacted to adrenaline and pilocarpine.

However, after five days, i.e., after one hundred and thirty hours, the fish removed had a somewhat strong odour of ammonia and the flesh was soft. On opening the abdomen the intestines were found to be only in fair condition. Strips removed from the stomach gave irregular spontaneous contractions of medium strength. The reaction to adrenaline and pilocarpine was poor. A fish removed twenty-four hours later was found to be in poorer condition and strips taken from the antrum of the stomach gave no spontaneous contractions and no reaction to adrenaline or pilocarpine.

SECTION II

THE EFFECT OF CERTAIN DRUGS

The effect of adrenaline, atropine, pilocarpine, acetylcholine and ergotoxine upon the cardia, pyloric sphincter, antral and fundic regions of the greater and lesser curvatures of the stomach, upon the upper and lower ends of the spiral intestine and valve, upon the colon and rectum were investigated.

STOMACH

ADRENALINE

This drug has a stimulating effect on all parts of the stomach except the antrum, where an ordinary dose is inhibitory, while a very minute dose is excitatory. The action of this drug can be described best under two heads:

(a) Its effect upon the base line from which contractions rise.

(b) Its effect upon the rhythmic contractions themselves.

In the case of the cardia, fundic region of the greater and lesser curvatures and the pyloric sphincter, the addition of 0.4 cc. of adrenaline causes a sharp rise of the base line, followed by a very gradual return to the previous base-line. This effect is least and lasts for the shortest time in the cardia.

The rate of the contractions is at first greatly increased, while their amplitude is usually decreased, but gradually as the base-line returns to normal they become slower and higher. Adrenaline in smaller or greater concentrations stimulates likewise.

In the case of the antrum, adrenaline in concentrations of 1:1,000,000 or more causes an inhibition in the strips taken from the antrum near the pyloric canal, whether from the region of the greater or of the lesser curvature. There is no change in the base-line, merely a cessation of the contractions, which in concentrations of 1:250,000 may last for 45 minutes. A concentration of 1:2,000,000 (i.e., 0.1 cc. of a 1 in 2,000 solution of adrenaline chloride) causes a stimulation which is of the same character as that found in the rest of the stomach except that it is of much shorter duration. Tissue from this region, that had been kept three or four days in saline at 0° C., was found to be stimulated by all effective doses of adrenaline.

The part of the antrum adjacent to the fundus reacts to adrenaline in a similar way to the fundus.

PILOCARPINE

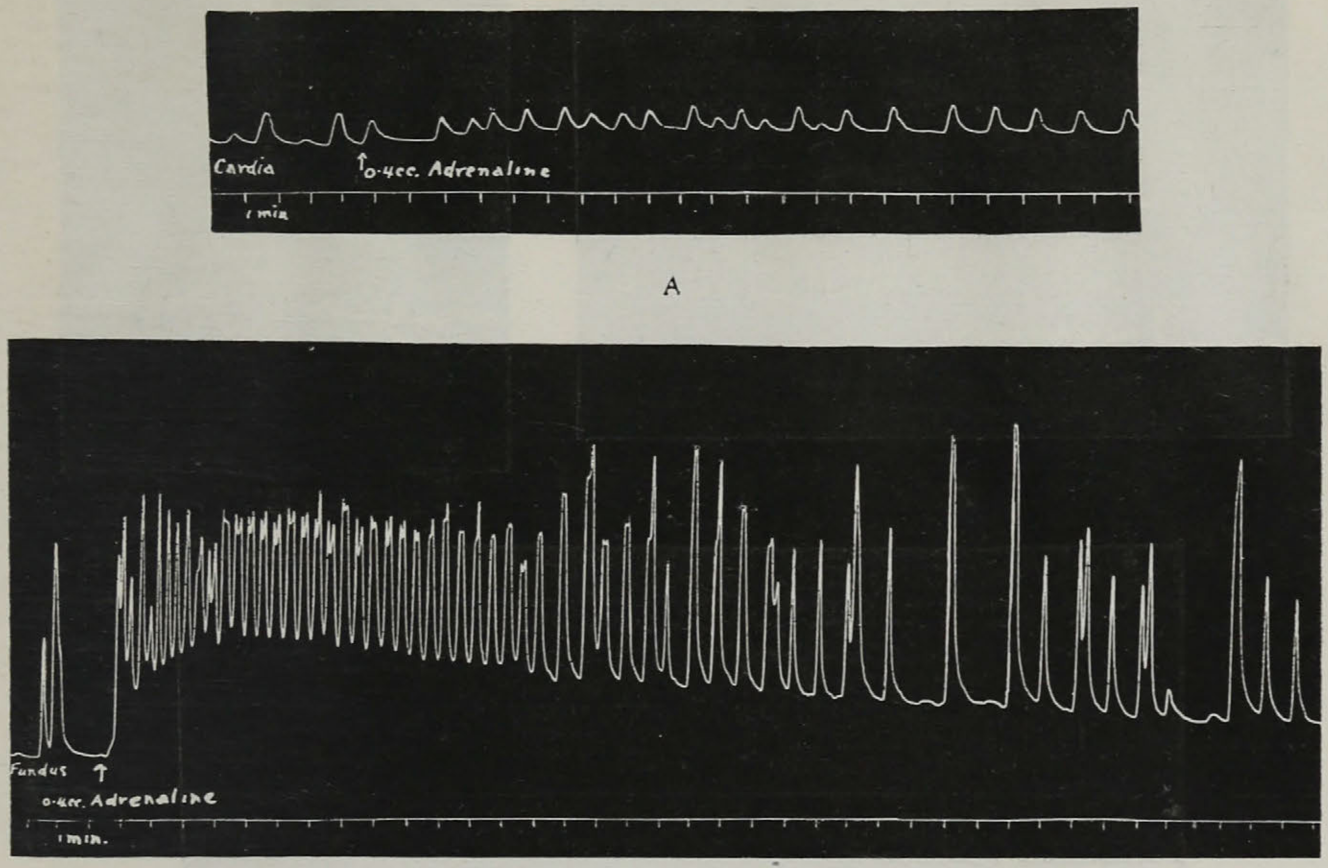
This drug stimulates all parts of the stomach. As before, its effects can be treated best under two heads:

(a) The effect upon the base-line.

(b) The effect upon the rhythmic contractions.

The addition of pilocarpine has no effect upon the base-line of the contractions of the cardia, fundic and antral regions of the greater and lesser curvatures. There is only an increase in the height and rate of the contractions. This effect usually lasts about 10 to 15 minutes.

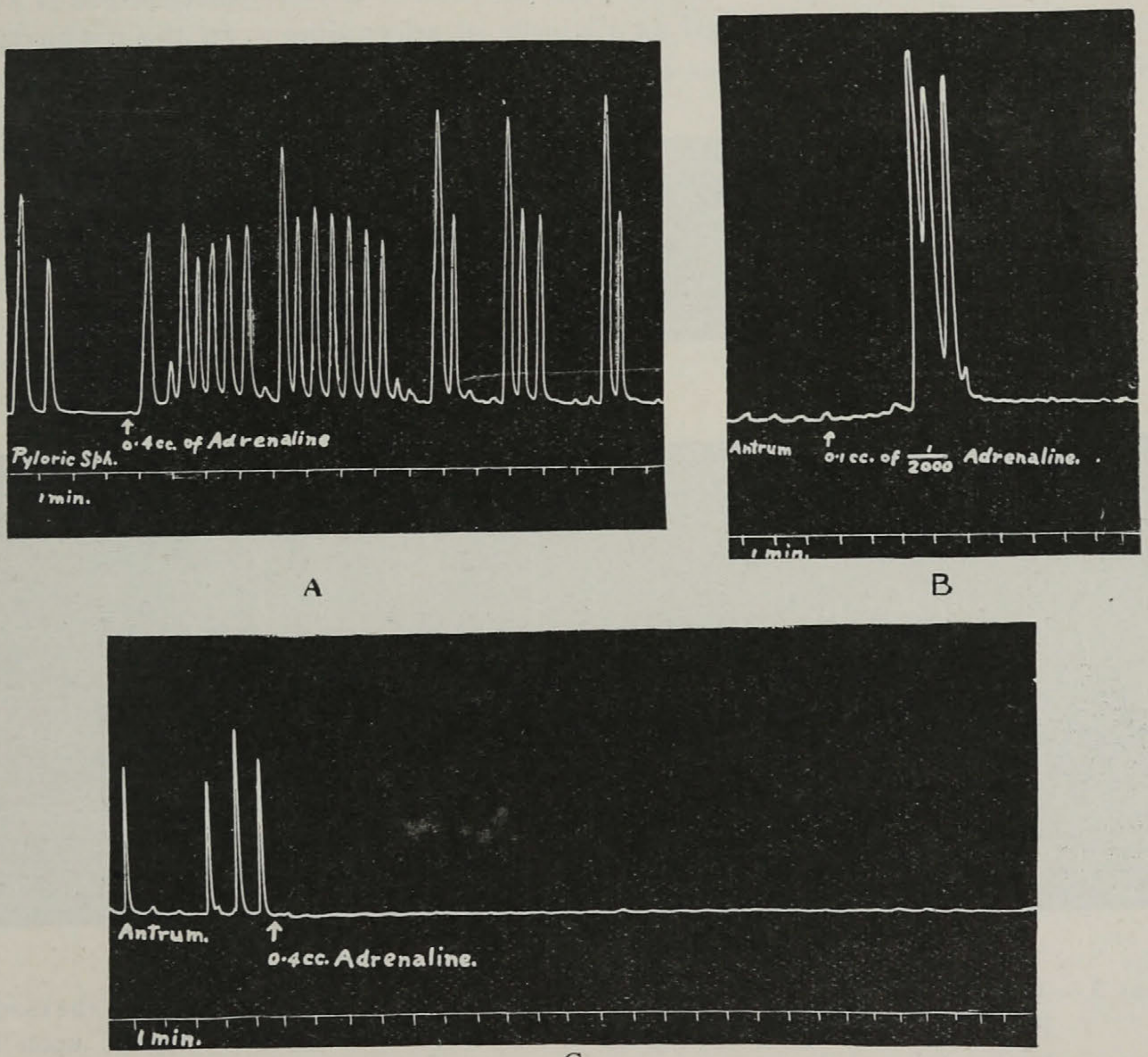
In the case of the pyloric sphincter, the effect of pilocarpine is more obscure. There is no change in the base-line, yet there are indications of stimulation. The normal



A

B

~~FIGURE 3~~.—A—record showing the effect of adrenaline (concentration 1: 250,000) upon the cardia; B—record showing the effect of adrenaline (concentration 1: 250,000) upon the fundic region of the greater curvature.

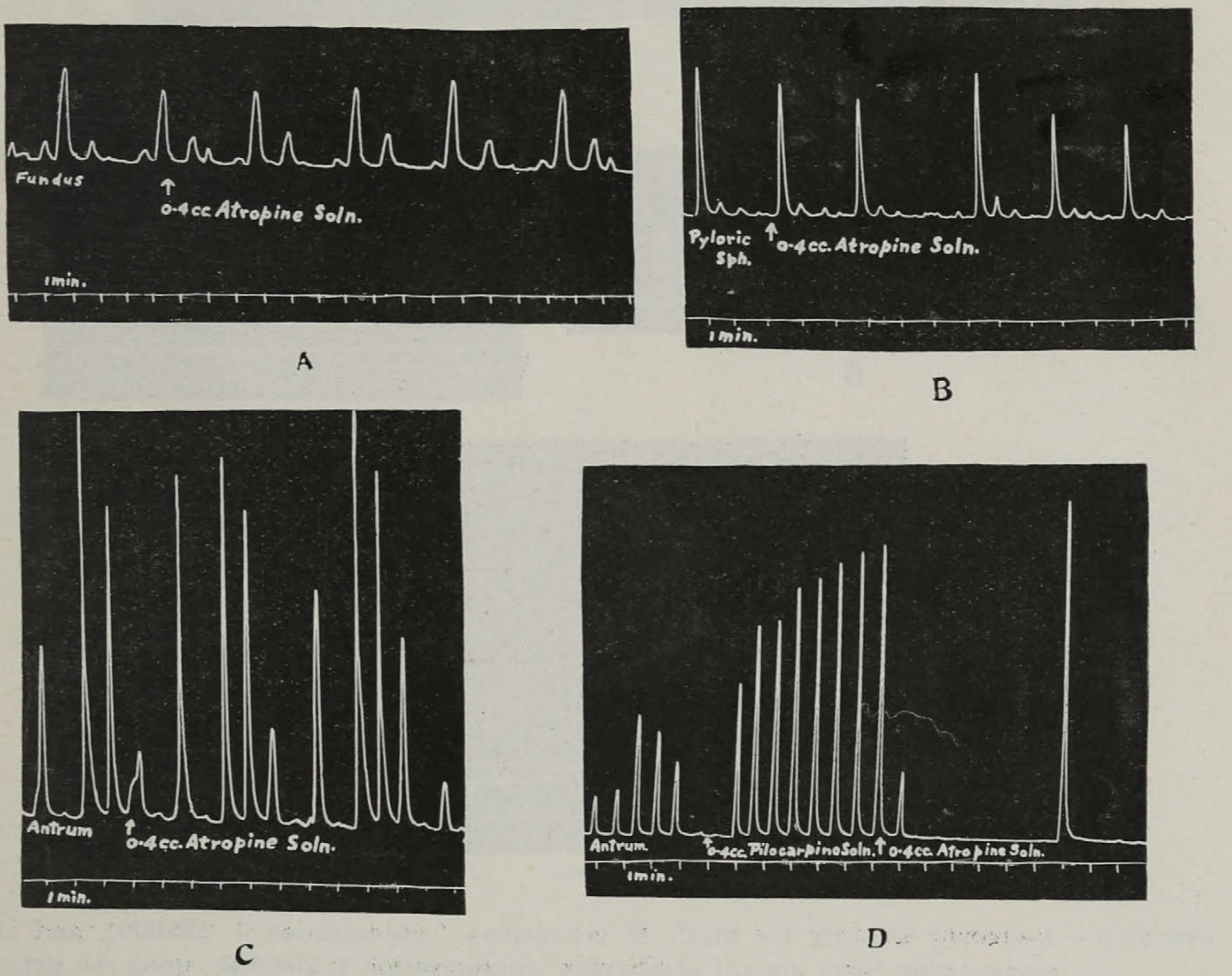


A

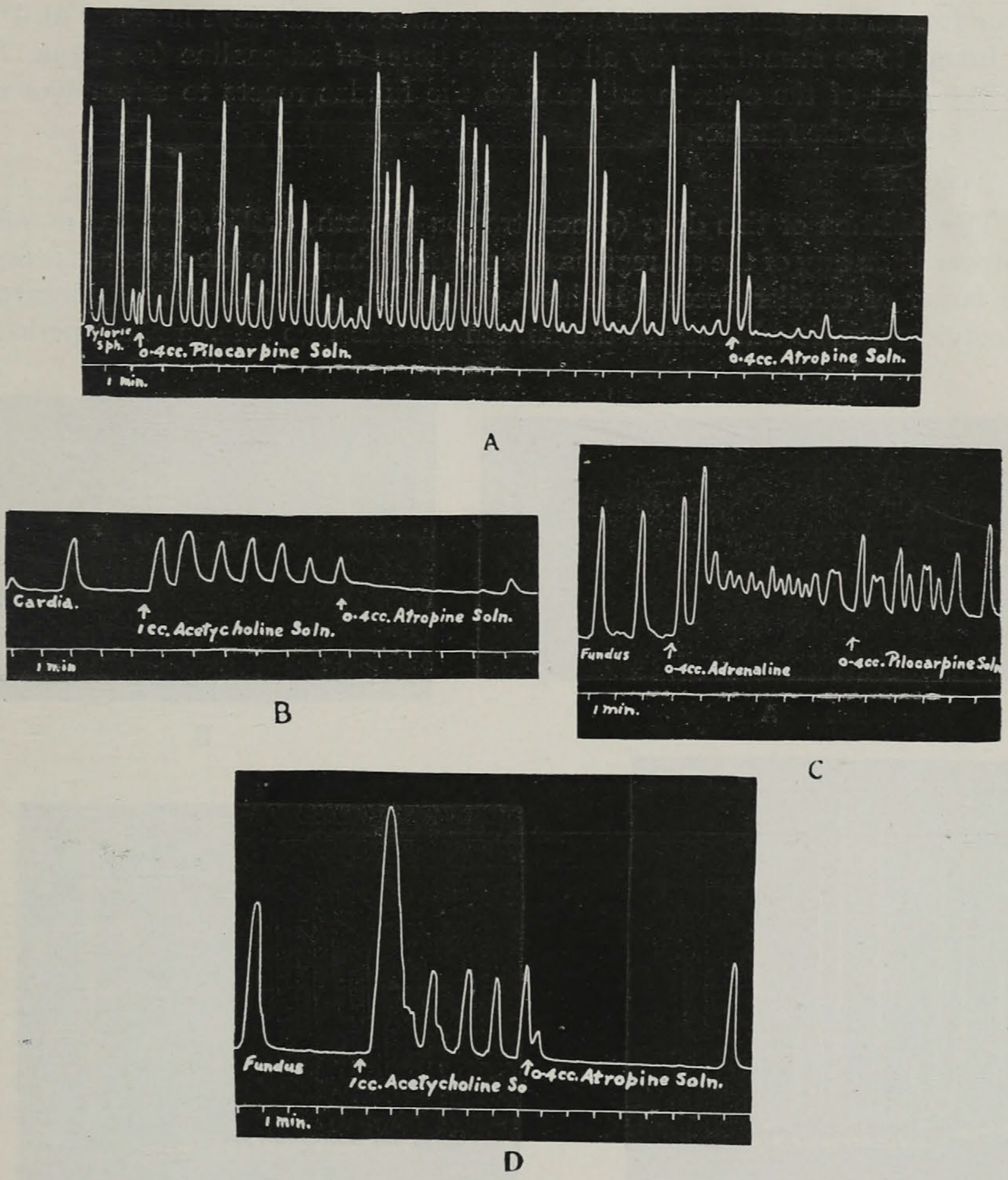
B

C

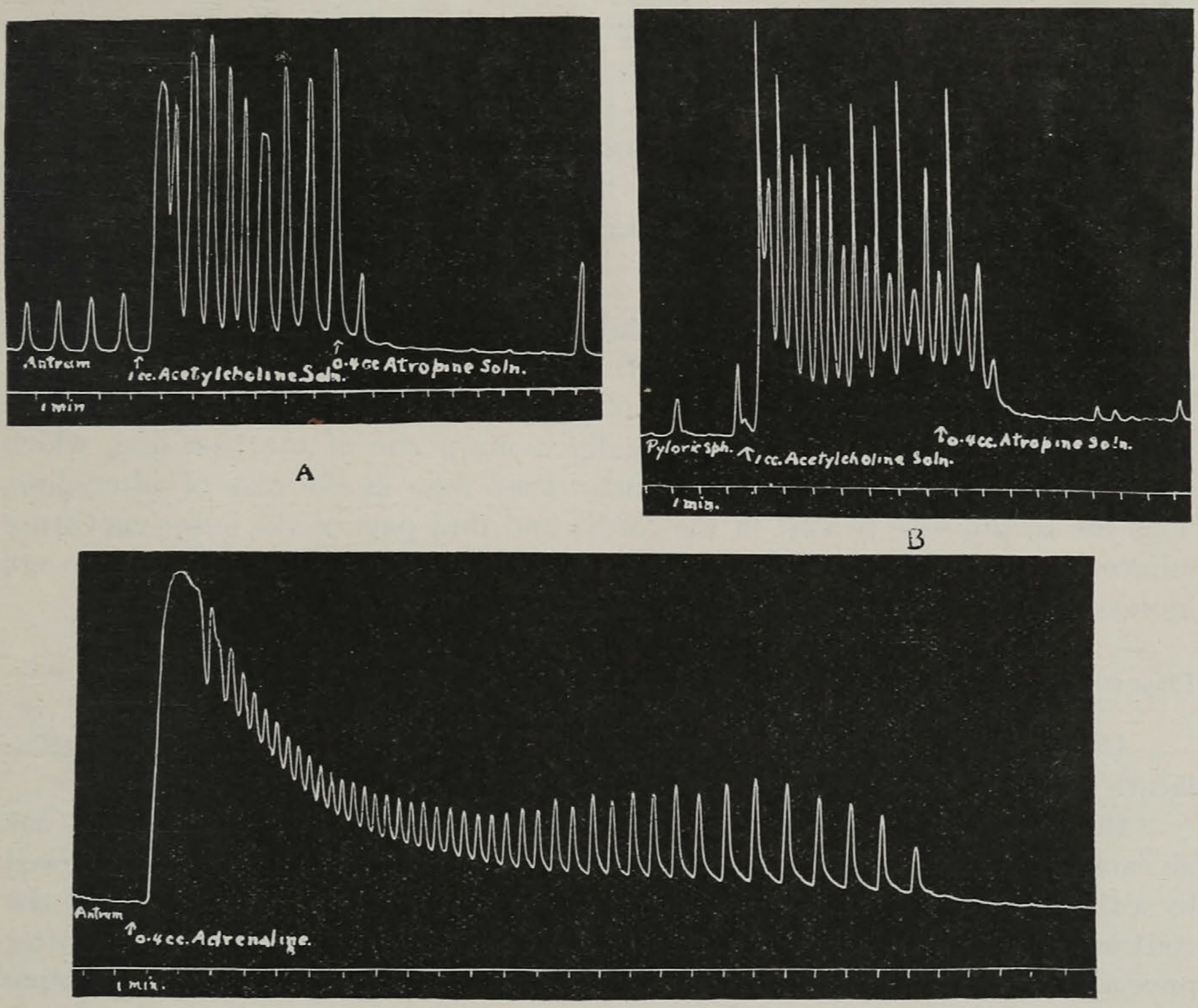
~~FIGURE 4~~.—A—record showing the effect of adrenaline (concentration 1: 250,000) upon the pyloric sphincter; B—record showing the effect of adrenaline (concentration 1: 2,000,000) upon the antral region of the greater curvature near the pyloric canal; C—record showing the effect of adrenaline (concentration 1: 250,000) upon the antral region of the greater curvature near the pyloric canal.



~~Figure 5.~~ A—record showing the effect of atropine (concentration 1: 250,000) upon the fundic region of the greater curvature; B—record showing the effect of atropine (concentration 1: 250,000) upon the pyloric sphincter; C—record showing the effect of atropine (concentration 1: 250,000) upon the antral region of the lesser curvature; D—record showing the effect of pilocarpine (concentration 1: 250,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the antral region of the greater curvature.



~~FIGURE 6~~.—A—record showing the effect of pilocarpine (concentration 1: 250,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the pyloric sphincter; B—record showing the effect of acetylcholine (concentration 1: 100,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the cardia; C—record showing the effect of adrenaline (concentration 1: 250,000), upon the fundic region of the lesser curvature. Also showing the additive effect of pilocarpine (concentration 1: 250,000); D—record showing the effect of acetylcholine (concentration 1: 100,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the fundic region of the lesser curvature near the cardia.



~~FIGURE 7~~.—A—record showing the effect of acetylcholine (concentration 1: 100,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the antral region of the lesser curvature; B—record showing the effect of acetylcholine (concentration 1: 100,000), and the antagonizing effect upon it of atropine (concentration 1: 250,000), upon the pyloric sphincter; C—record showing the effect of adrenaline (concentration 1: 250,000), upon the antral region of the lesser curvature near the pyloric canal. The tissue in this case has been kept for three days at 0° C.

type of contraction is usually a series of contractions of various heights. The addition of pilocarpine increases the number of contractions of great amplitude and diminishes the number of small ones. There is no change in the base-line and the contractions are slightly increased in rate. Furthermore, pilocarpine will initiate contractions in a quiescent pyloric sphincter. Hence the effect of pilocarpine on the pyloric sphincter is excitatory.

Pilocarpine following adrenaline has an additive effect also pilocarpine following adrenaline has an additive effect.

ACETYLCHOLINE

The addition of this drug stimulates all parts of the stomach. Its effect may be considered under the same two heads.

Its action is very similar to that of adrenaline, except that it stimulates all parts of the stomach. It causes usually a sharp rise of the base-line, which returns to normal in a somewhat shorter time than in the case of adrenaline. This rise of the base is least in the cardia and that part of the lesser curvature adjacent to the cardia. Both the rate and amplitude of the contractions are increased.

ATROPINE

The addition of this drug (concentration in bath, 1:250,000) has no effect whatever upon any of the six regions tested. No change in the response is found with larger or smaller doses. If, however, atropine be given after pilocarpine or acetylcholine, it will restore the normal contractions after a short period in which the contractions are completely inhibited. If the base-line has been raised, as is the case with acetylcholine, atropine restores it to its former level. If the base-line has been unaffected, as is the case with pilocarpine, atropine has no effect upon it.

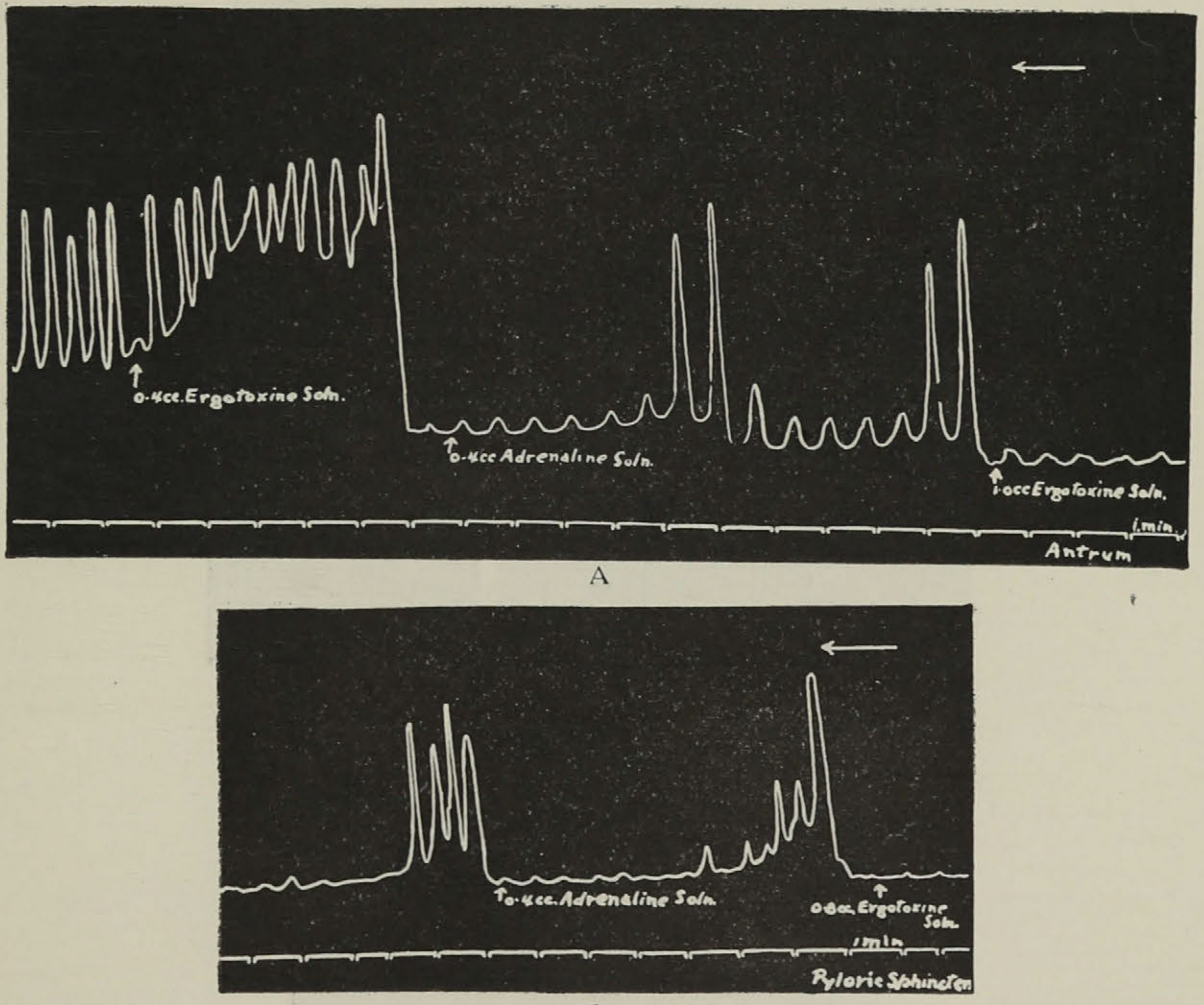
ERGOTOXINE PHOSPHATE

ANTRUM AND FUNDUS

Ergotoxine in doses of 0.4, 0.6 and 1.0 c.c. usually caused a rise of the base-line with increase in the rate and amplitude of the contractions.

PYLORIC SPHINCTER

The effect on this part was doubtful, though on one or two occasions there was definite evidence of an excitatory effect; after a delay of some 2 or 3 minutes there was a slight rise of the base-line and increase in the amplitude of the contractions.



~~Fig. 2.~~ A, showing the effect of ergotoxine (1 : 100,000) followed by adrenaline (1 : 250,000) upon the antrum of the stomach. B, showing the effect of ergotoxine (1 : 150,000) followed by adrenaline (1 : 250,000) upon the pyloric sphincter. This and one other experiment were the only ones in which ergotoxine had an excitatory effect; usually it had no effect.

ADRENALINE FOLLOWING ERGOTOXINE

ANTRUM

In general there was very little evidence that ergotoxine, even in massive doses, was antagonistic to adrenaline. Small doses of ergotoxine, such as 0.4 c.c., did not inhibit the effect of 0.4 c.c. of adrenaline. In cases where the muscle was in a weakened condition 0.2 c.c. of adrenaline did not stimulate. In one case when a massive dose of ergotoxine, 2.5 c.c., was given, there was a marked stimulatory effect which lasted fully 30 minutes. One-half c.c. of adrenaline given 25 minutes after this dose of ergotoxine had no effect. When an inhibitory effect occurred, it seemed to come on at least 10 - 15 minutes after the ergotoxine had been added to the bath.

FUNDUS

In the majority of instances small doses of adrenaline (0.2 - 0.5 c.c.) had an effect after 0.6 c.c. of ergotoxine or even larger doses. When antagonistic effects did seem to occur, the muscle was in a weakened condition.

PYLORIC SPHINCTER

The action of adrenaline in small doses was not inhibited by even massive doses of ergotoxine except in one or two instances.

INTESTINE

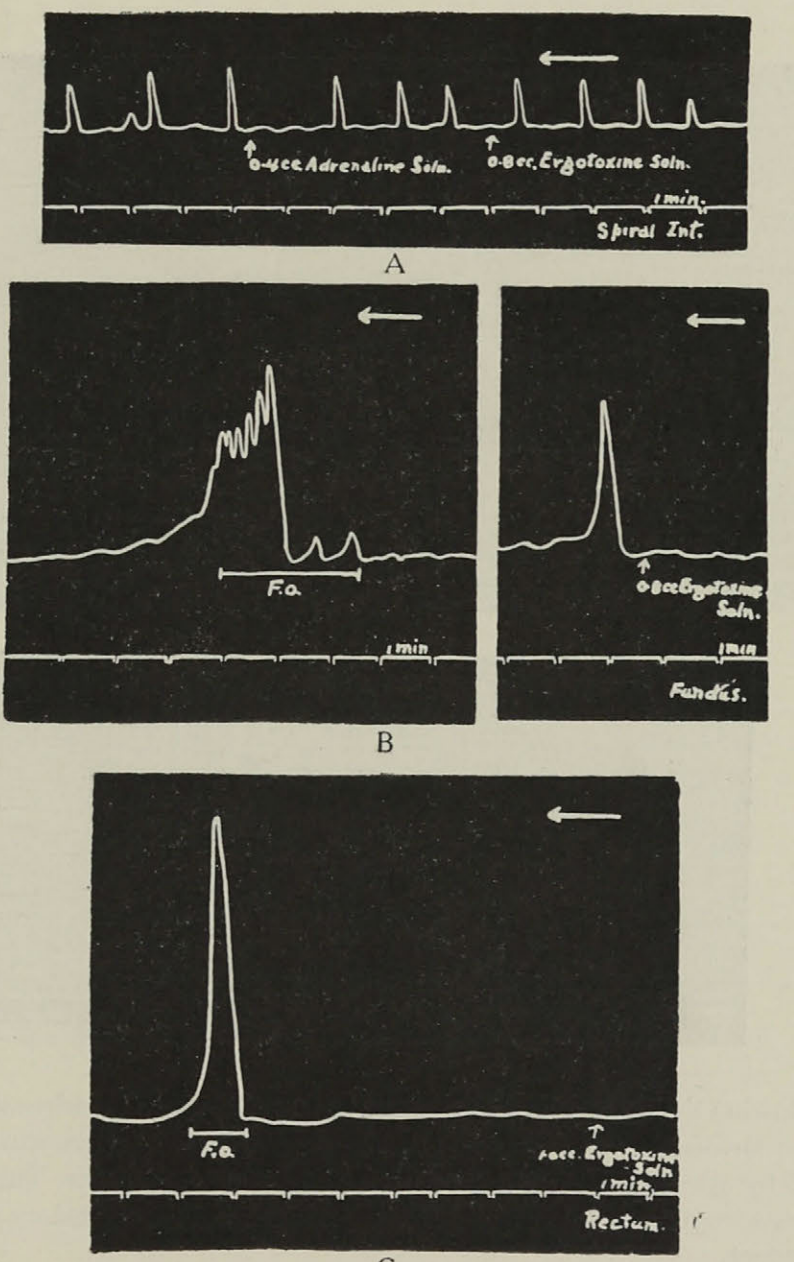
ADRENALINE

This drug in a concentration of 1:250,000 has an excitatory effect on all parts of the intestine. However, on two occasions an inhibitory effect was obtained in a strip taken from the spiral intestine.

SPIRAL INTESTINE AND VALVE

Adrenaline increases the rate and amplitude of the contractions of all parts of the spiral intestine and valve. The base-line gradually rises and again gradually subsides, returning to normal in about twenty minutes.

On two occasions an inhibitory effect was obtained on the spiral intestine, once on the upper end and once on the lower end, the contractions coming to a stop but the base-line remaining unchanged.



~~Fig. 3.~~ A, showing the effect of ergotoxine (1 : 150,000) followed by adrenaline (1 : 250,000) upon the spiral intestine. B, showing the effect of ergotoxine (1 : 150,000), followed by arterial stimulation (faradic, secondary coil at 0 cm.) 10 min. later, upon the fundus of the stomach. C, showing the effect of ergotoxine (1 : 100,000) followed by arterial stimulation (faradic, secondary coil at 0 cm.) upon the rectum.

COLON AND RECTUM

Adrenaline in a concentration of 1:250,000 has an exactly similar effect on both these parts of the intestine. Rapid rhythmic contractions of higher amplitude than normal are initiated, and the base-line is sharply raised, to return to normal after about fifteen minutes.

PILOCARPINE

This drug in a concentration of 1:250,000 has an excitatory effect on the spiral intestine, colon and rectum, but the effect on the spiral valve is very doubtful.

SPIRAL INTESTINE

Pilocarpine stimulates both the upper and lower ends of this part of the spiral intestine, increasing the rate and amplitude of the contractions. The base-line is gradually raised and again gradually returns to normal in about fifteen minutes.

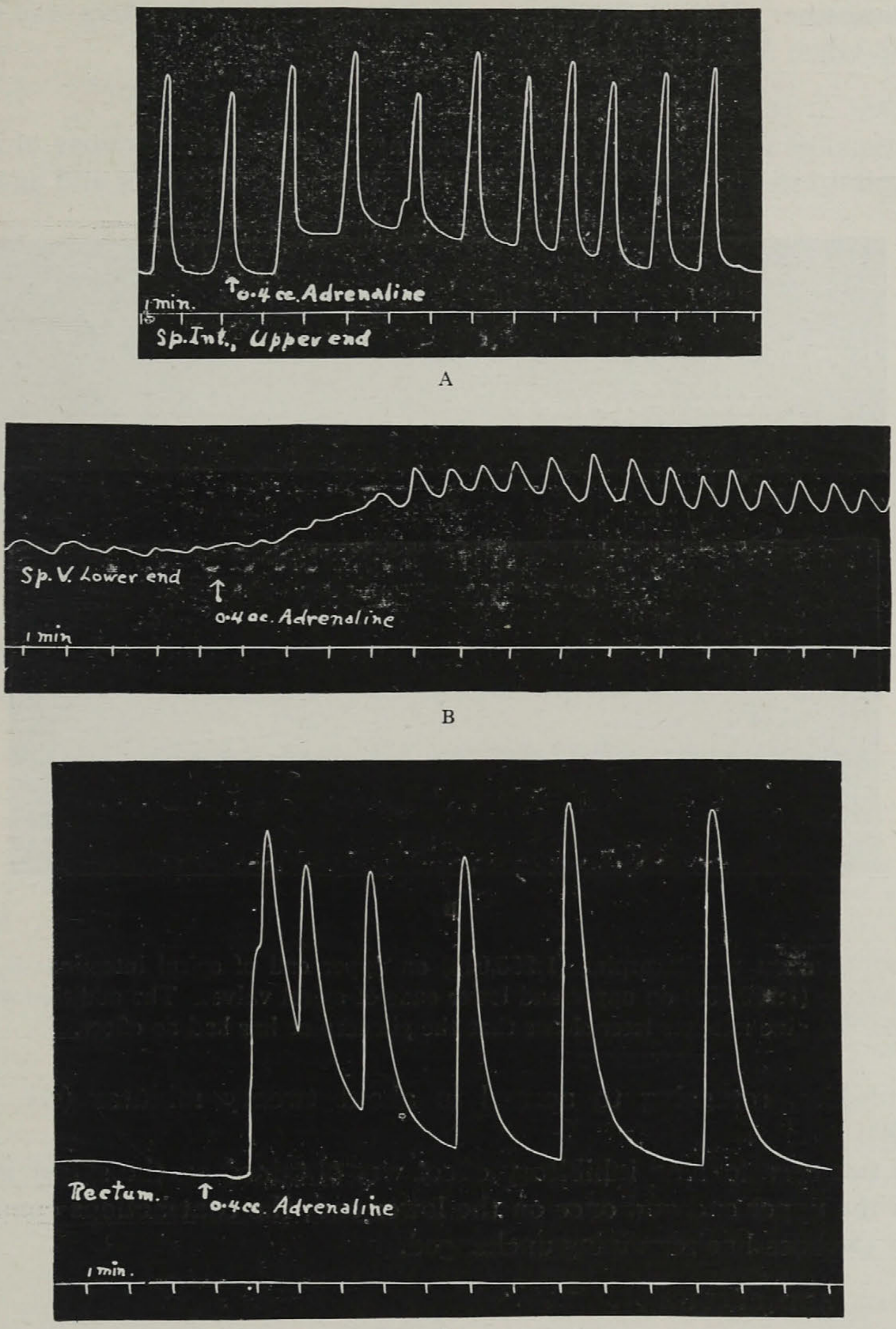
SPIRAL VALVE

The effect of pilocarpine on both the upper and lower ends of the spiral valve is very doubtful. There is no change in rate but the amplitude of the contractions seems to be slightly raised. There is no change in the base-line.

COLON AND RECTUM

Pilocarpine has an exactly similar effect on both these parts. It establishes rapid rhythmic contractions of higher amplitude than normal. The base-line is sharply raised and returns to normal in about ten minutes. See figure on top of page 53.

Pilocarpine following adrenaline or vice versa has an additive effect.



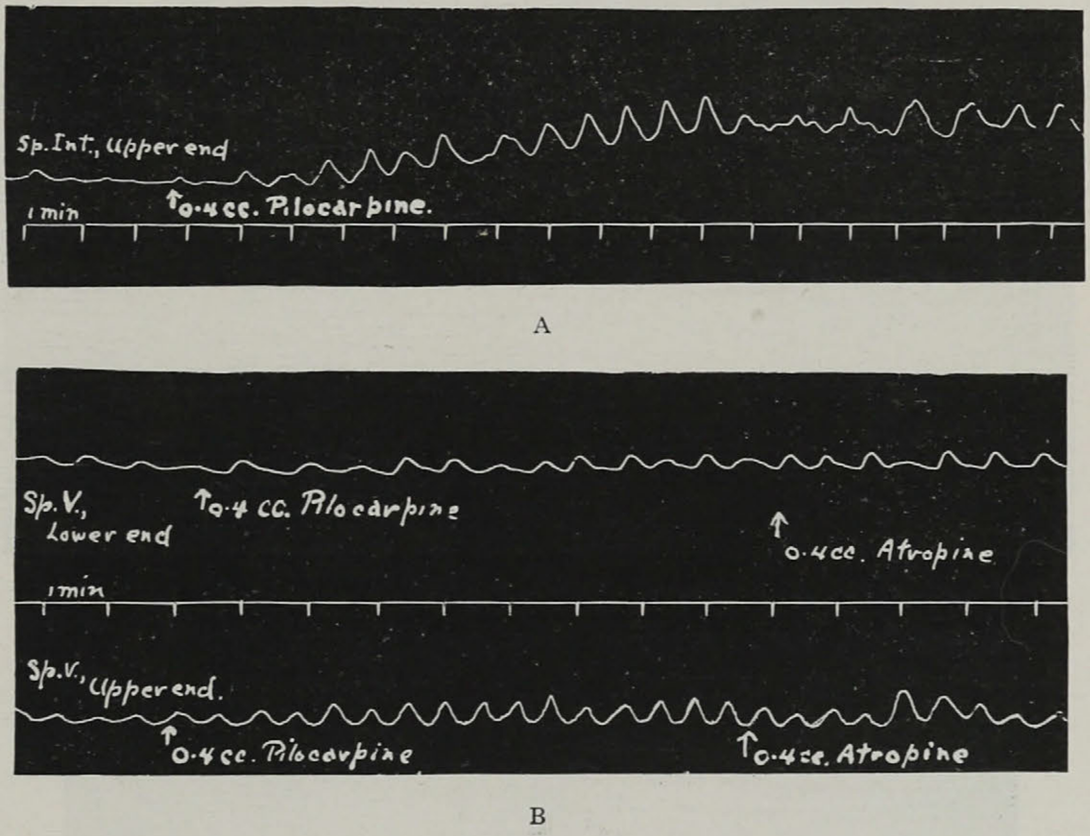
~~Figure 3~~.—A, effect of adrenaline solution (1:250,000) on upper end of spiral intestine; B, effect of adrenaline (1:250,000) on lower end of spiral valve; C, effect of adrenaline (1:250,000) on rectum.

ACETYLCHOLINE

This drug in concentrations of 1:100,000 stimulates all parts of the intestine.

SPIRAL INTESTINE AND VALVE

The contractions of the upper and lower ends of both these parts are increased in rate and amplitude. The base-line gradually rises and again gradually returns to normal in about fifteen minutes.



~~FIGURE 4~~.—A, effect of pilocarpine (1:250,000) on upper end of spiral intestine; B, effect of pilocarpine (1:250,000) on upper and lower ends of spiral valve. The addition of atropine (1:250,000) nine minutes later shows that the pilocarpine has had no effect.

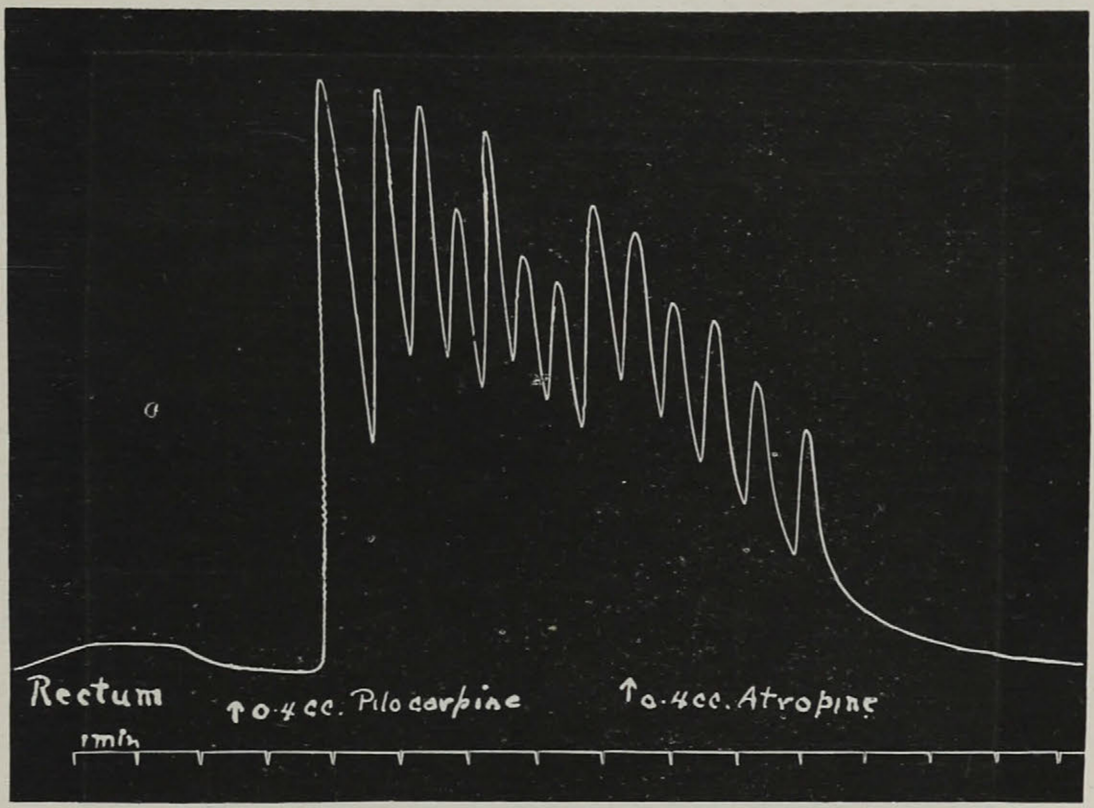
COLON AND RECTUM

Rapid, rhythmic contractions are established, of greater amplitude than normal. The base-line is sharply raised but gradually returns to normal in about fifteen minutes.

Acetylcholine following adrenaline or vice versa has an additive effect.

ATROPINE

This drug in all concentrations has absolutely no effect upon the normal contractions of the spiral intestine and valve, the colon and the rectum, but it restores the normal rate and amplitude, if given after pilocarpine or acetylcholine, and restores the base-line to normal.



~~Figure 5~~.—Effect of pilocarpine (1:250,000) on rectum, and antagonistic action of atropine (1:250,000).

ERGOTOXINE PHOSPHATE

SPIRAL INTESTINE

Ergotoxine in all doses had no effect.

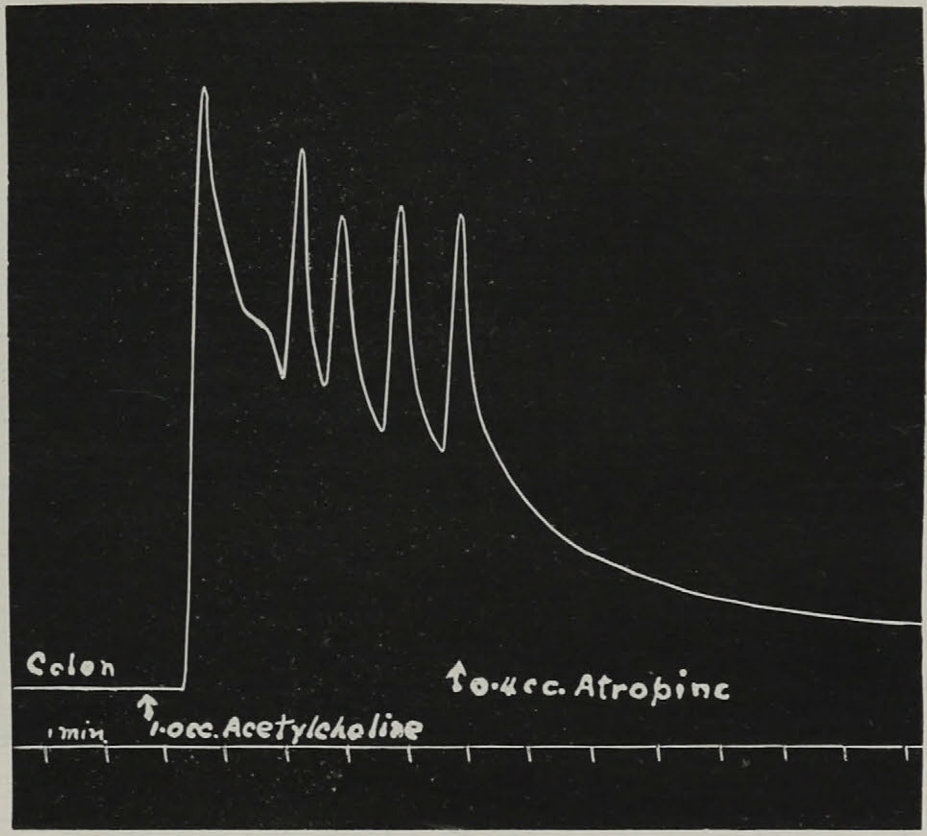
RECTUM AND COLON

Ergotoxine usually had no effect, but sometimes it caused a rise of the base-line with increase of rate and amplitude of contractions. This effect was most marked in the colon.

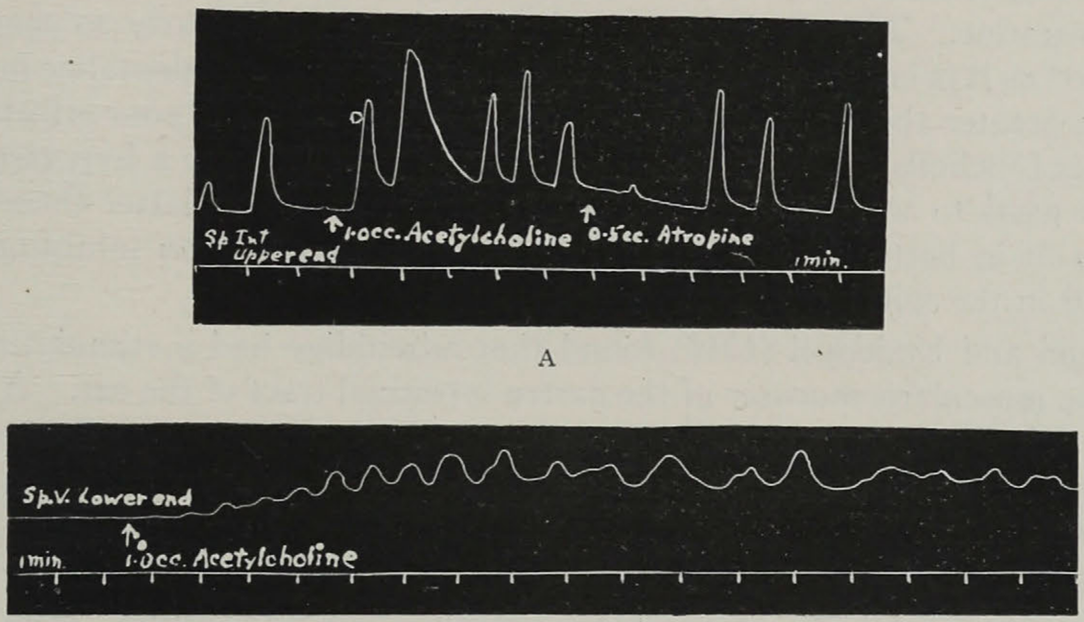
ADRENALINE FOLLOWING ERGOTOXINE

SPIRAL INTESTINE, RECTUM AND COLON

Doses of 0.6 c.c. or more of ergotoxine practically always inhibited small doses of adrenaline, such as 0.3 c.c. (see page 50).



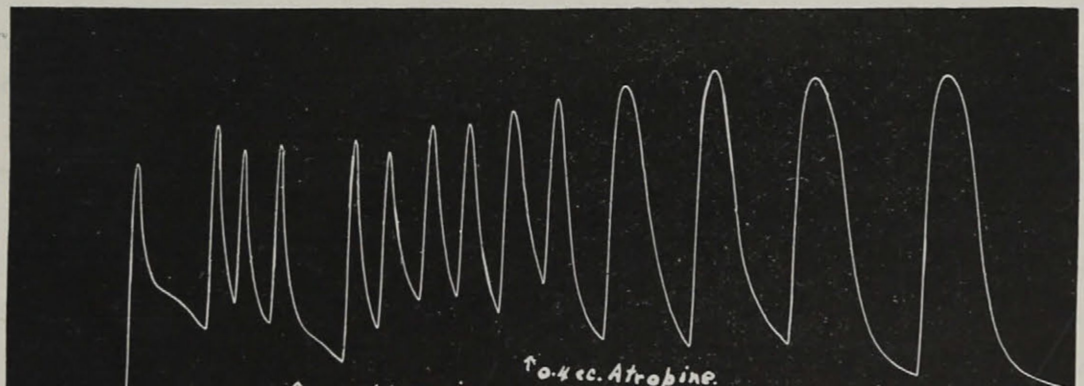
~~Figure 6~~.—Effect of acetylcholine (1:100,000) on colon, and antagonistic action of atropine (1:250,000).



A

B

~~Figure 7~~.—A, effect of acetylcholine (1:100,000) on upper end of spiral intestine, and antagonistic action of atropine (1:250,000); B, effect of acetylcholine (1:100,000) on lower end of spiral valve.



~~Figure 8~~.—Synergic effect of acetylcholine (1:100,000) and adrenaline (1:250,000) on colon. The addition of atropine (1:250,000) releases the adrenaline effect by dampening the effect of the acetylcholine.

SECTION III

STRUCTURE AND FUNCTION OF CERTAIN ARTERIES

The structure and reactions to stimuli of the ventral aorta, coeliac artery, superior mesenteric artery, anterior intestinal artery and conus arteriosus of the species *Raja diaphanes* and *stabuliforis* were also investigated. For the sake of completeness the whole of the results will be recorded here.

PART I. HISTOLOGICAL FINDINGS

For the study of the structure of the arterial walls in different species of Raja, tissue was obtained from the ventral aorta, from the coeliac axis, and from the superior mesenteric artery near its origin from the dorsal aorta and also near its connection with the valvular intestine. The tissue was fixed in Zenker's fluid and stained by special methods to demonstrate muscle, collagenous fibres and elastic fibres.

Sections of the ventral aorta show an elastic type of artery somewhat similar to that found in mammals. The first part of the aorta, immediately above the conus arteriosus, may be entirely free from muscle cells. Proceeding upward, circularly arranged muscle cells begin to appear adjacent to the tunica intima as a dark narrow band. They may become increased in number in this location to make up about one-fifth of the thickness of the tunica media. The other four-fifths of the media is practically free from muscle cells, and is composed of concentric layers of elastic tissue between which there is some collagenous connective tissue.

The coeliac and superior mesenteric arteries resemble the muscular type. The muscular layer may occupy from one-half to three-quarters of the thickness of the entire wall. Both inner circular and outer longitudinal muscles appear in the wall. The relative proportion of these two layers varies in the arteries from different specimens and also in different parts of the wall of the same artery. The longitudinal muscles often predominate.

The muscular layers are surrounded by a fairly wide zone of dense elastic and collagenous tissue and we have regarded this as being the adventitia. Benninghoff (1930) has described a somewhat similar adventitia in the superior mesenteric artery of man. It may be mentioned here, however, that, according to Maximow (1930), Schaffer describes a part of the superior mesenteric artery of man in which the concentric layers of elastic tissue encircling the muscle are regarded as part of the media and consequently would form the hybrid type of artery described by Argaud (1908).

For the study of the nerve supply to these arteries we found supravital staining with methylene blue to be the most satisfactory method. We adopted the method used by Irwin (1931) and that of Kreibich (Romeis 1928). The latter method requires the decolorizing of the methylene blue before injection, and we found it to be the most satisfactory. Some of the material was mounted on slides without cutting of sections, while some of it was embedded in paraffin before cutting. ~~Further details of methods and results will be reported elsewhere.~~

The various preparations revealed bundles of non-medullated nerve fibres accompanying the arteries on the outer layers of the adventitia, while at the zone of junction between the adventitia and the muscular media there is a very rich network of very fine non-medullated nerve fibres. These fibres divide, rejoin and divide again, forming a true network capable of conduction of impulses in all directions. We did not see anything to indicate free nerve endings on smooth muscle cells, and according to Busch (1929) a closed network without free endings is the typical sympathetic innervation of blood vessels in mammals. According to Woollard (1926) the innervation of the muscular coat of blood vessels by this fine network of non-medullated nerves is derived entirely from post-ganglionic fibres of the sympathetic system. He also states that vasoconstrictor nerve fibres are recognized to be exclusively sympathetic. Reasoning by analogy it would seem that the nerve net which we have seen on the mesenteric arteries in skates is a part of the sympathetic nervous system. We also observed individual typical sympathetic ganglion cells in the outer part of the adventitia of the coeliac and mesenteric arteries. These ganglion cells are not easy to locate but can be found on studying many serial sections of material fixed in Zenker's fluid and stained by hematoxylin and eosin or by acid fuchsin stains.

A large number of peculiar branching cells in the wall of the ventral aorta take the methylene blue stain. Woollard has described similar cells in the blood vessels of the cat. Their function is unknown.

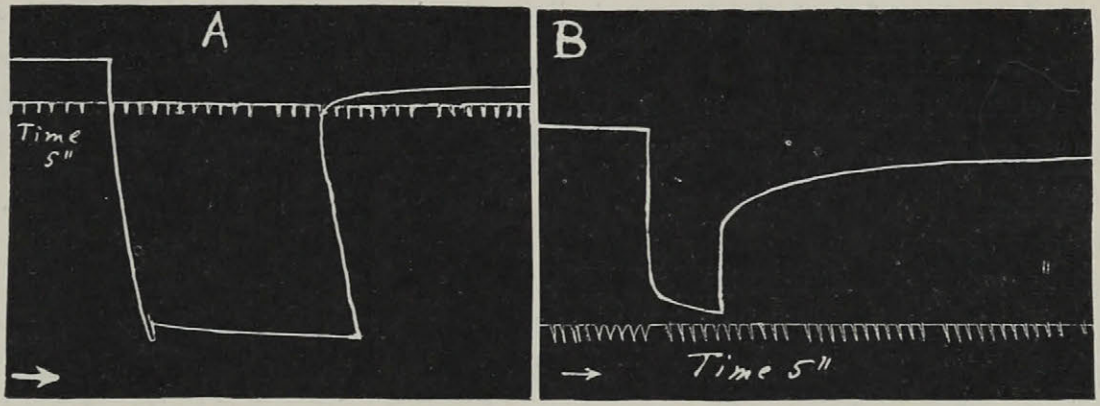
PART II. EXPERIMENTAL RESULTS

ARTERIES

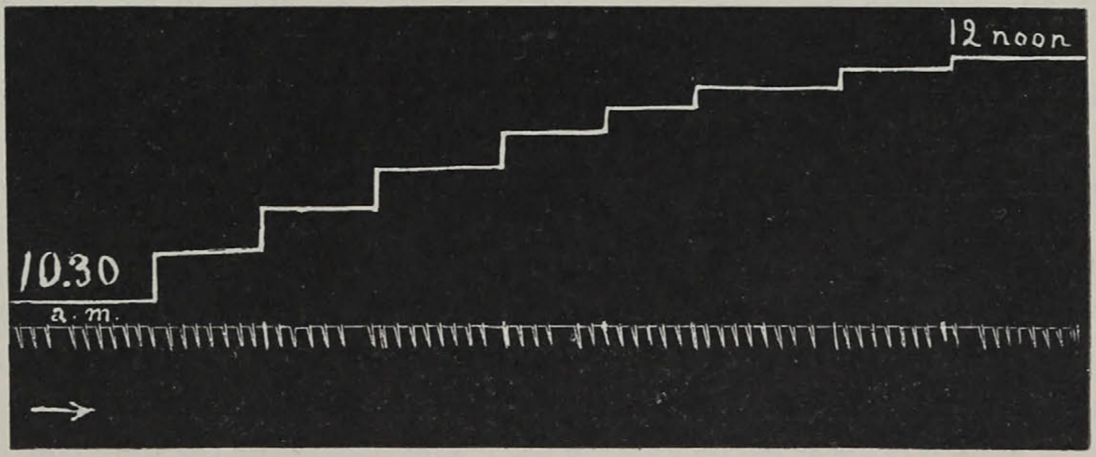
REACTION TO DISTENSION

No spontaneous contractions were noted under any conditions in the arteries investigated. This applies equally to the arteries suspended vertically (longitudinal layer of muscles) and to the rings of artery (circular layer of muscles).

Distension by the placing of an appropriate weight on the long arm of the lever produced a different reaction in different arteries. Like the arteries of mammals, the arteries of the skate may be divided into two general groups: the elastic type (ventral aorta) and the muscular type (coeliac, mesenteric superior and anterior, and intestinal arteries). After the application of a moderate weight to the lever connected with a single ring of artery of the elastic type (ventral aorta) in *Raja stabuliforis* the dilatation quickly reaches its maximum. When unloaded the artery quickly returns to its previous diameter, although the recovery is not quite complete. The distension of a circular layer of artery of the muscular type presents another picture. The distension on loading of the muscles is gradual. After unloading of the weight the lever returns very gradually to its previous level, drawing a rounded curve. The rate of recovery of the diameter of the ring depends on the weight applied; the greater the weight, the slower the return of the artery to its previous size. The same type of curve as depicted in figure B was obtained from long pieces of artery of the muscular type suspended vertically. This incomplete recovery of an isolated artery after distension does not occur in intact arteries in the body, which possess perfect elasticity. The reason for the long continuance of the distension in surviving preparations of arteries is that partly contracted muscles, when distended, do not regain their previous tonus after unloading (Fleisch, 1927). Observations analogous to those described above were made by Ducret (1930) on coronary arteries (elastic type) and mesenteric arteries (muscular type) in warm-blooded animals. Therefore the reaction of different types of arteries to distension is practically the same in elasmobranch fishes and in mammals.



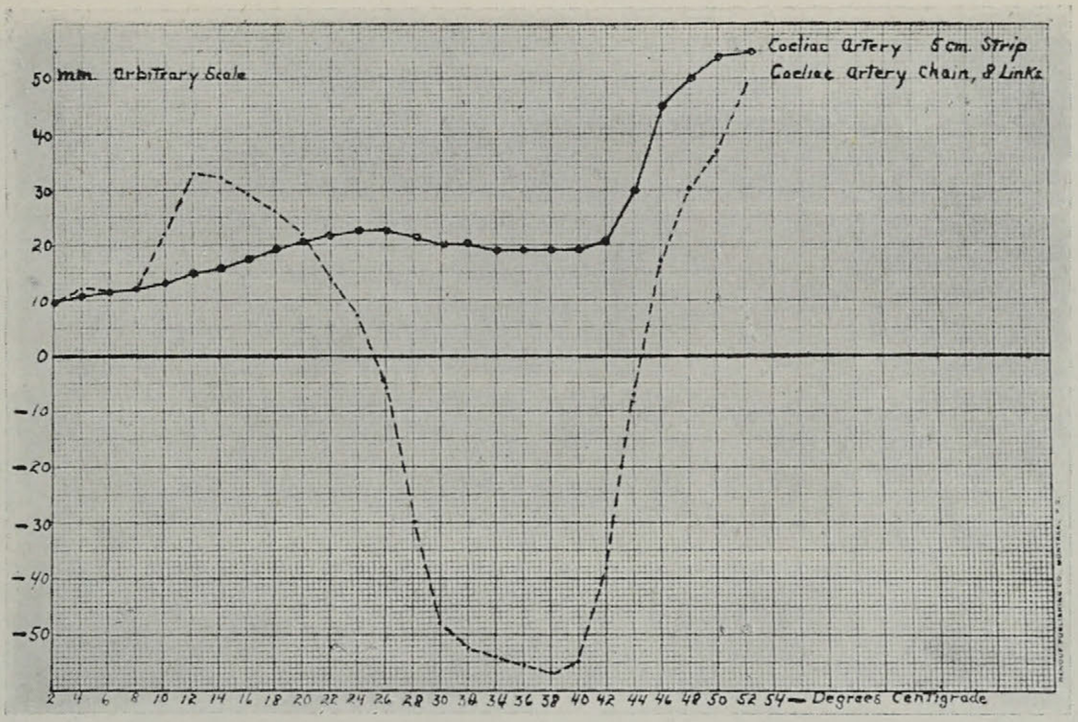
~~Figure 3~~.—A. Distension, by weight, of a ring from the ventral aorta of *Raja stabuliforis*. B. Distension of a chain of 9 rings from the superior mesenteric artery of *Raja stabuliforis*. The full effect of the weight, placed on the long arm of the lever, was prevented because the edge of the bath impeded the lever.



~~Figure 4~~.—*Exp. Aug. 17. Raja stabuliforis*, killed 9:30 a.m. Aug. 16, superior mesenteric artery preserved in cold saline (3° to 4° C.). Chain of 10 rings. Spontaneous recovery of tonus under slight distension at temperature of $9\frac{3}{4}$° to $10\frac{1}{4}$° C. Each step on the tracing represents an interval of 10 minutes. Experiment started, Aug. 17, at 10:40 a.m.; finished 12 noon.

TONUS

After being excised from the body and kept for two or more hours in the ice-cold nutritive solution, an artery of the muscular type is in a state of relaxation. On being placed, slightly weighted, in an oxygenated solution and kept at a temperature of 10° to 12° C., it regains its tonus. This capacity to regain tonicity is preserved for at least thirty hours. In the figure below is shown a slow recovery of tonus of circular muscles of the superior mesenteric artery in Raja Stabuliforis. The rate of recovery of the tonus is at first rapid, gradually slowing down.

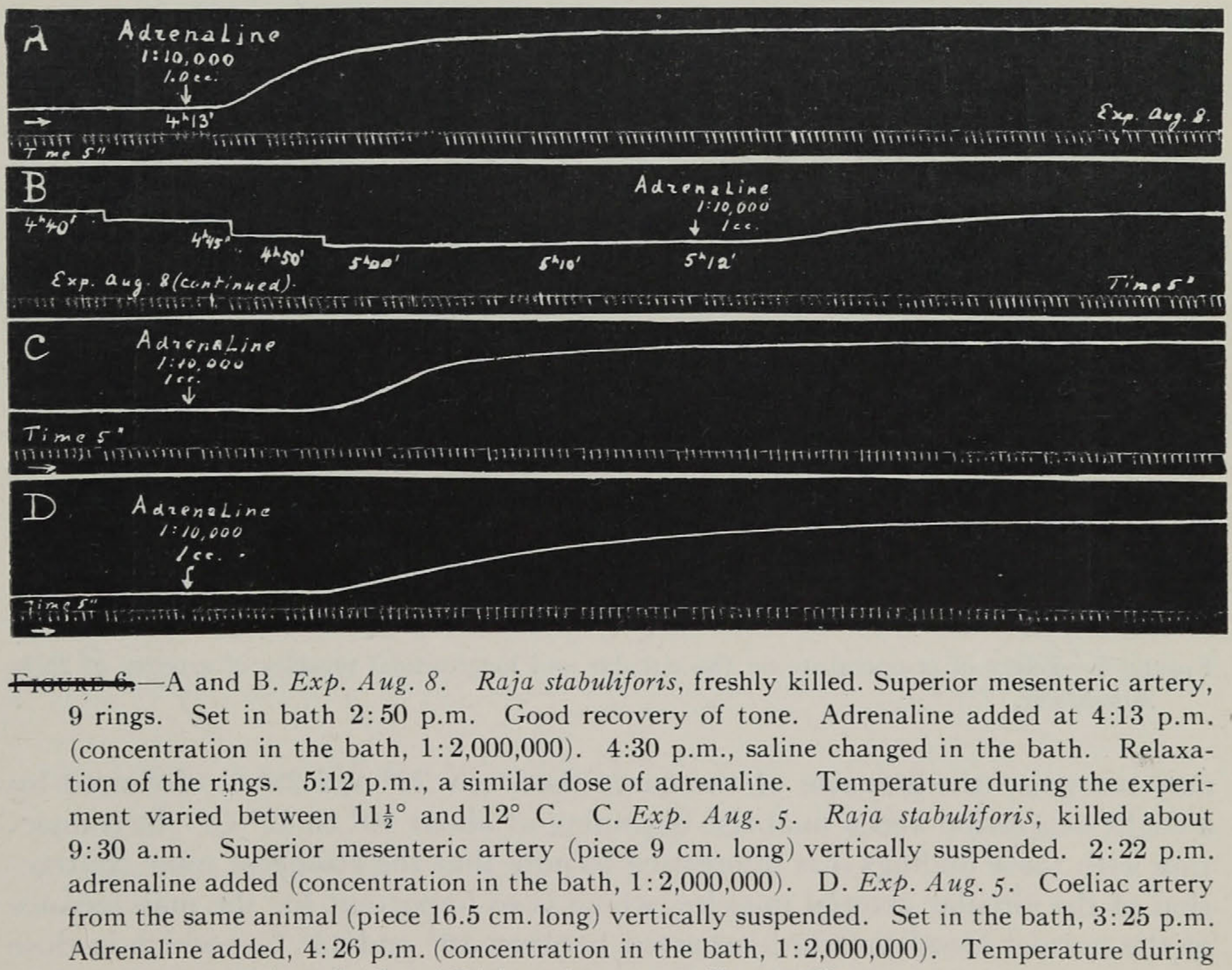


~~FIGURE 5.~~—Effect of temperature on the circular and longitudinal muscles of arteries of *Raja stabuliforis.*

Arteries set in the bath immediately after removal from the body did not show a similar recovery of tonus, perhaps because like mammalian arteries in similar circumstances they were in a state of spasm. This condition is not favourable for the action of drugs and the best results were obtained from blood vessels kept in ice-cold nutritive fluid for several hours before the experiment.

REACTION TO TEMPERATURE CHANGES

A chain of rings and also a longitudinal strip of coeliac and superior mesenteric arteries of Raja stabuliforis were subjected to changes of temperature in the bath. The artery was placed in solution with an initial temperature of 2°C. The temperature was gradually raised to 52° or 53° C. No substantial difference was noted in the reaction of corresponding preparations of the coeliac and superior mesenteric arteries to temperature. But the circular and the longitudinal layer of each artery gave somewhat different reactions, as may be seen in the figure below. The circular layer showed a maximal contraction between 12° and 14°C., which began rapidly to fall when the temperature of the bath reached 20° to 22°C. The greatest relaxation occurred at about 36° to 38° C., after which a second and final rise of the curve could be observed. The variations of the longitudinal layer of muscles were less marked,

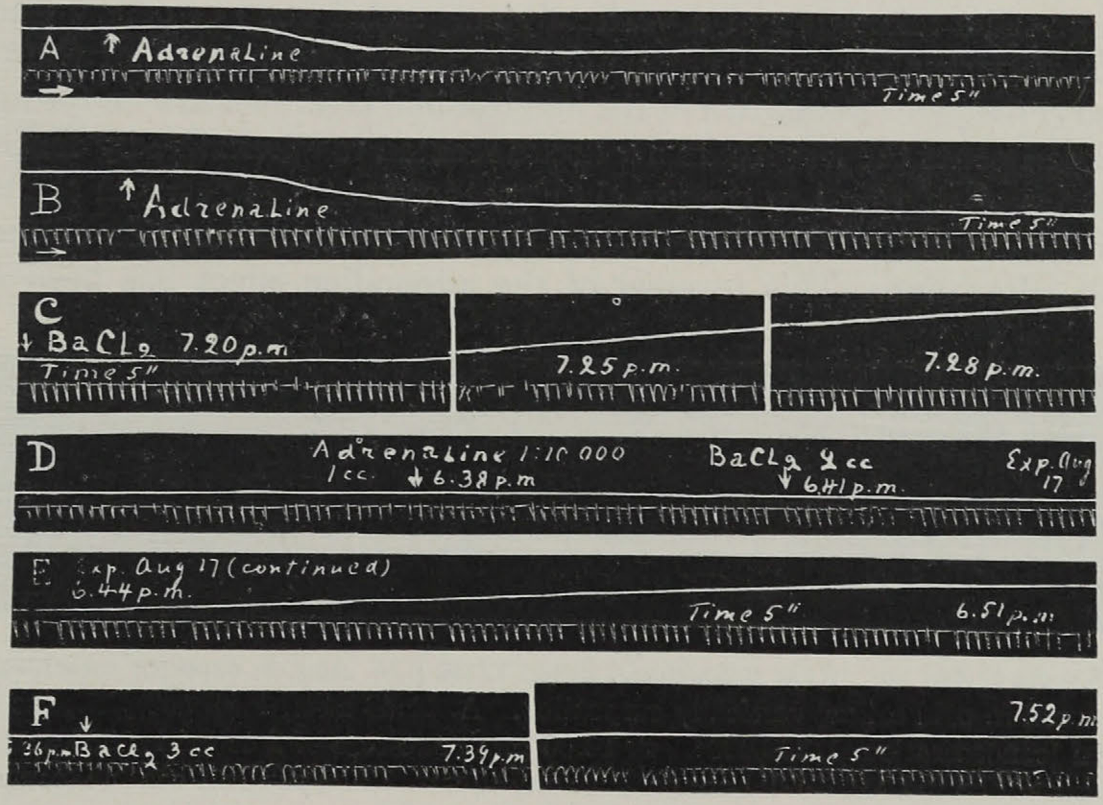


~~Figure 6.~~—A and B. *Exp. Aug. 8. Raja stabuliforis,* freshly killed. Superior mesenteric artery, 9 rings. Set in bath 2:50 p.m. Good recovery of tone. Adrenaline added at 4:13 p.m. (concentration in the bath, 1:2,000,000). 4:30 p.m., saline changed in the bath. Relaxation of the rings. 5:12 p.m., a similar dose of adrenaline. Temperature during the experiment varied between $11\frac{1}{2}$° and 12° C. C. *Exp. Aug. 5. Raja stabuliforis,* killed about 9:30 a.m. Superior mesenteric artery (piece 9 cm. long) vertically suspended. 2:22 p.m. adrenaline added (concentration in the bath, 1:2,000,000). D. *Exp. Aug. 5.* Coeliac artery from the same animal (piece 16.5 cm. long) vertically suspended. Set in the bath, 3:25 p.m. Adrenaline added, 4:26 p.m. (concentration in the bath, 1:2,000,000). Temperature during experiments of Aug. 5 (C and D) kept between $11\frac{3}{4}$° and 12°.

although here too we may recognize the first rise of the curve (between 22° and 28°C.) followed by a slight depression and a final rise beginning at about 40° to 42°C. In connection with these findings it is interesting to note that the strongest tonic contraction of the circular arterial muscles, which is so important for the maintenance of the normal blood pressure, occurred between 10° and 14°C. -- which is close to the temperature of the fish in summertime (8° to 12°C.) Cow (1911) describes the somewhat analogous behaviour of mammalian arteries under varying temperature conditions. The first contraction of the skate's artery on heating was undoubtedly due to an increase in the tone of its muscular tissue. The second contraction was merely a heat rigor. In this case the shortening of the preparation was probably due not only to the processes occurring in the muscles under the influence of high temperature but was determined to a greater degree by the coagulative changes in the connective tissue of the artery. That such is the course of events in structures formed of muscles and connective tissue was very convincingly demonstrated by Verbitzky (1923) on the cat's iris. The iris of this animal consists of an inner muscular ring and an outer ring of connective tissue. Heating of the former gave a curve with two moderately high rises; heating of the latter gave a single but very abrupt rise when the temperature reached about 60°C. In the case of the arteries of the skate, the second rise, due to heat rigor, occurred at a much lower temperature (38° to 42°C.) than in warm-blooded animals (about 60°C.).

INFLUENCE OF DRUGS

Both the chain of arterial rings and the artery vertically suspended responded by shortening to adrenaline in concentrations of 1:1,000,000 to 1:2,000,000. This applies to all the arteries which we investigated of *Raja stabuliforis*. Analogous, though much less pronounced results were obtained with the arteries of *Raja diaphanes*. The difference may be attributed to the insignificant development of the muscular layers of the arteries owing to the smaller size of these fish. The reaction of the circular layer of muscle to adrenaline was somewhat different from that of the longitudinal. The latent period, after adrenaline was first added to the nutritive fluid, was much shorter, the rise of the curve was more abrupt and the peak of contraction was

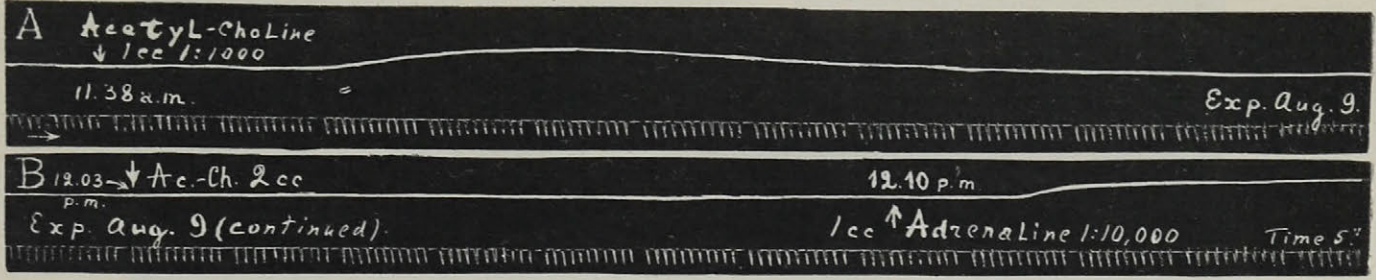


~~Figure 7~~.—A. *Exp. Aug. 11.* Relaxation, under the influence of adrenaline (concentration in bath, 1:2,000,000), of superior mesenteric artery suspended vertically. Temperature 11° C. *Raja stabuliforis,* killed 45 hours previously and artery preserved in cold saline. B. *Exp. Aug. 16.* Weakened preparation of superior mesenteric artery of *Raja stabuliforis,* killed only $6\frac{1}{2}$ hours previously. Relaxation under adrenaline (1:2,000,000). Temperature, $11\frac{1}{2}$° to $11\frac{3}{4}$° C. C. *Exp. Aug. 15.* Coeliac artery (9 rings) removed from *Raja stabuliforis* a few hours previously. The preparation reacted to adrenaline and acetylcholine. Effect of barium chloride (1:100,000). Temperature, $9\frac{3}{4}$° to 10° C. *Exp. Aug. 17.* D and E (direct continuation of D). Coeliac artery (8 rings) from a *Raja stabuliforis,* killed Aug. 16. Neither first nor second addition of adrenaline (1:1,000,000) has any effect. $BaCl_2$ (1:100,000) produces a contraction. Temperature, $10\frac{1}{4}$° C. F. *Exp. Aug. 17.* Aorta ventralis. Same animal as in D and E. Effect of $BaCl_2$ (1:75,000). Temperature, $10\frac{1}{2}$° C.

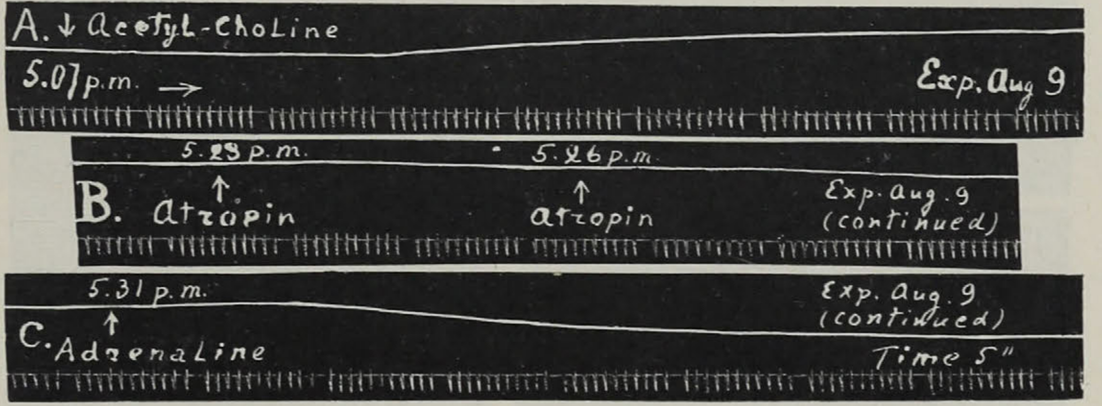
reached sooner with the circular muscles than with the longitudinal. On the average, in different experiments and for different arteries, it was 58 seconds for the circular muscles and 85 seconds for the longitudinal muscles. In the particular experiment shown in A of the figure on page 62, the latent period of the adrenaline effect on the circular layer of the superior mesenteric artery was only 25 seconds.

The addition of adrenaline at the height of the contraction produced by a previous application of the drug did not have any effect. However, if the nutritive solution containing adrenaline was replaced by fresh solution containing no adrenaline and the artery was allowed to relax, a second addition of adrenaline produced the usual, though a somewhat diminished, effect. One such experiment is represented in A and B in the figure on page 63.

A relaxation of the artery under the influence of adrenaline (1:2,000,000) was noted on two occasions only in both cases in longitudinal strips of the superior mesenteric artery forty-five and six-and-a-half hours respectively after removal from the body. Part of the anterior intestinal artery (9 rings) excised from the second animal did not react to acetylcholine, showed a very weak positive reaction to adrenaline, but gave a good contraction after the addition of 2% $BaCl_2$ solution. Therefore both superior mesenteric arteries seemed to be in a weakened state. This fact is worthy of mention because all mammalian arteries do not contract in response to adrenaline. For instance, in oxen, pigs and sheep, very small doses of ad-



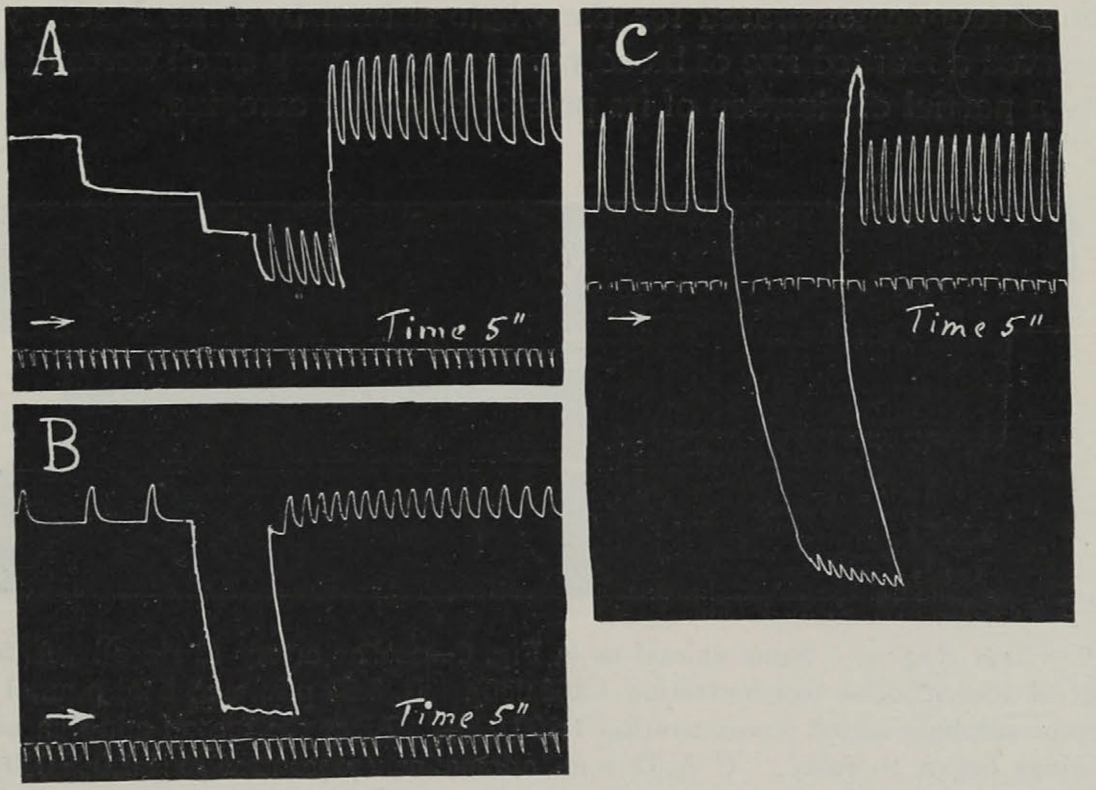
~~FIGURE 8~~.—*Exp. Aug. 9. Raja stabuliforis*, killed 12:30 p.m. Aug. 8, superior mesenteric artery preserved in cold saline, vertically suspended. A. 11:38 a.m., 1 cc. acetylcholine (1:1,000) added (concentration, 1:200,000). B. 12:03 p.m., addition of 2 cc. of 1:1,000 acetylcholine (concentration 2:200,000) did not produce any effect. 12:10 p.m., adrenaline (concentration 1:2,000,000) gave positive effect. Temperature 10½° C.



~~FIGURE 9~~.—*Exp. Aug. 9.* Same animal as in Fig. 6—coeliac artery (6 rings). A. 5:07 p.m., effect of acetylcholine (concentration 1:100,000). B. 5:23 p.m. and 5:26 p.m., 1 cc. 0.1% atropine sulphate added (concentration 1:100,000). After the second addition of atropine the rings began to relax. C. 5:31 p.m., adrenaline (concentration 1:1,000,000) did not produce any marked contraction but somewhat inhibited the relaxation. Temperature 10¾° to 11° C.

renaline cause constriction of the coronary arteries, but larger doses produce dilatation (Rothlin 1920a and b), which shows that the same muscles may respond differently to different doses of a drug. The relaxation of a vertically suspended strip of artery under the influence of adrenaline could not be due to strong contraction of the circular layer and consequent elongation of the longitudinal layer, because in the majority of the experiments the longitudinal muscles reacted to adrenaline by contracting. It is more probable that in certain circumstances changes occur in the muscles of surviving arteries of the skate, which reverse the usual reaction of the blood vessel to adrenaline. A conclusion which may be drawn from all the experiments with adrenaline is that in general there is no difference in its action on the arteries of elasmobranchs and of mammals.

Acetylcholine (1:100,000 to 1:200,000) had a different effect on the arteries of the skate than it has on mammalian arteries. Instead of relaxation, acetylcholine caused contraction of the longitudinal as well as of the circular muscles of the skate's arteries. The latent period averaged 85 seconds. A second addition of acetylcholine to the nutritive fluid, even



~~FIGURE 10.~~—A. *Exp. July 21.* Ring from conus arteriosus of *Raja diaphanes* (freshly killed). Effect of gradual distension on a ring, not yet spontaneously contracting. B. *Exp. July 22, 2 p.m.* Ring from conus arteriosus of *Raja diaphanes,* killed 9:30 a.m. Effect of distension on rarely contracting ring. C. *Exp. Aug. 2.* Ring from conus arteriosus of *Raja stabuliforis* (freshly killed). Stimulating effect of distension. The marked rise of the curve on release of the ring after tension was due to jerk of the lever.

if the dose was increased, did not produce the same effect, but adrenaline had still the effect of contracting the artery. Atropine caused relaxation of an artery contracted by the action of acetylcholine. If the dose of atropine was large enough (2:100,000), as in the experiment of August 9, adrenaline produced a very insignificant effect, only briefly retarding the relaxation of the artery. In another experiment (August 15) half a dose of atropine sulphate (1:100,000) did not depress the effect of adrenaline so greatly as in the experiment of August 9, although considerably diminishing it. It seems that in the skate the muscles of the arteries respond in the same way to adrenaline after poisoning with atropine as do the heart muscles. The only difference is that the arterial muscles respond more strongly to adrenaline and are more resistant to poisoning with atropine than the heart muscle. It was demonstrated by Miss Huntsman (1931) that after atropine adrenaline does not influence the beat of the isolated heart of Raja diaphanes and Raja erinacea.

The results which we obtained with acetylcholine on isolated arteries coincide with the effects demonstrated for the whole animal by Miss MacKay (1931), who observed a marked rise of blood pressure after very small doses of the drug and only a partial diminution of its pressor effect by atropine.

Barium chloride invariably produced a contraction of the muscular type of artery in the skate. The experiment of August 17 is interesting because the animal (Raja stabuliforis) was killed 30 hours before the experiment and the arteries preserved in ice-cold saline. In this particular case neither acetylcholine nor adrenaline produced any contraction of the coeliac artery. Nevertheless barium chloride gave the usual effect after a long latent period.

The effect of barium chloride on arteries of the elastic type (ventral aorta, F of figure on page 64) is rather doubtful.

Therefore barium chloride has the same effect in stimulating contraction of the arteries in elasmobranchs as in mammals. Its effect persists in spite of lack of response to drugs which are supposed to act on the nerve-endings or receptive substance.

CONUS ARTERIOSUS

A few experiments were performed with rings cut from the conus arteriosus of Raja diaphanes and stabuliforis. No difference was noted in the behaviour of rings whether cut from the proximal or from the distal part of the conus.

One of the characteristic properties of the conus ring is the way in which it reacts to distension. When set in the saline bath and appropriately loaded, it does not always start to contract immediately. The rhythmic contractions appear gradually. It first they are irregular; later they acquire a regular character and under proper conditions continue for several hours. Distension of a quiescent ring, by the placing of a weight on the longer arm of the lever, activates rhythmic contractions. A certain degree of distension is necessary to produce this effect. After unloading of the weight the contractions thus stimulated may continue for a while at a gradually diminishing rate, and may then stop or pass into rhythmic contractions. If a ring is already contracting, a brief distension greatly increases the rate of the contractions for a certain period. There is an after-effect of the distension in the form of somewhat decreased tonus, which gradually returns to normal.

Therefore one of the properties of the conus arteriosus is its reaction to tension, as expressed in an increased rate of contraction. It would be legitimate to suppose that under normal conditions the rise of the blood pressure inside the conus will also increase the rate of its contractions and thus facilitate the propagation of blood into the arterial system. Another feature of the muscular wall of the conus arteriosus is its reaction to changes of temperature. With rise or fall of the temperature the rate of the automatic contractions increases or decreases respectively.

Some experiments were performed with the object of determining the effect of very small doses of adrenaline (1:1,000,000 to 1:2,000,000 concentration in the bath) on the spontaneous contractions of a ring of conus arteriosus. Only one-half of the preparations showed any noticeable (though small) increase in the rate of the contractions (from 5% to 15%, and in one case 20%). Care was taken to keep the temperature of the saline in the bath constant.

SECTION IV

THE EFFECT OF NERVE STIMULATION

Finally, the reaction of the smooth muscle of the gastro-intestinal tract of the skate to stimulation of autonomic nerves was investigated. The procedure carried out in obtaining nerve muscle preparations has been fully explained above.

GASTRO-PANCREATICO-DUODENAL ARTERY

FUNDUS OR ANTRUM

Stimulation of the artery with single or multiple break shocks, or faradic stimulation with the secondary coil at from 0 to 14 cm. from the primary coil had no effect.

ANTERIOR GASTRIC ARTERY

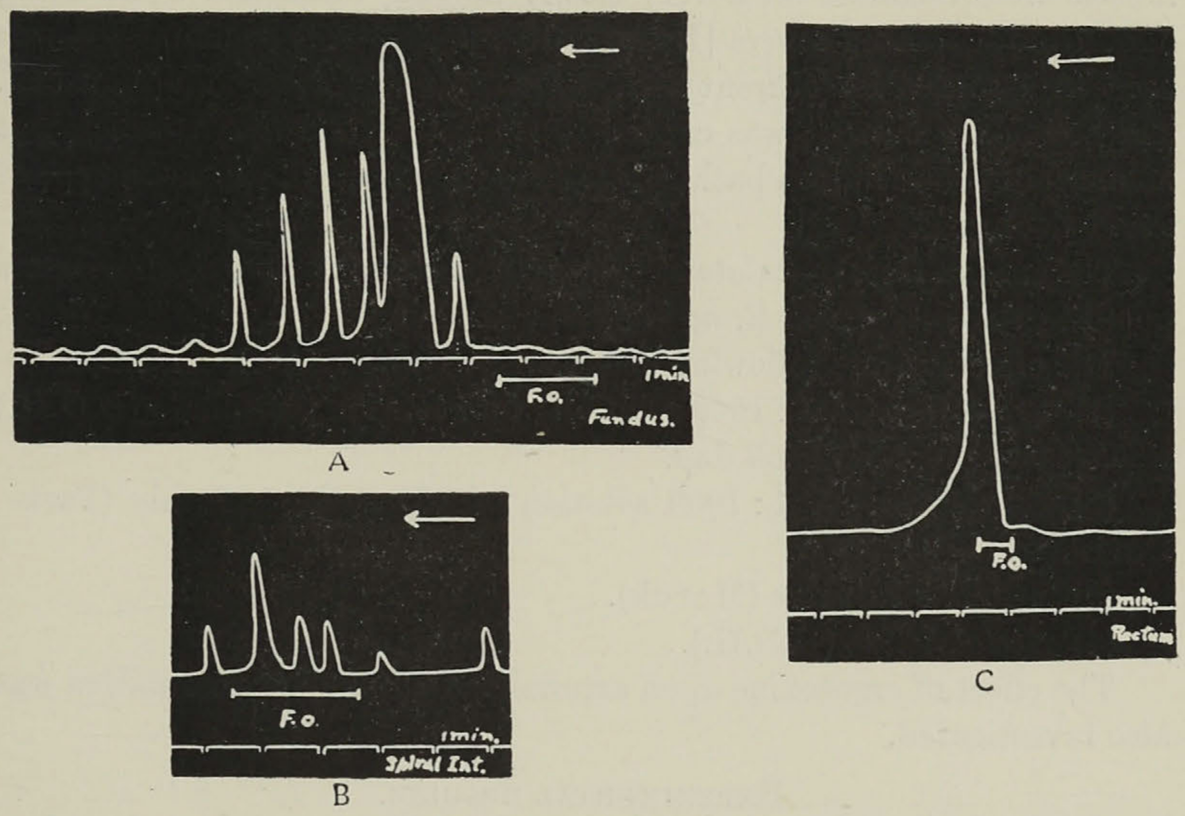
ANTRUM

Single or multiple break stimuli with the secondary coil at 0-14 cm. had no effect. Faradic stimulation lasting 2 min., with the secondary coil at 14 cm., sometimes produced excitation, the amplitude of the contractions being increased. When the secondary coil was at 0 cm. there was definite stimulation, the rate and amplitude being increased, and in fully half the experiments the base-line was raised. The latent period varied greatly, being never less than 1/3 min. and never more than 3 min. A loss of vitality in the muscle seemed to be the cause of the longer latent periods.

FUNDUS

Stimulation of the anterior gastric artery with single or multiple break stimuli with the secondary coil at 14 or 0 cm.

had no effect. Faradic stimulation with the secondary coil at 14 cm. had no effect, but when it was at 0 cm. the contractions showed an increased rate and amplitude, although the base-line was only sometimes raised. Again there was a variable latent period.



~~Fig. 1~~. A, muscle from fundic region of stomach. Shows the effect of stimulation (faradic, secondary coil at 0 cm.) of the anterior gastric artery. B, muscle from the spiral intestine. Shows the effect of stimulation (faradic, secondary coil at 0 cm.) of the superior mesenteric artery. C, muscle from the rectum. Shows the effect of stimulation (faradic, secondary coil at 0 cm.) of the inferior mesenteric artery.

SUPERIOR MESENTERIC ARTERY

SPIRAL INTESTINE

Single and multiple break shocks and faradic stimuli had the same effect upon the spiral intestine as had similar stimuli, applied to the anterior gastric artery, on the fundus.

COLON

The same results were obtained as in the spiral intestine, except that the muscle reacted to the arterial stimulation practically instantaneously.

INFERIOR MESENTERIC ARTERY

RECTUM

Single or multiple break stimuli with the secondary coil at 14 to 0 cm. had no effect. Faradic stimulation with the secondary coil at 14 cm. sometimes stimulated, and with the secondary coil at 7 or 0 cm. always stimulated, usually causing an increased rate and amplitude and a rise of the base-line, though sometimes there was only a single spasmodic contraction. The latent period was very short.

ARTERIAL STIMULATION AFTER ERGOTOXINE

Ergotoxine did not inhibit the response of the antrum and fundus of the stomach, the spiral intestine, colon, and rectum, to stimulation of the artery, and on two occasions it actually seemed to sensitize the fundus and antrum to arterial stimulation, as an enhanced effect was obtained after the addition of the drug. See figure on page 74.

VAGUS-MUSCLE PREPARATION

It was, as previously mentioned, only possible to obtain a vagus-muscle preparation near the cardiac end of the stomach. The nerve was stimulated by a single break shock when the secondary coil was at 0 cm., and by multiple break shocks when the secondary coil was at 0 and 14 cm. Increase in the amplitude of contraction was observed, but only that contraction was affected which coincided with the stimulation of the nerve.

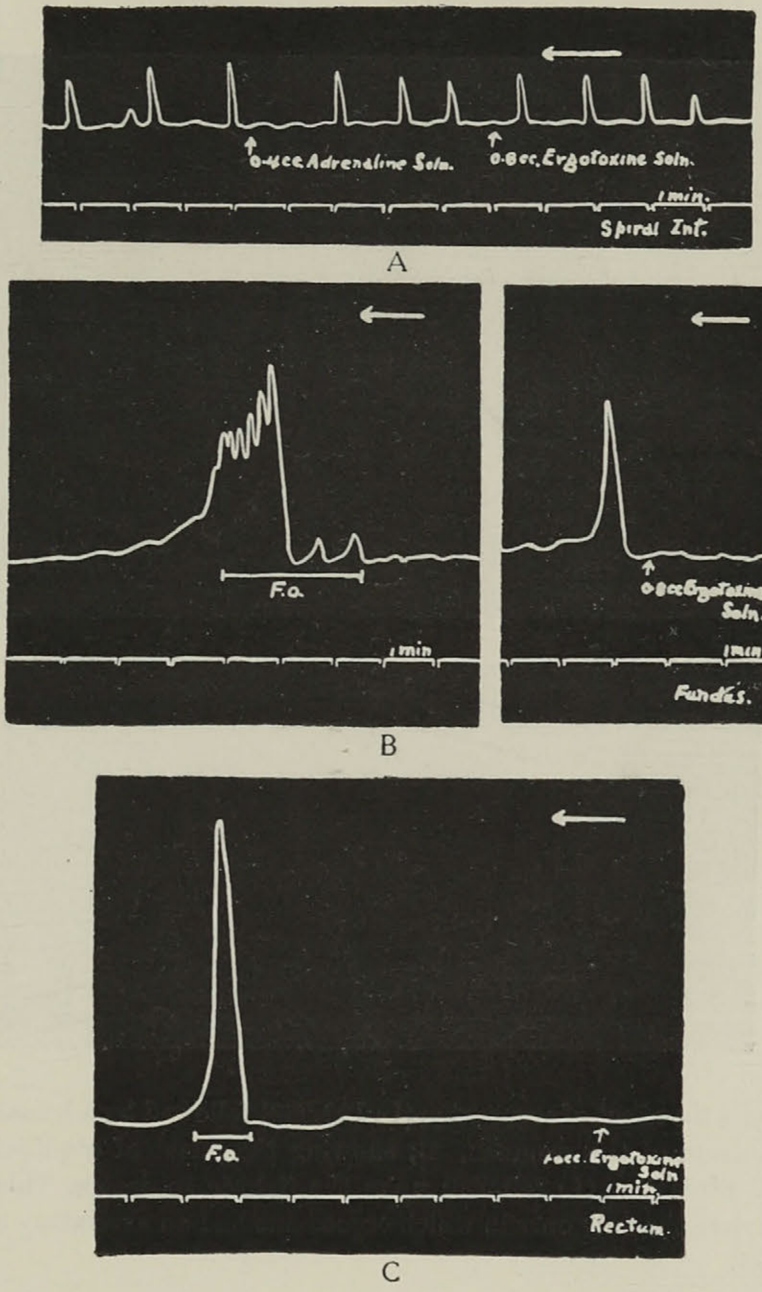
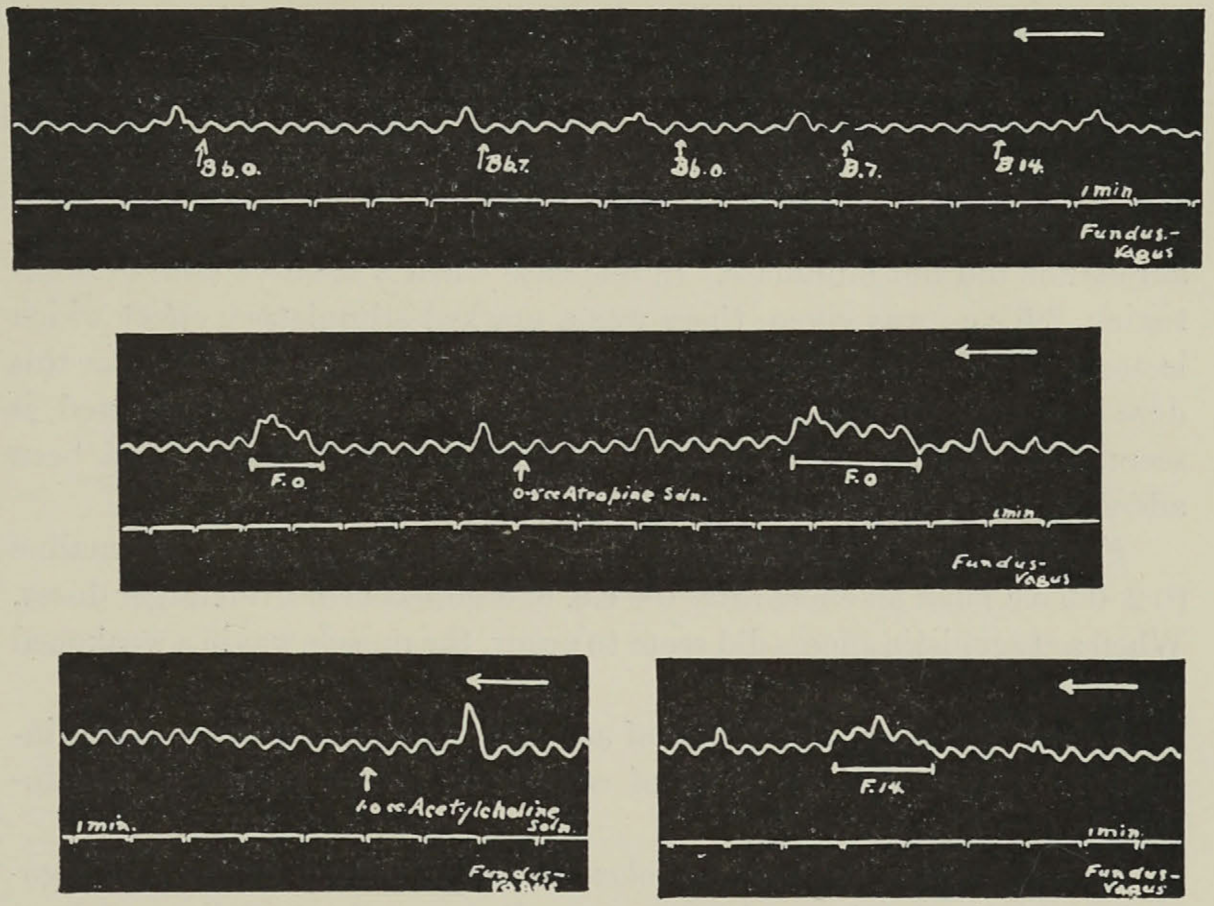


Fig. 3. A, showing the effect of ergotoxine (1 : 150,000) followed by adrenaline (1 : 250,000) upon the spiral intestine. B, showing the effect of ergotoxine (1 : 150,000), followed by arterial stimulation (faradic, secondary coil at 0 cm.) 10 min. later, upon the fundus of the stomach. C, showing the effect of ergotoxine (1 : 100,000) followed by arterial stimulation (faradic, secondary coil at 0 cm.) upon the rectum.

Faradic stimulation, with the secondary coil at 0 and 14 cm., caused a rise in the base-line, increase in the rate and amplitude of the contractions for the duration of the stimulation. The response was practically instantaneous. Atropine in doses of 0.4 - 1.4 c.c. (1:1000 solution) did not inhibit this excitatory effect, though if 1.0 c.c. of acetylcholine

(1:1000 solution) were now given it had no effect. Previous experiments have shown that 0.4 c.c. of atropine (1:1000 solution) will inhibit the effect of 1.0 c.c. of acetylcholine (1:1000 solution).



~~Fig. 4~~. Muscle from the fundus of the stomach. Shows the effect of stimulation of the vagus nerve, and the effect upon this of atropine (1 : 200,000). *B*, signifies single break stimulus; *Bb*, multiple break stimuli; *F*, faradic stimulation. The figures denote the distance in centimetres of the secondary coil from the primary coil.

CONTROL EXPERIMENTS

When a faradic current was passed through the electrodes, which were placed in the solution bathing the muscle preparation without touching it, no alteration in the contraction of the muscle was observed. Stimulation of an artery or nerve after its continuity had been destroyed between the point of stimulation and the muscle had no effect upon the motility of the muscle.

OTHER OBSERVATIONS

As mentioned above, it was the custom to put two strips of muscle in the same bath. This led to an accidental finding. When two strips of muscle taken from the stomach, whether from the fundus or the antrum, were set up together and the artery of one strip was stimulated faradically, there was on several occasions an excitatory response not only in this strip but also in the other strip. This peculiar effect of "transferred stimulation" took place only when both strips of muscle came from the stomach, and it was much more evident after a previous dose of ergotoxine.

In Raja naevus adrenaline in doses of 0.1 c.c. or more had an inhibitory effect upon the antrum of the stomach, decreasing the rate and amplitude of the contractions, while in smaller doses it had a stimulatory effect. This effect had been already found in Raja diaphanes and Raja erinacea (see above). In Raja clavata, however, adrenaline in all doses stimulates the antrum. Nevertheless, in both Raja naevus and Raja clavata faradic stimulation of the anterior gastric artery had an excitatory effect upon the antrum.

CHAPTER IV

DISCUSSION

We shall first discuss the effect of temperature upon the motility of the gastro-intestinal tract. It can be inferred from these experiments that a rise of temperature causes an increased rate of spontaneous rhythm in the gastro-intestinal musculature of the skate. A similar condition has been found by other workers to prevail in a large number of other physiological processes.

It has been shown by the above experiments that the lower temperature limit at which contractions exist varies for different parts of the stomach and intestine. Thus the minimum temperature for the cardia is 7° to 8°C., for the fundus 3.0° to 5.5°C., for the antrum 1.5° to 4.0°C., for the pyloric sphincter 1.5° to 3.0°C., and for the spiral intestine and valve 3.0°C. Hence it can be seen that as the temperature is lowered inactivity will first appear at the cardia and spread down to the pyloric sphincter and spiral intestine and valve. Complete inactivity will exist at about 3.0°C. On one occasion in this series of experiments, contractions were obtained in the antrum of the stomach at 0.9°C. The fact that contractions can still exist at such low temperatures indicates that the physico-chemical processes underlying muscular contraction in the skate must be of an extraordinary nature.

Alvarez (1928) believes that the gastro-intestinal contents in mammals pass in an aboral direction because there is a gradient of rhythmicity, excitability and metabolism in the tract. In various mammals he has found that the fundic region of the greater curvature of the stomach contracts more slowly than the lesser curvature in the same region, that the antral portion of the greater curvature contracts more slowly than the fundic part, that the pyloric sphincter contracts more slowly than the antral region of the greater curvature, and so down the alimentary tract. The experiments of Brown and McSwiney (1926) in dogs and cats along this line give indications of such a gradient in the former but not in the latter. A glance at the findings in our series of experiments will show that such a condition does not exist in the stomach of the skate, as the pyloric sphincter has the greatest rate of contraction. However, if one were to disregard the pyloric sphincter, a very definite gradient can be determined. The experiments on the intestine also show that a gradient exists here. It is suggested that, with the exception of the pyloric sphincter, a definite gradient does exist in the gastro-intestinal tract of the skate, and that the reason why the pyloric sphincter has a high rate of contraction is that its function is that of a barrier and not that of a propelling mechanism. Independently of us, McSwiney and Pyrah (1932) recently came to analogous conclusions. According to them the pyloric sphincter in the dog is normally contracted, and relaxes some 2 to 4 seconds after the onset of antral contraction for a period of some 6 seconds.

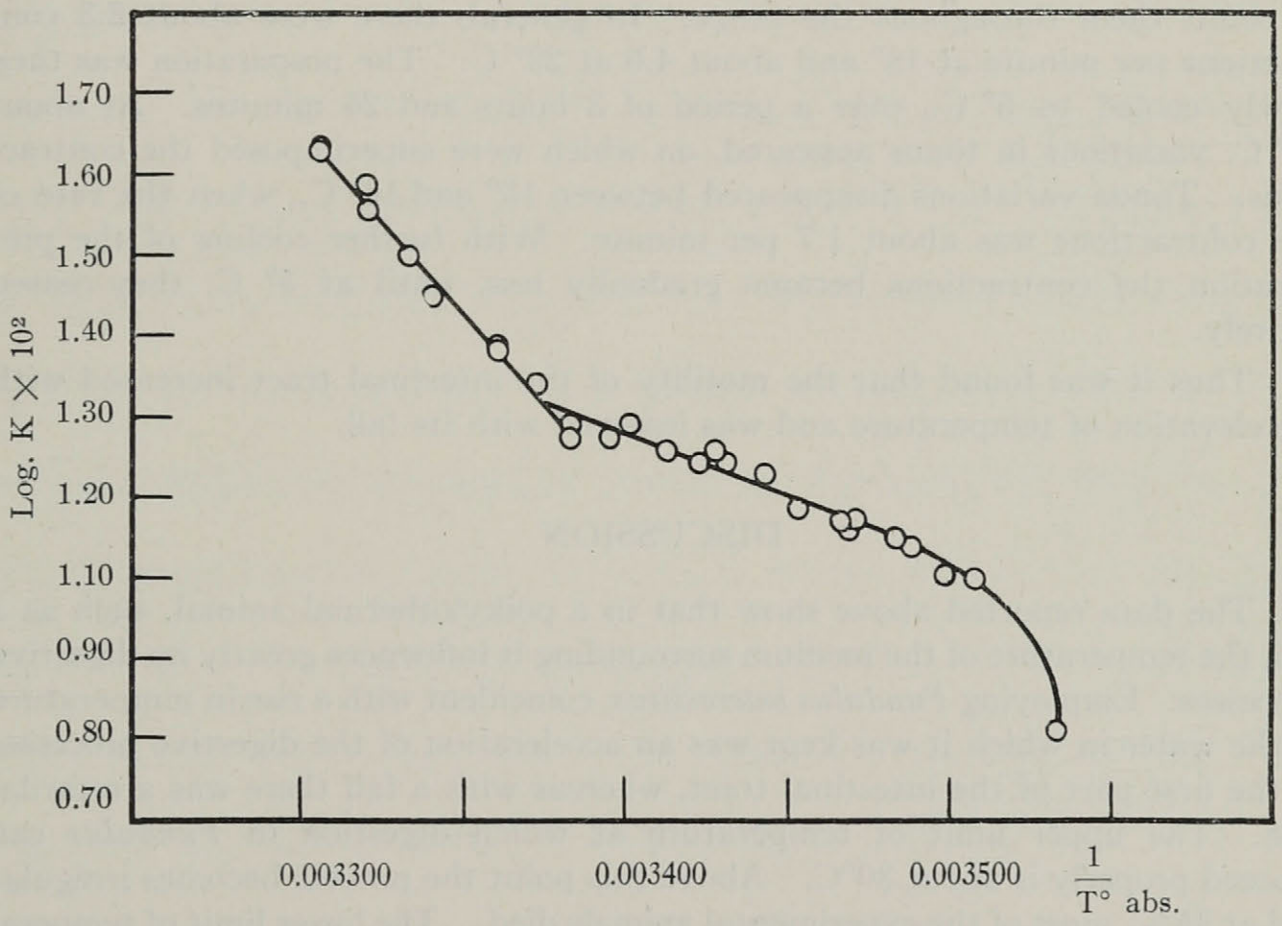
A point of interest is the fact that, whereas the upper end of the spiral valve has a more rapid rate of contraction than the lower end, yet the upper end of the spiral intestine has the same rate of contraction as the lower end. This fact may be explained by the hypothesis that the outer wall, i.e. the spiral intestine, has merely a churning function, whereas the spiral valve is the real motive force that drives the food material towards the rectum and colon.

Another point of interest is the fact that only sharp spasmodic contractions coming at intervals of about half-an-hour could be obtained in the colon and rectum. It is suggested that these two parts merely act as a receptacle for food residues, and contract spasmodically and powerfully every half hour or so. This contraction may be brought on by a mechanical stimulus such as stretching, which would tend to show that, as soon as the colon and rectum reach a certain degree of distension, they react by powerfully contracting and emptying their contents. These hypotheses are further supported by the fact that these parts are very distensible. It will be shown also in a subsequent part of this paper that these parts are remarkably sensitive to parasympathomimetic and sympathomimetic drugs, indicating a further influence on their activity.

A series of experiments carried out on Fundulus heteroclitus show that the whole process of digestion is also greatly influenced by temperature variations. Employing this fish, it was found that coincident with a rise in temperature of the water in which it was kept there was an acceleration of the digestive processes in the first part of the intestinal tract, whereas with a fall there was a retardation. The upper limit of temperature at which digestion in Fundulus can proceed properly is about 30°C. Above this point the process becomes irregular and at 35°C. most of the experimental animals died. The lower limit of temperature for digestion is between 7° and 6°C. At 5.8°C. almost unchanged food was aspirated from the duodenum more than 26¾ hours after feeding. The fish at this temperature were motionless and would not take food of their own accord. Analogous results were obtained by Turbin in the case of Rana esculenta.

In the matter of the interpretation of the temperature relationships of the digestive process observed in this investigation the van't Hoff or R.G.T. rule may be applied. The Q_{10} calculated from the data presented in the table on page 35 gave values varying between 1.44 and 3.06, using two valid points for each. The temperature quotient expressed by Q_{10} however, does not give a true picture of the relationship between temperature and the rate of a given biological process. The majority of investigators apparently prefer the formula of Arrhenius. As Crozier (1924) points out,--"For certain vital activities which may be taken as typical it is found that the underlying or controlling processes may be treated as systems of 'irreversible' first order reactions. To such processes the equation of Arrhenius should be applied."

For the above reasons the results obtained in this investigation have been interpreted according to Arrhenius, and in the graph given herewith log. K has been plotted against $\frac{I}{T^o}$. When this is done it can be seen that two straight lines are obtained. This is explained by the fact that digestion is a composite of reactions, both physiological and physico-chemical. It is known that in such cases the slowest reaction governs the rate of the whole. At low temperatures one reaction having a low-temperature coefficient is dominant. As the temperature rises dominance passes suddenly to another reaction having a higher temperature coefficient. The application of Arrhenius' formula seems to be justified by the results obtained in this investigation. Using valid points, μ, or the coefficient of



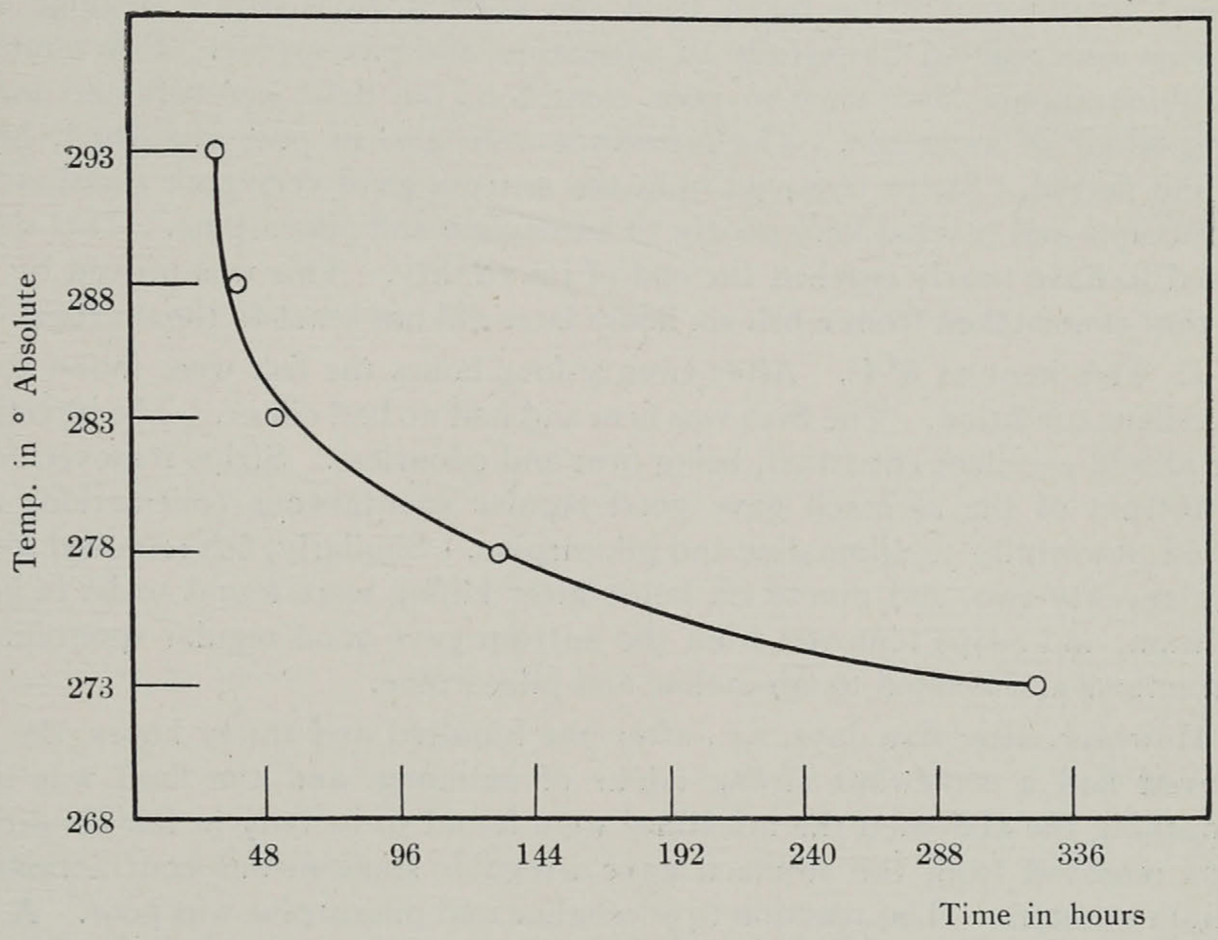
~~FIGURE 1.~~—The influence of temperature upon digestion in *Fundulus heteroclitus*.

activation, has been calculated for both high and low temperature ranges at 21,000 for the former and 7,000 for the latter. At the upper and lower ends of the temperature range the relationships do not hold. Probably there are three reasons for this; (1) inhibition of enzyme action; (2) cessation of secretion of the digestive fluids; (3) paralysis of the motility of the alimentary canal.

Finally, under the head of temperature relationships must be discussed the findings in the above series of experiments upon the survival time of tissue taken from the stomach and kept at different temperatures under different conditions. A glance at the results shows that the survival time at each temperature can be said to be exactly the same under these three vastly different conditions. Thus the tissue will live about 30 hours at 20°C., about 38 hours at 15°C., about 50 hours at 10°C., about 132 hours at 5°C., and about 324 hours at 0°C. (This last figure was only determined for strips of muscle preserved in the excised condition because of the great difficulty of obtaining such a low temperature steadily over a period of two weeks, with the apparatus available; but it would seem that one is justified in supposing that it would also apply to the other series of experiments, since the figures are exactly the same for the three series at 20°C., 15°C., 10°C., amd 5°C.).

In the matter of the interpretation of the above results the van't Hoff or R.G.T. rule may be applied. Q_{10} calculated from these has the following values: 1.66, taking the points at 293° and 283° absolute; 3.47, taking the points at 288° and 278°

absolute; and 6.48, taking the points at 283° and 273° absolute. It can be seen from these figures that the value of Q_{10} increases enormously as the temperature goes down. Similar results have been obtained in other like processes (A.G. Huntsman, personal communication). An adequate reason for such a tremendous increase in the value of Q_{10} is very difficult to find.



~~FIGURE 1~~.—Survival time of gastric musculature of *Raja* in relation to temperature.

Another feature appeared quite prominently in all these experiments. The tissue of the fish would remain firm and odourless until just about twenty-four hours before contractions could no longer be obtained. Then, regardless of the temperature at which the fish were being kept, the flesh would suddenly and rapidly show signs of disintegration, that is, it would become soft and give off a strong odour which at first was not unpleasant but soon became so. These rapid changes which culminate in a complete loss of vitality would seem to be explained by the findings of Reed (1925); if the bacterial growth in fish muscle was inhibited by toluol, there was a very slow, followed by a rapid hydrolysis of the proteins, as measured by an increase in non-coagulable and ammonia nitrogen. When bacteria were allowed to develop in muscle, although the increase in non-coagulable and ammonia nitrogen was approximately the same as in the autolytic reaction for the first sixteen to eighteen hours, from this time on the combined action of the autolyzing enzymes and the bacterial resulted in a very much more rapid protein transformation.

Of the two processes mainly concerned in the disintegration of tissue, autolytic and bacterial, the former would seem to be the more important, since if the latter were the more important, it would be difficult to conceive how tissue kept under such vastly different conditions would, nevertheless, have the same lease of life when kept at the same temperature. This conclusion supports the findings of Gibbons and Reed (1930) that autolysis is a necessary pre-existing condition for bacterial decomposition.

The results of the experiments dealing with the effect of adrenaline, pilocarpine, acetylcholine and ergotoxine have been summed up in the following tables. (See page 85)

Effects of drugs on gastric musculature in Raja

Region	Adrenaline 1:250,000	Acetylcholine 1:100,000	Pilocarpine 1:100,000
Cardia	Stimulation	Stimulation	Stimulation
Fundus	Stimulation	Stimulation	Stimulation
Antrum	Inhibition (minute doses stimulate)	Stimulation	Stimulation
Pyloric sphincter	Stimulation	Stimulation	Stimulation

Effects of drugs on intestinal musculature in Raja

Region	Adrenaline 1:250,000	Acetylcholine 1:100,000	Pilocarpine 1:250,000
Spiral intestine:			
Upper end.....	stimulates	stimulates	stimulates
Lower end.....	"	"	"
Spiral valve:			
Upper end.....	"	"	doubtful effect
Lower end.....	"	"	" "
Colon............	"	"	stimulates
Rectum...........	"	"	"

The effect of drugs on the stomach and intestine.

Region	Effect of Ergotoxine	Effect of Adrenaline after Ergotoxine	Effect of Acetylcholine or Pilocarpine after atropine.
I. Stomach (a) Fundus (b) Antrum (c) Pyloric sphincter	 Stimulation Stimulation Stimulation	 Stimulation Stimulation Stimulation	 nil nil nil
II. Spiral intestine	nil	nil	nil
III. Colon	nil	nil	nil
IV. Rectum	nil	nil	nil

We first shall discuss these effects upon the gastric musculature of the skate. It is interesting to compare these with the results obtained in mammals. Brown and McSwiney (1926) have dealt very comprehensively with these drug effects in the dog and the cat. They found that adrenaline has an excitatory effect upon the cardia, causing a sharp rise in the base-line followed by a slow return to normal. In the fundus there is a complete inhibition of the contractions, with a slight relaxation in tone, and if the drug is given after pilocarpine inhibition also occurs. In the antrum they found that adrenaline also causes inhibition, the contractions being reduced in height, but there being no change in the base-line. Relaxation only occurs after pilocarpine, which raises the base-line. In two preparations from the dog but never from the cat they found indications of stimulation in the antral region. The effect of adrenaline upon the pyloric sphincter is almost identical with that in the antrum.

The peculiar effect of adrenaline upon the antrum of the skate is difficult to explain. Gruber (1922) and others found that large doses of adrenaline inhibited the gastro-intestinal muscle of frogs, while minute doses stimulated it. Kaltreider (1930) found in the case of the pyloric sphincter of terrapin that in all cases but two adrenaline had an inhibitory effect on the rhythmic contractions initiated by pilocarpine, and in these two had no effect at all.

The significance of the data concerning the different reaction of various parts of the gastric musculature to adrenaline is evident. We are dealing here with the problem of the character of the response--excitatory or inhibitory--of the given tissue to the nervous or humoral stimulus. The interpretation of these facts is, however, difficult. Two tentative explanations may be suggested at the present time, but further investigation will be necessary to show which of these is correct.

The effect of adrenaline upon the antral region of the skate's stomach may be explained by the hypersensitivity of this region to the drug. The fact that tissue from this region that has been kept for three or four days at 0°C., is stimulated by all effective doses of adrenaline would seem to support this view.

This effect might also be explained by the hypothesis that two types of sympathetic innervation exist in the antrum near the pyloric canal, one type inhibitory in function, the other excitatory. With large doses of adrenaline the inhibitory effect is dominant, while with minute doses the excitatory effect predominates. In the skate the sympathetic nervous system is in a primitive state of development as compared with that of the higher animals. In the dog the inhibitory effect predominates throughout the stomach except in the cardia and pylorus. In other words, from the evolutionary standpoint the sympathetic nerves of the excitatory type appear first, and the sympathetic nerves of the inhibitory type develop later, appearing first in the antrum and finally spreading throughout the stomach and becoming dominant.

That adrenaline stimulates the muscular tissue taken from the antral region, after it has been kept for a few days, may be explained by the fact that the inhibitory nerves degenerate first.

Brown and McSwiney (1926) found that atropine had an inhibitory effect upon the normal rhythm throughout the whole stomach of the dog and cat, its effect being similar in many ways to that of adrenaline. If atropine is given before pilocarpine, the latter will have no effect, if given after, it will inhibit the action of pilocarpine. As above stated, in the skate atropine by itself has no effect, and if given before pilocarpine the latter has no effect. If given after pilocarpine or acetylcholine there is a slight inhibition followed by a restoration of the normal contractions. These results are interesting in the light of the theory of Magnus (1925) who claims that atropine acts upon the acetylcholine, neutralizing it in some chemical way, and not upon some part of the parasympathetic nerves themselves, as some investigators think.

Pilocarpine has a stimulating effect upon all parts of the stomach of the dog and cat. Brown and McSwiney (1926) found that under the influence of pilocarpine there is a considerable rise in the base-line in the cardia and fundic regions, with less rise in the antrum, and seldom any rise in the pyloric sphincter. These tonic changes are the only difference between the response of the stomach of the dog and cat to pilocarpine and that of the skate.

It is believed now that the effect of acetylcholine stimulates parasympathetic stimulation more exactly than that of pilocarpine. As far as the action of different drugs justifies the conclusion concerning the presence of a certain type of nervous system in an organ, the very definite and generalized effect of acetylcholine throughout the skate's stomach lends support to the idea that this organ possesses a well developed parasympathetic nerve supply.

It is difficult to reconcile the results of this series of experiments with those of Dreyer (1928-29), where he found that strips taken from the ascending limb of the skate's stomach were not affected by pilocarpine and atropine, but were stimulated by adrenaline. Perhaps the reason lies in the fact that he used a different nutrient solution, namely Mines', modified by adding

0.5 g. $NaHCO_3$ per litre. The fact that he found adrenaline to be always excitatory might be accounted for in that none of his strips came from the central region of the stomach.

A few experiments were carried out on strips of the muscular layer, cut so as to record mainly the contractions of the longitudinal layer of the stomach. The contractions of such strips were as a rule faster and more irregular in rate and shape than those obtained from strips cut so as to show mainly the contractions of the circular layer. Kuroda (1924) found no rhythmic contractions of any magnitude in such longitudinal strips cut from the fundus of the dog's stomach. He also found that acetylcholine caused contractions, but was without effect after atropine, and that adrenaline usually caused contractions.

From the results of the experiments dealing with drug action on the intestine, it can be seen, first, that adrenaline has a well-marked excitatory effect throughout, which is in confirmation of the findings of Dreyer (1928-29). This is further evidence of a well-developed sympathetic nerve supply to the intestine of the skate, a fact which was first shown by Bottazzi (1902). Babkin and MacKay-Sawyer (personal communication) also found that by stimulation of the superior mesenteric artery they were able to stimulate the contractions in the spiral intestine. Lutz (1931) found that adrenaline caused a decrease in the tone and an inhibition of the motility of the posterior end of the intestine and rectum. The results of our series of experiments do not agree with this but do agree with his further finding that stimulation of the posterior splanchnic nerves caused an increased motility of these parts. Just how to explain these two different results is very difficult. Roth (personal communication) has been able to confirm the fact that adrenaline has an inhibitory effect on the rectum and colon. He used a temperature of 17°C. and a different nutrient solution. Perhaps the explanation lies in these two facts.

On two occasions adrenaline caused inhibition instead of stimulation in the spiral intestine. This double effect of adrenaline is not nearly so sharply demarkated as it is in the antral region of the stomach, where adrenaline in concentrations greater than 1:1,000,000 inhibits, but stimulates in concentrations less than this. This double effect may be due to a hypersensitivity of these parts to adrenaline under certain conditions. This latter theory would seem to fit in better with the fact that only sometimes is an inhibitory effect obtained in the spiral intestine (vide supra).

Gunn and Underhill (1915) found that adrenaline had a stimulating effect upon the muscularis mucosae of the gastro-intestinal tract of the cat. A directly comparable result was found in this series of experiments on the effect of adrenaline upon the spiral valve, since this part is supplied with muscle derived from the muscularis mucosae of the spiral intestine.

The marked excitatory effect of acetylcholine on all parts of the intestine tends to show that there is a well-developed parasympathetic motor nerve supply to these parts. Bottazzi (1902) first showed that stimulation of the vagus nerve had a strong excitatory effect upon the spiral intestine, but no effect upon the colon and rectum. However, if he stimulated the 45th to 48th segments of the spinal cord, he could obtain marked stimulation of these latter two parts, an effect which took place, he thought, through a sympathetic outflow. The results of our investigation on excised strips of colon and rectum showed that acetylcholine has a marked excitatory effect, which would lead one to believe in the existence of a parasympathetic nerve supply to these parts. Bottazzi's experiments show that this supply does not come from the vagus. One may speculate on the existence of a parasympathetic outflow from the lower end of the spinal cord comparable to the sacral outflow in mammals. Perhaps Bottazzi stimulated this outflow when he stimulated the 45th to 48th segments of the spinal cord, rather than a sympathetic outflow as he thought.

Gaskell (1920) expressed an analogous opinion as regards the nerve cells lying close to the surface of the cloaca of <u>Ammocoetes</u>. Dohrn (1888) called these cells sympathetic, but Gaskell points out that Dohrn used the term "sympathetic" vaguely and that the cells in question correspond in position to the cells of the pelvic plexus in higher vertebrates and therefore indicate that group.

It is very desirable that a special morphological examination of the innervation of the hind part of the intestine should be made.

The results obtained with pilocarpine in this series of experiments, in which it was found that the drug had an excitatory effect on the spiral intestine, rectum and colon, but an indefinite effect upon the spiral valve, do not agree with those of Dreyer (1928-29), who found that the drug had absolutely no effect on the spiral intestine and valve. The very indefinite effect of pilocarpine on strips taken from the spiral valve may be explained by the fact that strips taken from this part are covered on both sides with mucosa, which may make it difficult for the drug to penetrate.

Atropine by itself was found, as in the stomach, to have no effect, but if it were introduced after pilocarpine or acetylcholine, it restored the contractions to normal.

Another point deserves mention. Pilocarpine and acetylcholine followed by adrenaline or vice versa has an additive effect on the gastro-intestinal contractions. This fact indicates that in the gut of elasmobranchs the parasympathomimetic and sympathomimetic drugs have not an antagonistic but a synergic effect. An interesting case of the synergic effect of epinephrine and parasympathetic drugs on isolated strips of guinea-pig's small intestine was demonstrated also by Bernheim and Blocksom (1932).

The effect of ergotoxine in the skate was interesting in that it differs markedly from that in the mammal, where it has first a stimulatory and then a paralysing effect upon the sympathetic nerve endings. In this investigation an excitatory response to ergotoxine was usually obtained in the fundus and antrum of the stomach. In the spiral intestine, colon and rectum the drug usually had no apparent effect, and in the pyloric sphincter gave indications of stimulation.

The effect of adrenaline following ergotoxine is also very interesting. In the spiral intestine, colon and rectum these drugs are antagonistic in their action, 0.6 c.c. of ergotoxine practically always inhibiting 0.2 to 0.3 c.c. of adrenaline. But in the stomach no such antagonistic action occurred, even when massive doses of ergotoxine were given, except where the vitality of the muscle was low, in which case the lessened response to adrenaline was probably due to lack of vitality and not to the action of the ergotoxine.

These differences between the mammal and the elasmobranch, moreover, the irregularities in response of the different parts of the gastro-intestinal tract of the fish itself, are difficult to explain.

We now come to the experiments dealing with the function and structure of the large arteries of the skate. The findings from these investigations demonstrate the fact that the large arteries of Raja diaphanes and stabuliforis are of two main types, elastic and muscular. Both these show a characteristic reaction to distension. However, the muscular type react by contraction to the presence of drugs like adrenaline, acetylcholine, and barium chloride, whereas the elastic type do not. It also has been shown by these experiments that both the circular and longitudinal layers of muscle enter into the response of the muscular type of artery to these drugs.

When considering the response of blood vessels to adrenaline, two facts should be kept in mind; first, the presence of a muscular layer; second, the innervation. In our series of experiments it was observed that adrenaline provoked a greater response in arteries of the muscular type in Raja stabuliforis than in diaphanes, where the muscular layer is thinner; and there was no response from the ventral aorta to adrenaline in either variety of skate, the aorta containing only a very thin muscular layer. It is interesting to note that it has been demonstrated by Cow (1911) that strips of the mammalian pulmonary artery taken near the periphery reacted to adrenaline weaker than those taken nearer the lumen.

Great controversy has and is still raging concerning the point of action of adrenaline. It is most commonly believed, now, that this drug acts peripherally on structures having sympathetic innervation. The work of Schmitt (1922) on the placental blood vessels is usually quoted as proving that the reaction of the blood vessels to adrenaline depends on the presence of nerves to these vessels. Schmitt showed that the placental blood vessels react in the same way as other blood vessels to electrical or thermal stimulation and to barium chloride and histamine. However, adrenaline even in high concentrations produced no effect, or else a very insignificant one. Nevertheless,, it is not as yet clear on what part of the neuro-muscular apparatus of a blood vessel that adrenaline acts.

This investigation of ours has shown that in elasmobranchs the arteries of the muscular type (coeliac, superior mesenteric and anterior intestinal arteries) are surrounded by a network of nerve fibres. Since the great sympathetic trunks follow these arteries, it is very probable that their nervous network is derived from the sympathetic nervous system. Therefore the positive motor effect of adrenaline in elasmobranch fishes may be due to the presence of sympathetic innervation of the arteries.

The fact that in the skate many of the arteries arising from the system of the dorsal aorta react to adrenaline by constriction explaines somewhat, at least, the rise of blood pressure in this fish after intravenous injection of adrenaline (MacKay 1931). Further investigation is necessary to show whether or not arteriolar constriction takes part in this phenomenon.

We have mentioned above that in certain conditions, when the preparations were presumably weakened, a dilator effect was obtained. A similar condition has been observed by many workers in mammalian arteries which usually react to this drug by constriction (Krawkow, 1922, found this occurred in inflammation; Ogawa, 1912, observed it after prolonged perfusion of mammalian organs with adrenaline; and, Schilf and Feldberg, 1925, found it occurred after repeated use of adrenaline on frog preparations.)

After careful consideration of all the above facts, one may safely say that the reaction of the muscular arteries of elasmobranch fishes to adrenaline is analogous to the reaction of mammalian arteries.

The effect of acetylcholine on the blood vessels of the skate is different from that obtained in mammals. Dale (1914), Dale and Richards (1918) and Hunt (1918) demonstrated that mammalian arteries dilate under the influence of acetylcholine. The effect of this substance on the blood vessels is purely peripheral and is independent of any nervous connection. Dale and Richards in their perfusion experiments used the superior mesenteric artery of the cat with its branches and fine ramifications passing on to the small intestine. The addition of 0.001 mg. to 0.002 mg. of acetylcholine to the perfused blood greatly increased its outflow from the fine arteries. Recently Gaddum and Holtz (1933) showed on cat and dog lungs perfused with blood that "small doses of acetylcholine produced vaso-dilation in cats and sometimes also in dogs." Larger doses produced vaso-constriction. In cats this occurred both on the inflow and outflow, but in dogs the action was mostly on the inflow.

Small doses of acetylcholine injected into the frog produce after a brief vaso-constriction a marked dilatation of the arterioles accompanied in most cases by a slight dilatation of the capillaries, while the venules remain unaffected (Doi 1920). Acetylcholine in a concentration of 0.005 per cent. applied to the frog's web produces a powerful dilator effect on the arteries from the largest visible down to the arterioles (Krogh 1921). Quite different results were obtained in frogs whose blood vessels were perfused with Ringer solution. Acetylcholine in dilutions of 1:10,000 to 1:100,000 (Amsler and Pick 1920) and even in a dilution of 1:1 billion (Teschendorf 1921) produced a strong vaso-constriction. Atropine abolished this effect of acetylcholine (Kolm and Pick 1920). No analysis was made of the discrepancy between the mode of action of acetylcholine in a whole frog and in a preparation with blood vessels perfused with Ringer solution. However, it seems that the presence of blood in the circulatory system is of great importance in regard to the type of vascular reaction to acetylcholine.

Both the circular and longitudinal layers of the isolated elasmobranch arteries reacted to acetylcholine by contraction rather than by dilatation. This fact may explain, in part at least, the pressor effect of intravenous injections of acetylcholine in the skate observed by MacKay (1931). In this latter case the possibility that acetylcholine stimulates the ganglionic cells of the sympathetic nervous system is not excluded by our findings.

It is not easy to interpret the stimulating influence of acetylcholine on the blood vessels of elasmobranchs. Since the arteries in the skate are presumably innervated by the sympathetic system alone, it would be logical to suppose that acetylcholine acts, not on the nerve endings or on some kind of myo-neural junction in them, but on the muscle cells themselves. In relation to this it is interesting to note that some investigations indicate that the muscles contain two kinds of muscular fibres--"tonic" acetylcholine-positive and "tetanic" acetylcholine- negative (Sommerkamp 1928). The muscles of invertebrates react the better to acetylcholine, the greater the tendency of the individual muscle to "tonic" contraction (Riesser 1931). According to Rückert (1930), reaction to acetylcholine has been noted in the phylogenetically younger muscles, whereas the phylogenetically older muscles are not stimulated by this drug.

These explanations are very interesting when one considers the results of our series of experiments. One hesitates to definitely apply them, all that can be said is that they are highly suggestive. However, this much is clear. As shown by our above experiments on the gastro-intestinal tract, and upon the blood vessels in elasmobranch fishes these tissues give a positive motor reaction to adrenaline and acetylcholine. These same two drugs, when applied to similar structures in mammals give opposite effects.

Finally, let us consider the effect of autonomic nerve stimulation upon the motility of the gastro-intestinal tract. This has been summed up in the following table on page 100.

In all cases where the artery was stimulated faradically an excitatory response in the muscle was obtained, except when the gastro-pancreatico-duodenal artery was stimulated, whereas single break or multiple break stimuli (i.e. ten or twelve break stimuli as fast as the hand could make them) did not give any response. A possible explanation is that what McSwiney and Robson (1929b) call "peripheral summation of stimuli," is necessary before an excitatory effect is evidenced by the methods used in this series of experiments. These writers believe that this phenomenon probably represents a summation of nerve impulses in Auerbach's plexus.

The presence of a periarterial plexus of nerves in the skate, probably sympathetic in origin, has been demonstrated (vide supra). It is probable that these fibres are derived from the coeliac plexus, and as well as carrying sympathetic fibres to the artery itself, carry fibres in some cases to smooth muscle, as occurs in the mammal. Evidence of the existence of such a system has been found by Babkin and MacKay-Sawyer (personal communication) who on stimulation of the coeliac plexus

Region	Effect of Artery Stimulation		Effect of Vagal Stimulation	
	Break	Faradic	Break	Faradic
I. Stomach				
(a) Fundus	nil	Stimulation	Stimulation	Stimulation
(b) Antrum	nil	Stimulation	*	*
(c) Pyloric sphincter	*	*	*	*
II. Spiral intestine	nil	Stimulation	*	*
III. Colon	nil	Stimulation	*	*
IV. Rectum	nil	Stimulation	*	*

Region	Effect of Art. Stim. after Ergotoxine	Effect of Atropine on Vagal Stimulation
I. Stomach		
(a) Fundus	Stimulation	nil
(b) Antrum	Stimulation	*
(c) Pyloric sphincter	Stimulation	*
II. Spiral intestine	Stimulation	*
III. Colon	Stimulation	*
IV. Rectum	Stimulation	*

*These experiments were not carried out as it was impossible to obtain a nerve muscle preparation.

obtained contraction of the duodenum. The present series of experiments indicates that the stomach is supplied by sympathetic fibres carried along the anterior gastric artery and that the gastro-pancreatico-duodenal artery does not form part of the system, probably because it is a distant branch of the coeliac axis; that the spiral intestine and colon are supplied by fibres carried along the superior mesenteric artery, and the rectum by fibres along the inferior mesenteric artery.

The results of Babkin, Friedman and MacKay-Sawyer (in press) give ample verification of the results of these experiments on nerve stimulation. They also found, by entirely different methods, that both vagal and sympathetic nerve stimulation cause increased contractions of the skate's stomach. Moreover, they found that the sympathetic and parasympathetic systems were synergetic in their action on the stomach and not antagonistic as they are in the mammal.. Several others have obtained similar results (Bottazzi, 1902; Babkin and MacKay-Sawyer, 1931; Lutz, 1931). Similarly our results obtained with parasympathomimetic and sympathomimetic drugs gave indications of such a state of affairs. Müller and Liljestrand (1918) found that vagal stimulation subsequent to sympathetic stimulation sometimes inhibited the excitatory effect of the latter. The other workers do not confirm this, and the bulk of the evidence shows fairly conclusively that the two parts of the autonomic nervous system are synergetic in their action. These findings are not easy to explain. Müller (1920) believes that the "gastric plexus" in <u>Squalus acanthias</u> (a Selachian) is a true nerve net in which neurofibrils pass from one nerve cell to another, similar to the nerve net in the sea anemone. This nerve net is connected with nerve ends which Müller believes are of vagal origin. He does not make clear the relation of sympathetic fibres to the "gastric plexus." But if the sympathetic nerves are in relation with it, it is easy to understand how both vagal and sympathetic stimulation have similar effects. Dale (1933) has suggested that the autonomic nervous system in mammals be not divided into sympathetic and parasympathetic systems, but into "cholinergic" and "adrenergic" depending on whether stimulation of an autonomic nerve causes effects similar to that of acetylcholine or adrenaline. Looked at from this point of view, the conditions found in the skate at once become less difficult to explain. It would seem that in the skate the autonomic nervous system is so primitive that division into two parts is not possible, at least on functional grounds. We cannot distinguish "cholinergic" and "adrenergic" fibres. The neuromuscular system reacts practically in an identical manner to acetylcholine and adrenaline. Independently of each other Young (1933), and Babkin, Friedman and MacKay-Sawyer also came to the conclusion that there was little evidence in the fish for division of the autonomic nervous system into antagonistic parts.

A further difference in the effect of ergotoxine on mammalian and skate muscle (besides that mentioned above under drug effects) was observed in that nowhere in the gastro-intestinal tract was an antagonistic action on arterial stimulation obtained, even when very large doses, such as 2.5 c.c., were placed in the 100 c.c. of nutrient solution bathing the tissue. In fact in one or two cases ergotoxine seemed to make the antrum and fundus of the stomach more sensitive to arterial stimulation.

The effect of vagal stimulation of the cardiac end of the stomach has one or two points of interest. Here single break as well as faradic, excitation is effective, as McSwiney and Robson found in the mammal. Atropine even in large doses does not inhibit the effect of vagal stimulation, though these doses will inhibit much larger doses of acetylcholine. This is very interesting in the light of the theory of Magnus (1925) who believes that atropine acts upon acetylcholine, neutralizing it in some chemical way, and not upon some part of the parasympathetic nerves themselves, as some investigators think.

This is interesting in the light of evidence that has come up in recent years. Loewi (1921) showed that when the vagus is stimulated a substance like or identical with acetylcholine is released. He believed that it was this substance, acting directly on the muscle cells, which produced the effect of parasympathetic stimulation. In 1925 Magnus put forward his theory that atropine inhibited the action of parasympathetic stimulation not by paralysing the nerve endings, as was then thought, but by reacting chemically with the acetylcholine released it produced an inert substance. This theory has received confirmation in the results from ~~some later~~ experiments carried out by Loewi and Navratil (1924) when they found atropine did not prevent the release of acetylcholine but merely prevented it acting. Any evaluation of our results is necessarily very speculative until more work is done. But it would seem that owing to some peculiarity of the skate muscle cell, such as peculiarities in the permeability of the cell membrane, the acetylcholine dialyses into the cell before it is inactivated by the atropine. One thing is certain, our results are not due to some peculiarity of our technique, because exactly similar results, using an entirely different technique, were obtained by Babkin, Friedman, MacKay-Sawyer, (in press).

Control experiments were performed which showed that the excitation must have been carried by nerves and not by conduction through other tissue or through the solution. These experiments make the phenomenon of so-called "transferred stimulation," spoken of above, of greater significance. The fact that it occurred only when two strips of stomach were in the bath is further evidence that it was not due to the passage of an induced current through the solution. It is possible that some "excitor substance" was liberated from the sympathetic nerve endings of the stimulated strip and this reached a sufficient concentration in the solution to affect the other strip of muscle. If this hypothesis is true, we have here an interesting example of the humoral factor in nervous stimulation.

CHAPTER V

SUMMARY

I. TEMPERATURE RELATIONSHIPS

1. Effect of temperature variations upon the motility of the gastro-intestinal tract of elasmobranch fishes.

A rise of temperature causes an increase in the rate of the spontaneous contractions of all parts of the gastro-intestinal tract.

The upper temperature limit at which contractions exist is about the same for all parts of the stomach and intestine, viz., about 24.5°C. The lower limit varies for different parts, being for the cardia 7° to 8°C., for the fundus 3.0° to 5.5°C., for the antrum 1.5° to 4.0°C., for the pyloric sphincter 1.5° to 3.0°C., and for the intestine about 3.0°C.

The rate of the contractions at any one temperature is different in one part of the stomach from that of another. The parts named in order of descending rate are: pyloric sphincter, antral and fundic regions of the lesser curvature, fundic portion of the greater curvature and antral portion of the greater curvature.

The upper end of the spiral intestine has the same rate of contraction as the lower end, whereas the upper end of the spiral valve has a more rapid rate than the lower end. The colon and rectum only give spontaneous spasmodic contractions at intervals of about half-an-hour.

If one disregard the rapid rate of contraction of the pyloric sphincter, Alvarez's theory of gradients is upheld by the results of these experiments.

2. Effect of temperature variation upon the rate of digestion (in Fundulus heteroclitus).

The rate of the digestive processes is increased by elevation of the temperature of the water in which it is kept, and decreased by its lowering.

The upper temperature at which digestion stops is about 30°C. The lower limit is between 7° and 6°C.

Apart from the general influence of the temperature of the environment upon all vital processes, the influences of temperature on enzyme action and upon the motility of the alimentary canal are causes of these variations in the digestive process of Fundulus.

The data obtained in this investigation can be interpreted according to the formula of Arrhenius.

3. Effect of temperature upon the survival time of gastric muscle of the skate.

Three series of experiments were carried out:

(a) One set of strips of gastric musculature was kept in nutrient saline at 0°C., and others at 5°C., 10°C., 15°C. and 20°C. respectively.

(b) Four sets of starved fish were taken and killed. One set was kept at 5°C., and others at 10°C., 15°C. and 20°C. respectively.

(c) Four sets of freshly caught fish were killed and kept at 5°C., 10°C., 15°C. and 20°C. respectively.

The survival time of strips taken from the antrum of the stomach in these three series of experiments was the same for each temperature. Thus the survival time at 0°C. was 324 hours, at 5°C. 132 hours, at 10°C. 50 hours, at 15°C. 38 hours, at 30°C. 30 hours.

These results have been interpreted according to the R.G.T. rule. Thus Q_{10} between 293° and 283° absolute equals 1.66, between 288° and 278° absolute equals 3.47, and between 283° and 273° absolute equals 6.48.

It is concluded that the autolytic process is more important than the bacterial process in the causation of tissue death.

There seems to be a very definite critical point in the processes leading to tissue death, after which they become much more rapid.

II. THE EFFECT OF CERTAIN DRUGS

Adrenaline stimulates all parts of the stomach except the antral region near the pyloric canal, where it stimulates in concentrations of 1:2,000,000, but inhibits in concentrations of, or greater than, 1:1,000,000. Adrenaline also stimulates all parts of the intestine, though on two occasions inhibition occurred in the spiral intestine.

This double effect of adrenaline might best be explained by a hypersensitivity of these regions to the drug.

Pilocarpine stimulates all parts of the stomach and all parts of the intestine, except the spiral valve where the effect is doubtful.

Acetylcholine stimulates all parts of the gastro-intestinal tract.

Atropine has no effect itself on any part of the stomach or intestine, but counteracts the effect of pilocarpine and acetylcholine.

Ergotoxine phosphate usually stimulates the antrum and fundus of the stomach, and usually has no effect upon the spiral intestine, colon and rectum, while it has a doubtful stimulatory effect upon the pyloric sphincter.

Small doses of adrenaline, such as 0.2 c.c. (concentration in bath, 1:500,000) stimulate the fundus, antrum and pyloric sphincter even after massive doses of ergotoxine, such as 2.5 c.c. (concentration 1:25,000), while in the spiral intestine, colon and rectum adrenaline in doses of 0.2 to 0.3 c.c. will not act after 0.6 c.c. or more of ergotoxine. (It should be mentioned that these last experiments were carried out on Raja maculata and not on Raja diaphanes or erinacea like the majority were).

It is concluded from these experiments that there is a well developed antonomic nerve supply to the gastro-intestinal tract of the skate.

III. STRUCTURE AND REACTIONS TO STIMULI OF ARTERIES

In Raja diaphanes and stabuliforis the ventral aorta is of the elastic type, while the coeliac and superior mesenteric arteries are of the muscular type.

The large abdominal arteries--coeliac, superior mesenteric and anterior intestinal--contain in their walls a nervous network of unmedullated fibres belonging probably to the sympathetic nervous system.

Arteries of the elastic type show a different reaction to distension from those of the muscular type, the effect reaching its maximum quickly on loading, and disappearing quickly on unloading.

Changes in the tonus of the circular and of the longitudinal layer in arteries under the influence of rising temperature run a different course, the circular alone contracting (and to a maximal extent) at the comparatively low maximum temperature of the water in which it lives, i.e., below 14°C.

The circular and longitudinal layers of muscles in the arteries contract in response to adrenaline, acetylcholine and barium chloride. Atropine relaxes an artery contracted by acetylcholine.

The conus arteriosus reacts both to distension and to rise in temperature by showing increased rhythmic activity. In several instances adrenaline increased the rate of the rhythmic contractions of the conus arteriosus.

IV. REACTION OF THE GASTRO-INTESTINAL TRACT TO AUTONOMIC NERVE STIMULATION

Faradic stimulation, but not single or multiple break shocks, of the anterior gastric artery will stimulate the antrum and fundus of the stomach, of the superior mesenteric artery will stimulate the spiral intestine and colon, and of the inferior mesenteric artery will stimulate the rectum.

This stimulation is probably carried by periarterial sympathetic nerves.

Ergotoxine, even in massive doses has no effect upon arterial stimulation of any part of the gastro-intestinal tract.

Single and multiple break shocks, and faradic stimulation of the vagus stimulate the cardiac and fundic regions of the stomach.

Atropine, even in large doses, such as 1.5 c.c., i.e. doses that will inhibit much larger doses of acetylcholine, will not inhibit this effect of vagal stimulation.

* * *

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Addendum:

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