THE RELATION BETWEEN THE EXCITATORY AND THE CONTRACTILE MECHANISMS OF MUSCLE

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MUSCLE responds by contraction or relaxation when it is stimulated, the response being produced through the mediation of the excitatory system. The type of response will also be conditioned by the properties of the contractile system. Though the excitatory system may be responsive, the contractile system may not be " excitable "; the contractile system may be unable to contract or relax, though the excitatory system may be functioning " normally". To know the behaviour of the contractile system in a response, it is therefore necessary to exclude the excitatory system.

Szent-Gyorgyi has introduced the glycerinised muscle to study the contractile mechanism. In another method, substances are injected directly into the fibres of striated muscle (Heilbrunn, 1952). By the latter method, it has been found that the contractile system of striated muscle is very sensitive to calcium (Heilbrunn and Wiercinki, 1952); barium ions act like calcium ions in shortening of the muscle protoplasm. Magnesium acts very weakly. Adenosine triphosphate also causes contraction. According to Heilbrunn, muscular contraction is akin to coagulation of blood.

The method of injecting substances into the striated muscle fibre to determine the response of the contractile mechanism is not free from objection. It is quite possible that the muscle may be responding *via* the excitatory system and not by direct action of the injected substance on the contractile mechanism. In fact, there is evidence, that the muscle may be excited by substances acting on the muscle membrane from within the fibres (Singh, 1938, 1939, 1942, 1944, 1945). Thus, when the osmotic pressure of the saline is increased suddenly, the muscle may contract. Increase in osmotic pressure of the saline increases the concentration of ions within the fibres, chiefly potassium (Gokhale and Singh, 1945), and this sudden increase in contraction of ions within the fibres acts like increase outside and causes contraction or inhibition. Thus the muscle membrane is sensitive from without as well as from within the cells, though it appears to be less sensitive from the inside.

The contractile mechanism of unstriated muscle has been studied by two methods (Singh and Singh, 1949, 1950, 1951, 1954, *a*, *b*, *c*). In the first method, the muscle is allowed to die in the experimental solution, which

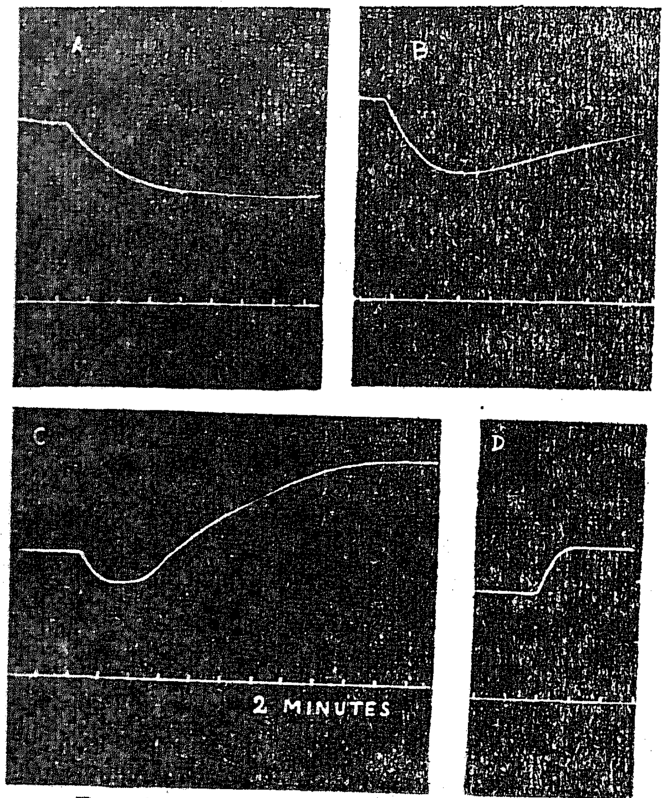

FIG. 1. Heat killed dog's stomach muscle.
A. Effect of 40 per cent. hydrochloric acid. B. Effect of 50 per cent. C. Effect of 70 per cent. D. Effect of 90 per cent.

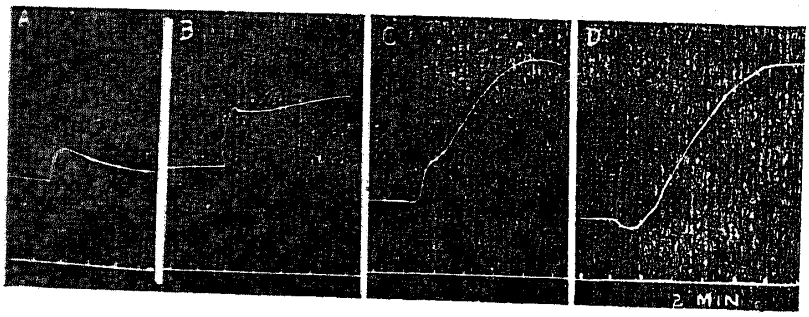

FIG. 2. Heat killed dog's stomach muscle.
A. Effect of 100 per cent. hydrochloric acid. B. Effect of 90 per cent. C. Effect of 80 per cent. D. Effect of 70 per cent.

is not oxygenated. It is well known that the normal permeability of the muscle depends upon metabolism, so that as the muscle dies, it becomes inexcitable as well as fully permeable to the substances; the excitatory system then ceases to function and the contractile system acts without interference. In the second method, the muscle is killed by heat. Most of the

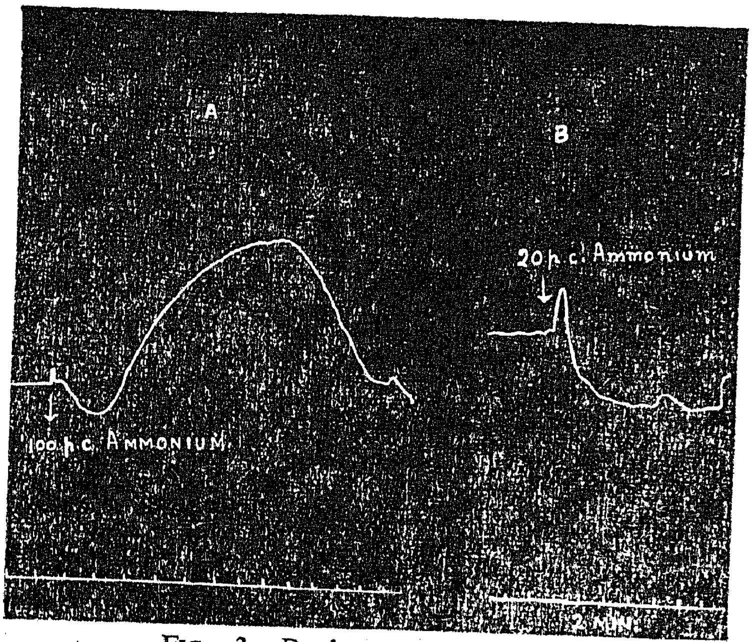

FIG. 3. Dog's stomach muscle.

Effect of ammonium saline. A. Effect of 100 per cent. ammonium. B. Effect of 20 per cent. ammonium.

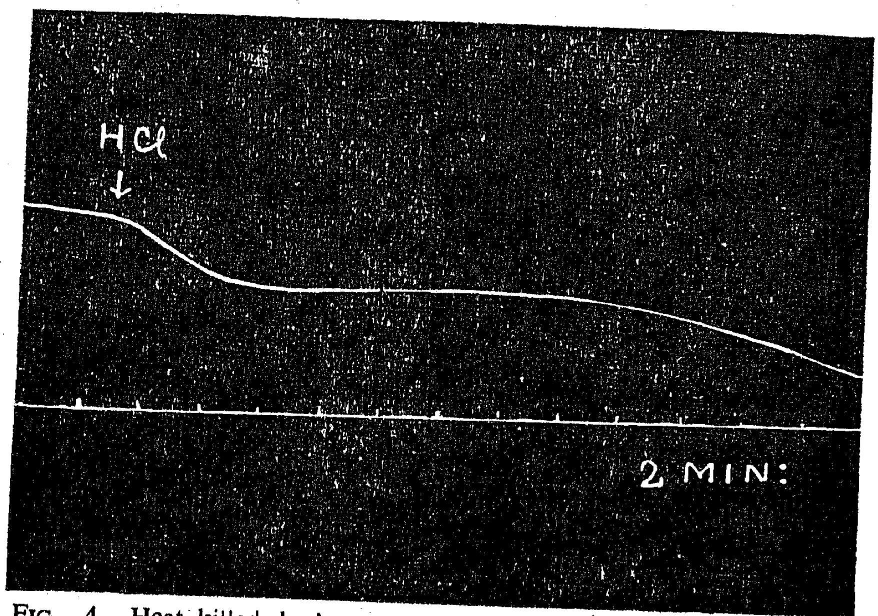

FIG. 4. Heat killed dog's muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.

results obtained by the former method have been substantiated by the latter method. It has been found that substances which denature proteins cause active or passive relaxation of unstriated muscle, and protein coagulants cause contraction.

Some of the results obtained by the dying muscle method agree with those obtained in striated muscle by the mioroinjection method. Thus

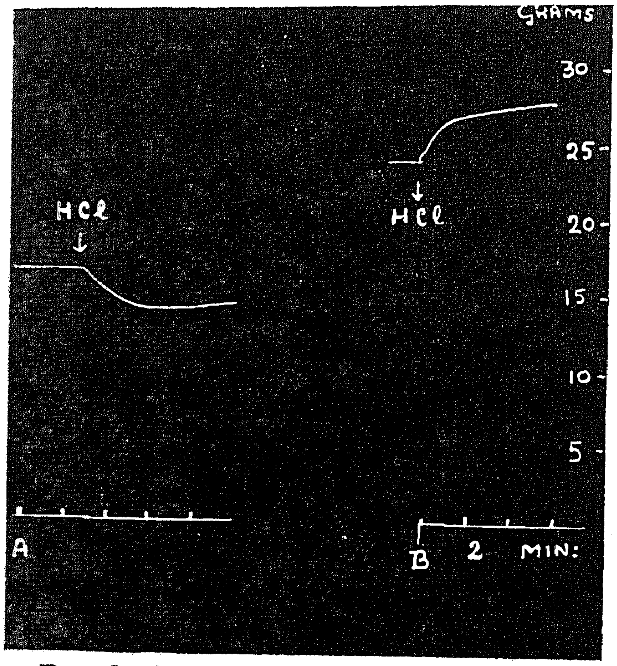

FIG. 5. Heat killed frog's rectus abdominis.

A Effect of 70 per cent. hydrochloric acid, with initial length 23 mm. B. Same as in A with initial length 27 mm.

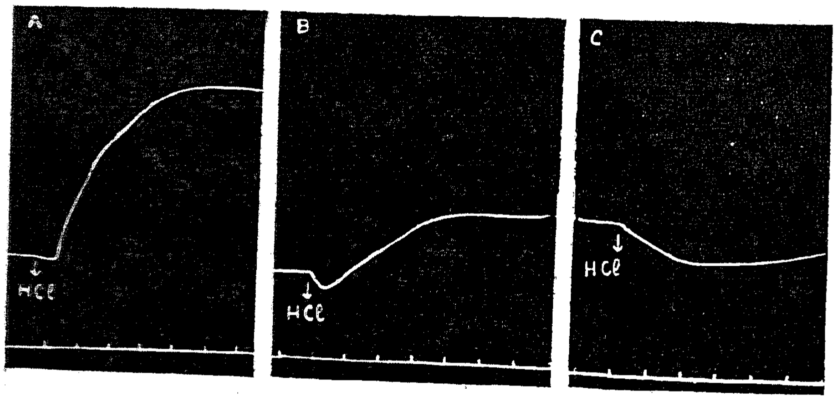

FIG. 6. A. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid.

A. From the cardiac end., B. From the middle part. C. From the middle part but near the pylorus.

in *Mytilus* muscle, both calcium and barium cause contraction of the contractile mechanism, as found in striated muscle. In frog's stomach muscle, barium causes relaxation, so that the contractile mechanism of *Mytilus* muscle, resembles that of striated muscle; a common physiological property of both is the absence of active relaxation.

In the present research, contraction, relaxation and latency relaxation have been studied, the response being produced by direct action on the

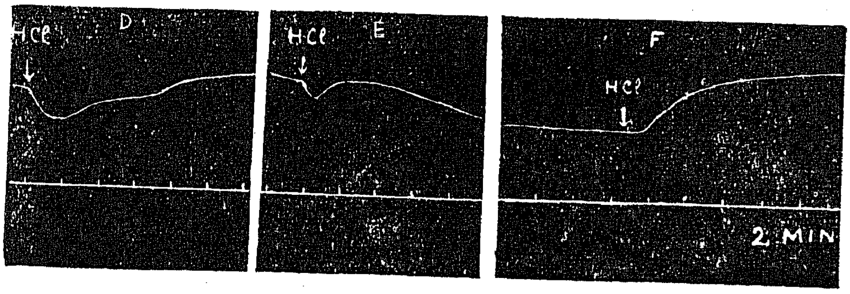

FIG. 6. B. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid. D. From the middle but nearer the pylorus than C. E. From nearer the pylorus than D. F. From the pylorus.

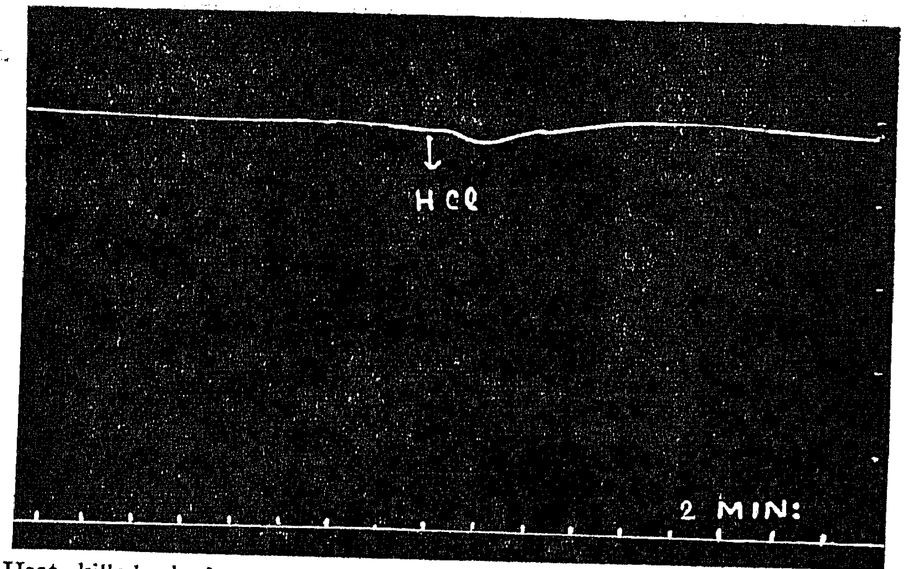

FIG. 7. Heat killed dog's stomach muscle from the pylorus. Effect of 70 per cent. hydrochloric acid.

contractile mechanism; these responses were compared with those produced through the mediation of the excitatory system.

EXPERIMENTAL

These experiments were performed on heat killed muscles (Singh and Singh, 1954, *a*, *b*, *c*). Pieces were obtained from the circular muscle of dog's stomach and stomach of the frog *Rana tigrina*. For experiments on striated muscle, the frog's rectus abdominis was used. For experiments

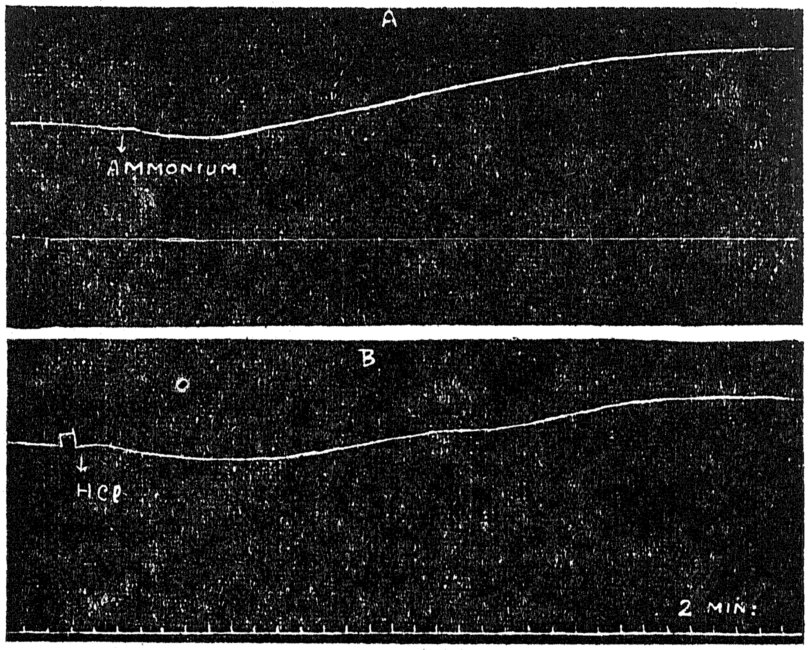

FIG. 8. A. Dog's stomach muscle from the cardiac end. Effect of ammonium. B. Heat killed dog's stomach muscle from the cardiac end. Effect of 70 per cent. hydrochloric acid.

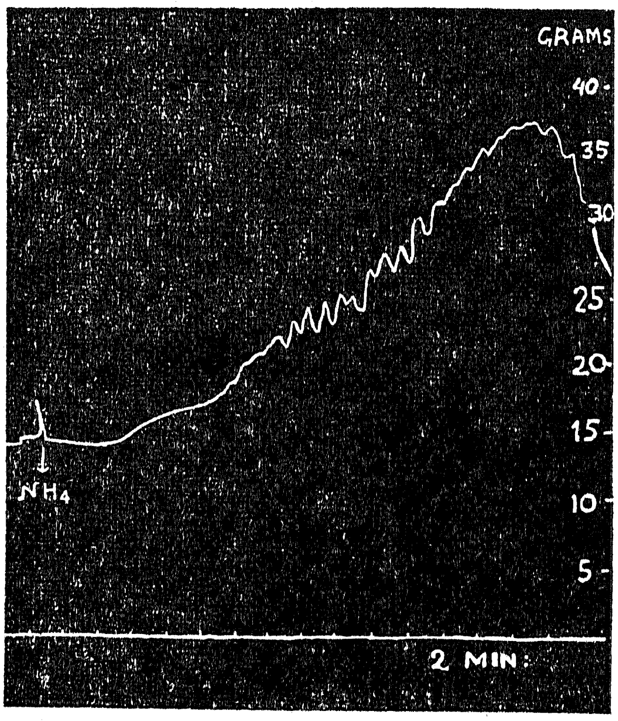

FIG. 9. Dog's stomach muscle from the pyloric end. Effect of ammonium.

on cardiac muscle, pieces from dog's ventricle were employed. From dog's heart, rings of ventricular muscle were removed and cut across at one place, so as to prepare an oblong piece for experimental use. In such a strip, the muscle bundles would be placed in various directions, but still a good contraction was produced.

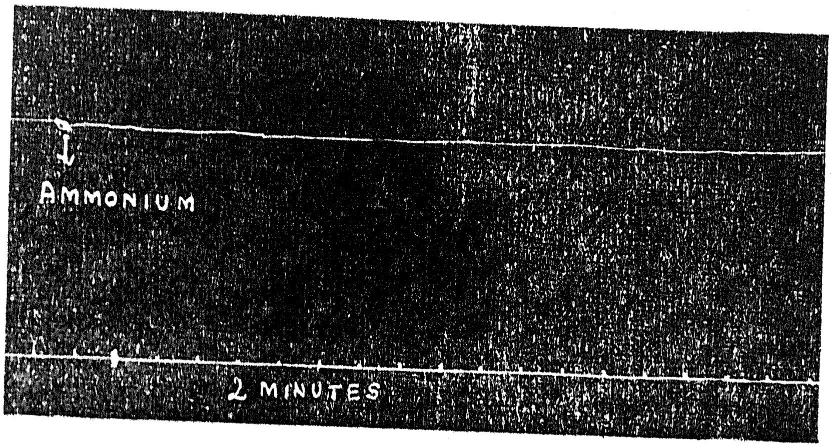

FIG. 10. Dog's stomach muscle from the pyloric end. Effect of ammonium.

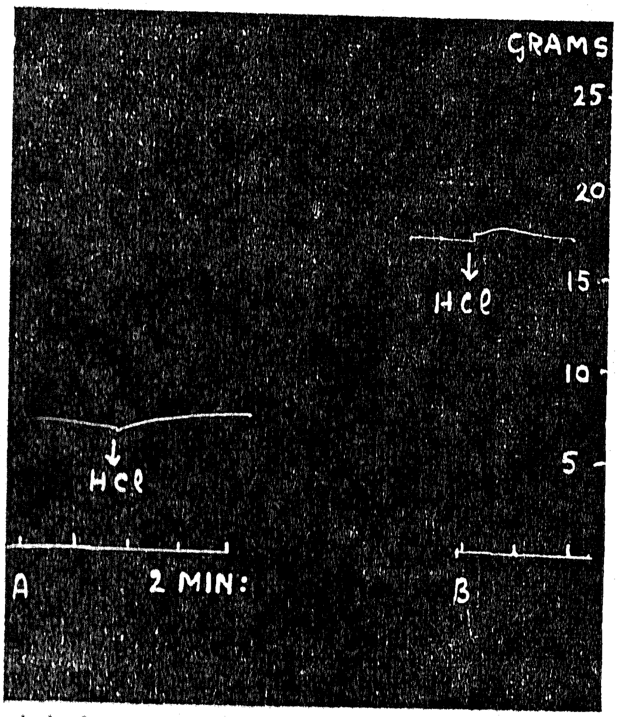

FIG. 11. Heat killed dog's muscle from the middle part of the stomach. Heated to 70° C. for 10 minutes. Effect of 70 per cent. hydrochloric acid.
A. Initial length 33 mm. B. Initial length 38. mm.

Heat killed muscles are contracted by some protein coagulants. A convenient substance formed to cause contraction of loaded muscles was strong hydrochloric acid. Varying volumes of concentrated hydrochloric acid (A.R., B.D.H.) were dissolved in water, and in this paper the strength of hydrochloric acid used is expressed as the percentage of concentrated hydrochloric acid so dissolved, the strength of the concentrated acid being

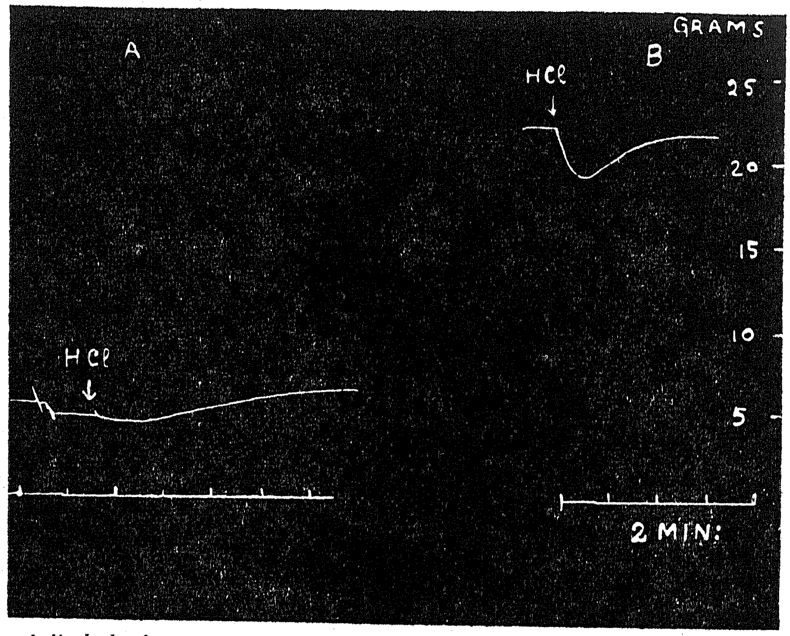

FIG. 12. Heat killed dog's stomach muscle from the middle part of the stomach. Heated to 60° C. for 10 minutes. Effect of 70 per cent. hydrochloric acid.
A. Initial length 32 mm. B. Initial length 37 mm.

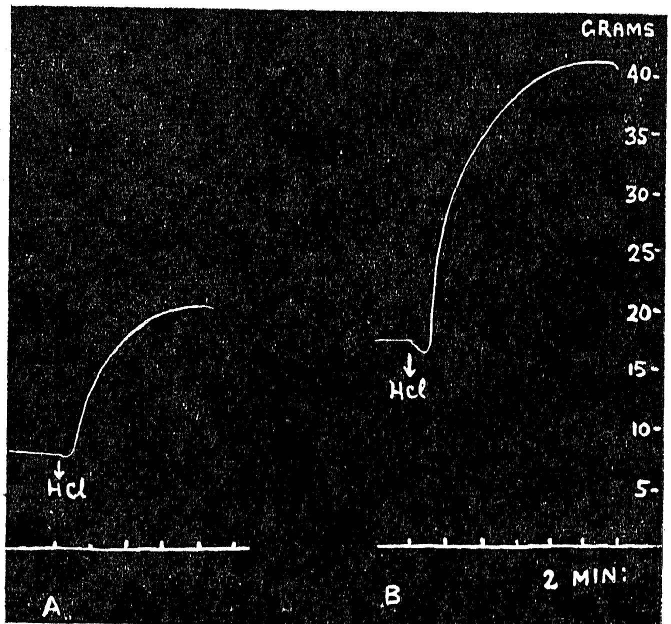

FIG. 13. A. Heat killed dog's stomach muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.
A. Initial length 33 mm. B. Initial length 38 mm.

considered as 100 per cent. Though strong hydrochloric acid is anything but a physiological stimulus to the contractile mechanism, yet the results

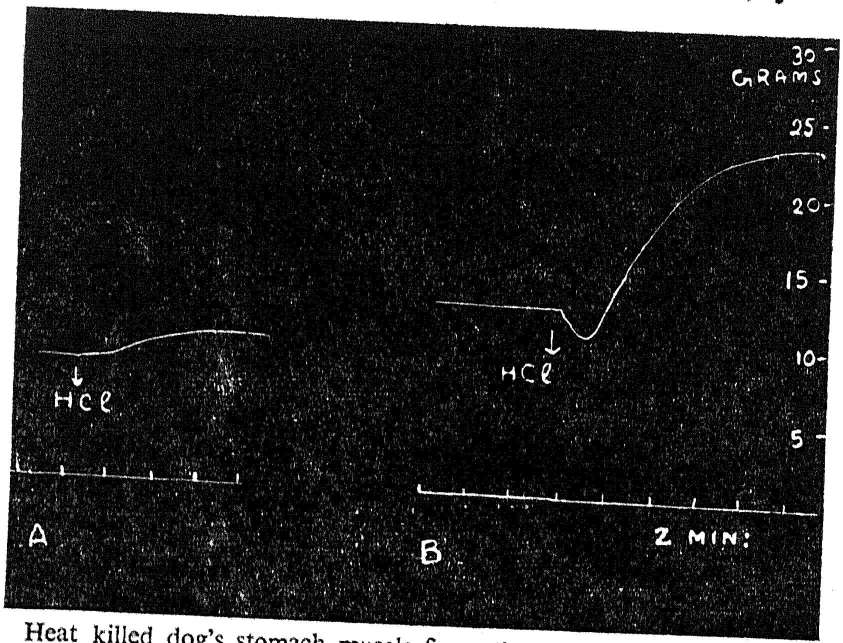

FIG. 13. B. Heat killed dog's stomach muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.
A. Initial length 30 mm. B. Initial length 34 mm.

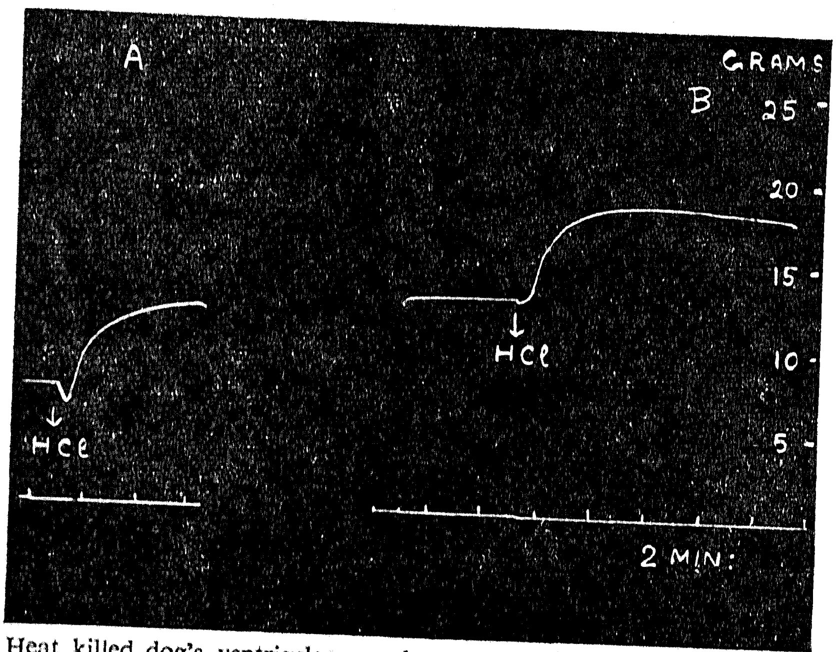

FIG. 14. Heat killed dog's ventricular muscle. Effect of 70 per cent. hydrochloric acid.
A. Initial length 72 mm. B. Initial length 77 mm.

obtained stimulated the physiological responses very closely. To produce the physiological response through the excitatory mechanism, the living muscle was stimulated with saline, the sodium of which had been replaced with ammonium, as with this substance, the latency relaxation is easily produced in unstriated muscle of dog's stomach (Singh, 1953). The strength of ammonium saline is expressed as percentage of sodium replaced; if all the sodium had been replaced, it is just termed as ammonium saline.

With use of strong hydrochloric acid solutions, a piece of muscle can be used only once. To produce comparable and consistent results, identical pieces of equal length of breadth have to be taken from the same segment of the stomach, as the sensitivity of the contractile mechanism varies in different parts of the organ.

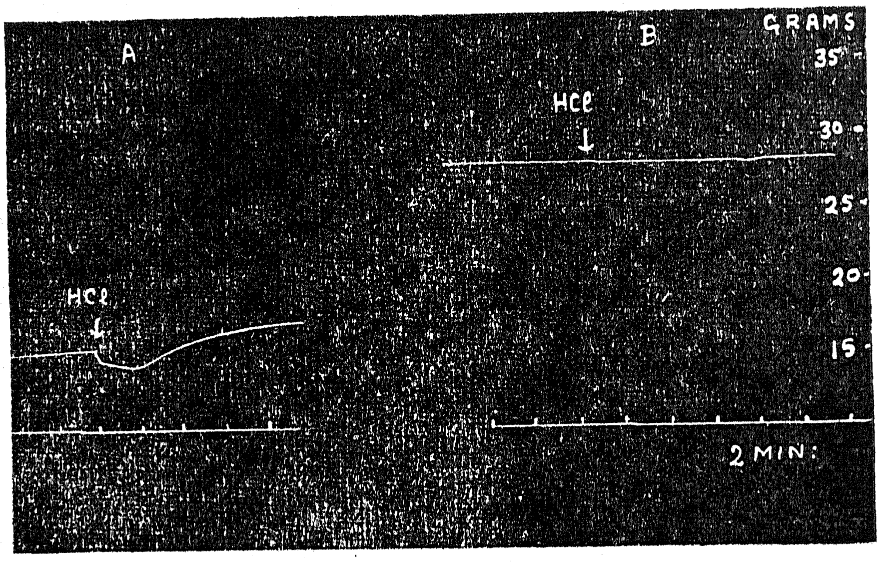

FIG. 15. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid.

A. Initial length 27 mm. B. Initial length 31 mm.

The muscles were killed by gradually raising the temperature of the saline from room temperature to 50° C. and keeping at that temperature for 5 minutes, if dog's stomach muscle was used. This minimum period is sufficient to make the muscle inexcitable to strongest electrical or chemical stimuli, such as saturated solutions of potassium or calcium chlorides; it is therefore presumed to be dead. This presumption is supported by the fact, that heating for a longer period, such as 10, 20, 30, 40 minutes does not make significant difference in the results; with such prolonged periods the muscle is certainly dead. The muscle may be heated to 55° C. for similar periods, usually 10 minutes being used in these experiments, without altering the nature of the results. Frog's stomach muscle was heated to 50° C. and kept at that temperature for 10 minutes. Frog's rectus abdominis and dog's heart muscle were similarly treated. With such treatment, striated muscle enters into a pronounced rigor.

Concentrations of hydrochloric acid used in these experiments were varied in steps of 10 per cent. Most of the experiments were performed on dog's stomach muscle, unless otherwise stated.

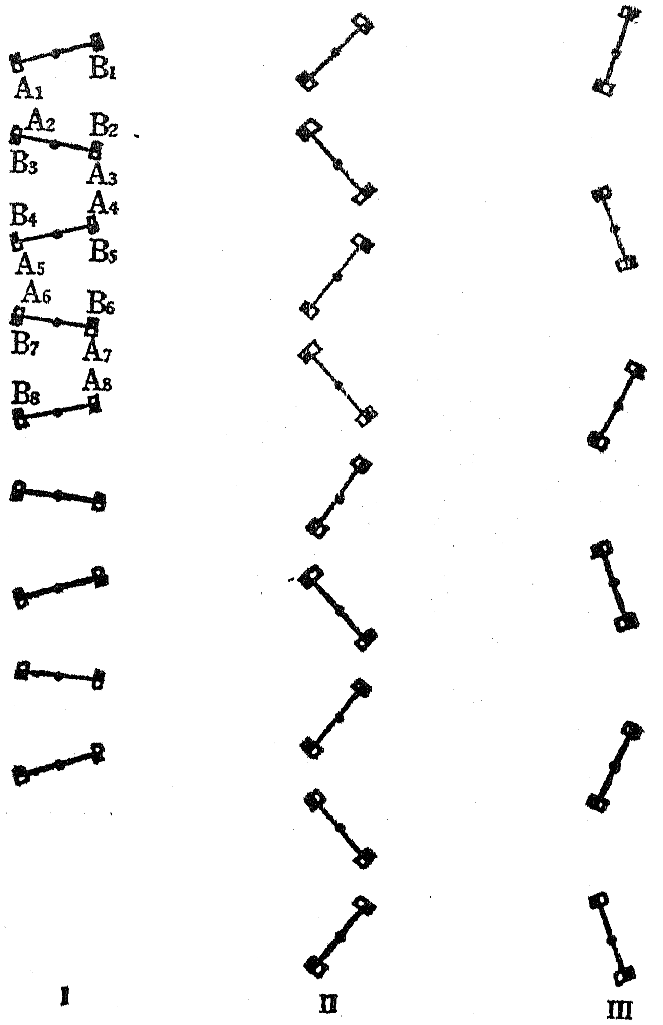

Fig. 16. Diagrammatic representation of the effect of increase in initial length on tension. I. Representation at a certain length. II. Effect of stretching so that the points of attraction come nearer. III. Effect of further stretching, so that the points of attraction move further apart.

Results

Effect of hydrochloric acid concentration.—Small concentrations of hydrochloric acid, 10 to 40 per cent., produce relaxation only of dog's stomach muscle. Maximum tension is produced by 70 per cent.; as the concentration of hydrochloric acid is increased, the relaxation is succeeded by contraction, so that with high concentrations, it becomes less and less and ultimately disappears with 80 to 100 per cent. hydrochloric acid (Figs. 1, 2).

Ammonium produces similar results on living muscle. Small concentrations, 10 to 50 per cent. produce relaxation only; with higher concentrations, relaxation becomes less and less, so that it may ultimately disappear altogether (Fig. 3). This is a well-known result in unstriated muscle. Substances which cause contraction, produce relaxation in small concentrations. In *Mytilus* muscle, small voltages of alternating current produce relaxation, whilst larger voltages produce contraction. Similarly, small concentrations

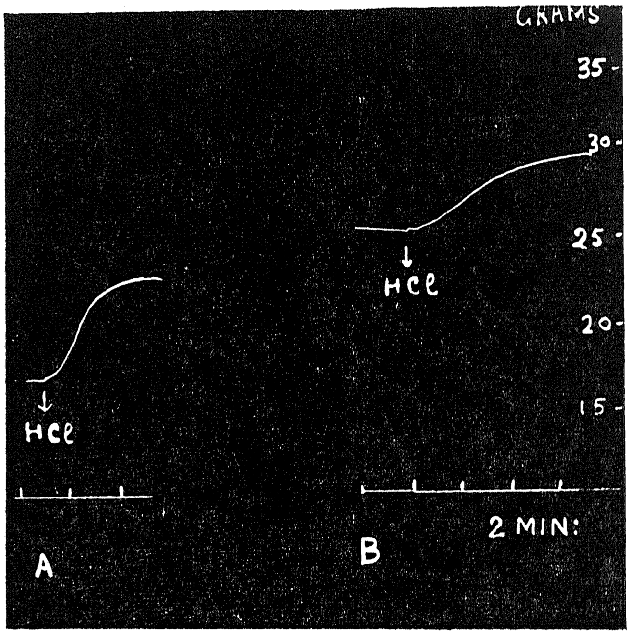

FIG. 17. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid.
A. Initial length 32 mm. B. Initial length 37 mm.

of ammonium, potassium, calcium, strontium, bromide, nitrate, iodide, thiocyanate, adrenaline, acetylcholine, caffeine, veratrine, cause relaxation, whilst larger concentrations cause contraction. It is known that small concentrations of adrenaline may cause vasodilatation, whilst larger doses cause vasoconstriction. Similarly, weak stimuli applied to nerves may produce dilator effects, and strong stimuli, constrictor effects.

Effect of sensitivity of muscle.—The sensitivity of dog's stomach muscle is variable. If the muscle from the middle part of the stomach does not contract, then it only relaxes (Fig. 4). Ammonium produces similar results on living muscle. This is also a well-known result on unstriated muscle.

Thus in *Mytilus* muscle small voltages of alternating current cause relaxation; if the excitability of the muscle is decreased by excess of lithium, ammonium, potassium, bromide, nitrate, iodide, thiocyanate, diminution of the calcium concentration of the saline, by excess of adrenaline, acetylcholine, veratrine, caffeine, strontium, barium, then stronger stimuli cause relaxation. Fatigue, as it diminishes excitability, may also convert a vasoconstrictor effect into a vasodilator one; thus whilst the first injection of pituitrin produces rise of blood pressure, a second dose soon after produces a fall of blood pressure.

Effect of tonus.—Dog's stomach muscle, if it shows much contraction on heating, is relaxed by hydrochloric acid. Similarly frog's rectus abdominis goes into rigor when heated; hydrochloric acid causes it to relax. If the muscle is stretched so as to undo the heat contraction, then hydrochloric acid produces contraction (Fig. 5).

Ammonium produces similar effects on living muscle. This is a well-known property of unstriated muscle. Thus when a stimulus, electrical, chemical or nervous, which when applied to the relaxed muscle, causes contraction, will when applied to the tonically contracted muscle, often provokes a rapid relaxation.

Sensitivity of dog's stomach muscle.—Pieces from various parts of the dog's stomach may vary in their response to hydrochloric acid. Those from the cardiac end are most sensitive; further towards the pylorus, the sensitivity decreases (Fig. 6), though very near the pylorus it may again increase. The muscle from near the pylorus does not relax appreciably; it may or may not contract (Fig. 7). Latency relaxation is best obtained from the middle part of pyloric region, and feeble near the cardiac end (Fig. 8).

Ammonium produces identical results on living dog's stomach muscle. The pyloric end does not relax, it may or may not contract (Figs. 9, 10). The latency relaxation is best marked in muscle from middle part of the stomach; it is feeble at the cardiac end and absent at the pylorus (Figs. 8, 9). The experiments with hydrochloric acid and ammonium should be performed on pieces from the same stomach.

The response of the middle and cardiac parts of the stomach muscle to hydrochloric acid can be made to resemble that of the pyloric region, if the former are heated to 70° C. (Fig. 11). The muscle then does not relax, and contraction is feeble. Further heating up to 80 or 90° C. produces similar results. Heating to 60° C. is only partially effective (Fig. 12). Heating up to 55° C. does not change the response.

Effect of initial length on tension.—Increase in initial length up to a certain point increases the tension produced by the action of hydrochloric acid on heated muscles; this has been found with dog's stomach muscle (Fig. 13), frog's rectus abdominis (Fig. 5), dog's heart muscle (Fig. 14). Thus this important property of muscle is a feature of the contractile mechanism.

Effect of initial length on relaxation.—Relaxation of heated dog's stomach muscle by hydrochloric acid increases with increase in initial length up to a certain extent; thereafter it decreases (Fig. 15). Ammonium produces similar effects on living muscle. Increase in initial length may abolish the inhibitory effect of ammonium and other inhibitory substances in living muscle (Singh, 1942, 1953).

Effect of initial length on latency relaxation.—Latency relaxation in heated dog's stomach muscle increases with increase in initial length to a certain extent (Fig. 13), and then decreases. Ammonium produces similar effects in living muscle (Singh, 1953). As in living muscle, latency relaxation increases with subsequent increase in tension, or decreases.

Effect of other acids.—Other acids like nitric, sulphuric, acetic, also cause contraction of muscle.

DISCUSSION

Strong hydrochloric acid solutions can hardly be considered as physiological stimuli to the contractile mechanism of muscle, yet the effects produced by them on dead muscles resemble the responses of living muscles so closely, that it suggests a close relationship between the two phenomena. Normal contraction, therefore, resembles coagulation of proteins, this resemblance presumably existing in the development of cross linkages in both (Singh, 1954, *b*, *c*).

Other experiments also suggest the similarity between the processes of normal contraction of muscle and coagulation of protiens. The pyloric end of the stomach shows greater tone than the cardiac; the contractile mechanism of the cardiac end can be made to respond with hydrochloric acid, like the contractile mechanism of the pyloric end, if the former is heated to 70° C., that is, if it is more coagulated.

These experiments suggest that the contractile mechanism of unstriated muscle is a variable entity, so that some of the variations in the response, which may be presumed to be due to the excitatory mechanism, are really due to variations in the contractile mechanism. An important variation of the contractile mechanism is its inability to relax, though the excitatory system may be functioning normally. Absence of relaxation therefore does not exclude the presence of an inhibitory nerve supply or inhibitory receptors on the cell surface.

With increase in initial length, muscle responds by greater tension during contraction; this property is resident in the contractile mechanism, as shown by the effect of hydrochloric acid on heated muscle. An explanation may be offered for this increase of tension (Fig. 16). It may be presumed that points of attraction are located on parts of the folded polypeptide chains these parts are represented diagrammatically as oblique lines. At the ends of each line, there are two points of attraction, represented by empty and dark squares respectively; these squares are on opposite sides of the line, a pair at each end, and exert their attractive forces in opposite directions. It is presumed that the attractive force exerted by each square, empty or dark, exists only on one side of the line, so that in the figure, A_1 attracts A_2, A_3 attracts A_4, and so on. Similarly, B_1 attracts B_2, B_3 attracts B_4, and so on.

When the muscle is stretched, two things happen. First, these lines are straightened out by stretching of the muscle, so that they rotate at their centre and bring the points A_1 and A_2, A_3 and A_4, etc., nearer together, and the points B_1 and B_2, B_3 and B_4, etc., more further apart. If the attraction between the points A_1, A_2, A_3, A_4, etc., is responsible for contraction, then increase in the length of the muscle would increase the contractile force. Secondly, this effect may be counterbalanced by increase in the distance between individual lines as a whole as an effect of stretching, so that the result will depend upon the net effect of these two opposing forces (Fig. 17). Thus it sometimes happens that stretching only decreases the tension produced as a result of stimulation (Singh, 1938).

A very interesting phenomenon takes place in unstriated muscle. Though twitch contraction may be enhanced on increase of initial length tone may decrease; similarly, other tonic contractions, such as produced by potassium, may also decrease (Singh, 1938). This may be explained by the above model if the points B_1, B_2, B_3, B_4, etc., represent attractive forces necessary to produce tonic contraction. These experiments, therefore, suggest that different bonds are formed in the muscle for the production of tone and twitch contraction respectively. Further, bonds for tonic contraction may be of different kinds (Singh and Singh, 1954, *b*, *c*).

The action of hydrochloric acid in reproducing so many phenomena which are produced through the excitatory system suggests that a chemical substance is liberated as a result of action of a stimulus on the excitatory system. Small concentrations of this substance produce relaxation. It takes time for the substance to reach the maximum concentration required by the stimulus, hence the occurrence of latency relaxation, which being produced whilst the concentration of this hypothetical substance, presumably ATP, is low.

The rate of release of the hypothetical substance which activates the contractile mechanism, depends upon the excitability. If the excitability is increased, the latency relaxation decreases and is ultimately not recordable by ordinary apparatus. Such long duration of the latency relaxation in unstriated muscle suggests that the rate of release of the activating substance is very small compared to that in striated muscle, in which it is comparatively an explosive phenomenon.

The early tension relaxation is of two kinds in unstriated muscle (Singh, 1953). In one kind as the early tension increases, the subsequent tension decreases. In the other kind the latency relaxation and the subsequent tension vary in the same direction. This has been ascribed to a mixture of responses; one is inhibition and the other latency relaxation proper. These results produced through the excitatory system, have been exactly reproduced by the action of hydrochloric acid on the contractile mechanism. It is possible that these are due to the presence of two systems in unstriated muscle, one of which only relaxes, and the other contracts after a preliminary relaxation.

These experiments show that the basis of Starling's law of the heart involves some change in the proteins of the contractile mechanism. Two factors are responsible for increase in the strength of contraction, increase in the initial length and increase in the concentration of the chemical substance acting on the contractile mechanism. The former probably acts by diminishing the distance between the points of attraction in the molecules of the contractile proteins and the latter increases the attractive forces. In heart failure a derangement of both these factors may occur; the treatment of the failing heart would therefore be either to increase the attractive forces or to diminish the distance between them by some modification of the molecular pattern.

SUMMARY

1. Muscles were killed by heat and their contraction studied by the use of strong hydrochloric acid solutions.

2. Though strong hydrochloric acid solutions are anything but a physiological stimulus, yet the responses produced resemble those produced on

the living muscle as the result of a physiological stimulus so closely, that there must be a close relationship between the normal response of the contractile mechanism and that produced by hydrochloric acid.

3. This resemblance between the two phenomena probably depends upon the formation of cross linkages in both.

4. The contractile mechanism of unstriated muscle is a variable entity, so that the response to a stimulus is not only conditioned by the state of the excitatory system, but also by that of the contractile system.

5. The increase in tension produced when the muscle is stimulated with increasing initial length, is also produced by hydrochloric acid on heat killed muscle, so that it is a property of the contractile mechanism.

6. Small concentrations of hydrochloric acid produce relaxation; physiologically weak stimuli produce relaxation of unstriated muscle.

7. A contracted heat killed muscle is relaxed by hydrochloric acid, and a relaxed muscle contracted. This again simulates the physiological response very closely.

8. The sensitivity of the contractile mechanism to hydrochloric acid varies in different parts of the stomach. The cardiac end is most sensitive, and pyloric, the least.

9. The pyloric end does not relax. This also simulates the physiological response.

10. The contractile mechanism has an optimum length for relaxation. This also simulates the physiological response.

11. Hydrochloric acid produces latency relaxation in the heat killed muscle. This latency relaxation closely resembles that produced in living muscle.

12. The mechanism of contraction of unstriated muscle is discussed.

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