EXCITATION IN UNSTRIATED MUSCLE

BY INDERJIT SINGH, F.A.SC.
(*From the Department of Physiology, Medical College, Hyderabad, Sind*)

Received June 5, 1944

EXCITATION phenomena in unstriated muscle are very complicated. Various kinds of contractions and inhibitions occur (Singh, 1936; 1937; 1938 *a, b, c, d, e, f*; 1939 *a, b*; 1940; 1942 *a, b*; 1943 *a, b, c, d, e, f, g, h, i*; 1944 *a, b, c*; Rao and Singh, 1940; Singh and Mrs. Singh, 1943, 1944; Gokhale and Singh). It would be very helpful if the inter-relation between these contractions is made clear by the help of a comparatively simple hypothesis or model.

In unstriated muscle there are certain salient features concerning excitation. These are: (1) The mechanisms of excitation produced by electric current and by ions appear to be different. Increase in permeability decreases the sensitivity to the former and increases that to the latter. (2) The excitation by ions depends upon the production of a difference in their concentration on two sides of the muscle membrane (Straub, 1903, 1907) or increase in permeability, or their attachment to sensitive patches (Clark, 1935). Certain substances, however, act in too small a concentration to produce such a difference. (4) Certain ions may produce inhibition at one time and excitation at the other. (5) Calcium may produce effects similar to those of increase of osmotic pressure. (6) Certain agencies affect the two excitabilities similarly and the others oppositely. (7) Change of length produces contraction. (8) The muscle shows withdrawal contractions. There are several other phenomena, which need elucidation.

It is the object of this paper to correlate these phenomena.

Excitation in unstriated muscle appears to be produced by difference in concentration of ions within and without the fibres.

In considering difference in ionic concentration on two sides of the muscle membrane causing excitation, the next question is whether it is the difference or the ratio. After the tension has subsided with a certain concentration of potassium, addition of a greater concentration produces further tension, and if the concentration of potassium in successive doses increases in arithmetical progression, the tension in successive stimulation is less than the preceding. This would be evident from consideration of the ratio R_o/R_i, where R_o is the concentration of ions outside, R_i that of the ions inside. A numerical increase in the numerator and the denominator by the same amount would result in decrease of the ratio.

Difficulty arises in explanation of certain phenomena. If the tension produced by addition of potassium to the outside of the muscle fibres depends upon the ratio R_o/R_i, then the less permeable the membrane of the fibres, not only should it become more excitable to alternating current but also to potassium, and the tension would be maintained longer. But generally in *Mytilus* muscle when the excitability to alternating current was high, it was inexcitable to potassium (0·1 M KCl). Addition of potassium did not produce any tension, as if it was not added at all, apparently something prevented potassium from coming into a zone where a difference in concentration of ions would be produced without and within the fibres. This would be explained if the muscle was surrounded by a second membrane with properties more or less similar to the first, dividing the muscle into two zones, inner and outer. Increase in tension would result only if there was a difference in concentration of ions in the outer and inner zones respectively. The outer membrane would require to be more permeable than the inner. Two zones have been described by Roskin 1925, 1926; Carleton (1943).

The next question is whether the outer zone is aqueous or non-aqueous. The postulation of a sodium space and a potassium space has been described by Singh (1939 *a*). The outer membrane would then be permeable to sodium and potassium, but the inner membrane impermeable to sodium. The outer zone must however be non-aqueous; this assumption is based on the previous assumption that the sodium contraction would be caused by the entrance of sodium or chloride ions in the outer zone, thus disturbing the normal ionic equilibrium. If the concentration of sodium ions is already equal to that in the medium than no such increase can take place. Hence, this outer zone must be non-aqueous, otherwise the membranes would have to support a large difference of osmotic pressure.

This is in agreement with the views of Beutner, Osterhout (1936) who have postulated a non-aqueous zone for the muscle.

The above idea is also supported by the fact that the stimulating power of the monovalent cations varies in the same order ($Li < Na < NH_4 < K$) as their velocity in protoplasm as found by Osterhout. The stimulating power of potassium is much greater than that of ammonium, and though its velocity in aqueous solutions is practically equal to that of ammonium, in protoplasm it is 40 times greater. This suggests that the outer zone is non-acqueous.

As the outer membrane is more permeable than the inner, anything that increases the permeability of the muscle, would at first make the latter more excitable to potassium and less to alternating current. With great increase in permeability the muscle would become inexcitable to both. Thus in the absence of calcium *Mytilus* muscle becomes less excitable to alternating current and more to potassium. With time like frog muscle, it becomes inexcitable to both.

The presence of the outer zone is also suggested by the action of barium and sodium (Singh, 1944 *b*).

The presence of the outer zone also explains the increase in excitability following increase in permeability to ions. The more permeable the muscle to an ion, more quickly its concentration will be raised in the outer zone.

If the muscle be immersed in a potassium rich solution, then R_i will be increased; to rise to the stimulating concentration, more time will be required for R_o. Thus potassium will diminish the response and increase the latent period, as happens with barium and sodium contractions, as well as the stretch and release contraction; which is presumably caused by sodium chloride (Singh, 1938 *b*).

The relative impermeability of the inner membrane would make it comparatively impermeable to ions with lesser penetrating power, such as sodium chloride, anions, barium, compared to faster moving ions such as potassium and ammonium. The former ions will therefore produce continuous contractions, as is found experimentally.

Ions will not only affect R_o/R_i, but also the permeability of the membranes. Thus calcium has effects resembling that of other ions, that is producing contraction, potentiating the response to potassium and decreasing that to alternating current. At the same time it has other effects that affect the two excitabilities similarly. If calcium decreases the permeability, potassium has probably an opposite effect, accounting for the lasting inexcitability in excess of potassium. This explains the two factors affecting excitability (Singh, 1939*b*).

Interaction between Excitation and Inhibition

For the sake of simplicity, the excitatory and the inhibitory action of ions may be termed excitation and inhibition respectivily. As the potassium contracture is antagonised by increase of osmotic pressure and alternating current, excitation inside is antagonistic to excitation outside and *vice versa.* Similarly the two inhibitions are antagonistic (Singh, 1942 *b*). As inhibition is the opposite of excitation, it follows then that increase of inhibition outside will increase excitation inside. This is realised by experiment. In frog stomach ammonium has an inhibitory action. In the presence of ammonium the contraction produced by increase of osmotic pressure is greater (Singh, 1939 *b*).

It thus follows that inhibition inside will increase excitation outside. Diminution of excitation inside will also have the same effect. In *Mytilus* muscle, this can be done in three ways: (1) by diminishing the concentration of potassium by immersion in hypotonic solution; (2) by replacement of potassium by sodium; and (3) by immersion in lithium saline (Singh, 1944 *b*). All these procedures increase the excitability to potassium and diminish that to alternating current. The presence of sodium or chloride inside the muscle fibres is the basis of all tonic contractions.

Tonic Contractions

Tonus.—This would be due to the presence of sodium in the outer zone as well as the inner. Plain muscle would then contain more sodium or chloride than striated muscle. Dog stomach exhibits greater tone than frog stomach and is comparatively richer in sodium (Gokhale and Singh).

Sodium will have a second action, that on muscle viscosity (Singh, 1943 *h*). This is in agreement with the fact that plain muscle is more viscous than striated muscle. Continued stimulation increases the sodium content of skeletal muscle (Fenn, 1936) as well as viscosity (Levin and Wyman, 1927).

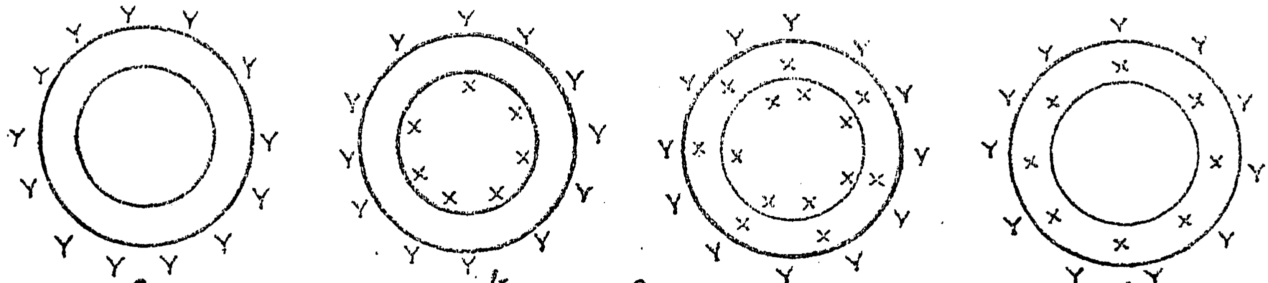
Tonic contractions produced by alternating current.—Increased sensitivity to ions outside during stimulation, or their entrance in the outer zone will have the following effects: (1) Diminish primary tension as with A.C. and D.C. contractions; (2) account for the optimum voltage for A.C., as with increased voltage ions outside begin to antagonise the alternating current; (3) diminish adaptation, as the anions are antagonistic to the action of calcium, producing adaptation to adaptation or accommodation to accommodation (Singh, 1943 *d*); (4) may actually produce a tonic contraction or inhibition with passage of current for long duration (Singh, 1942 *b*) (5) diminish the rate of relaxation either by causing after-excitation or increasing the viscosity. Some of these effects will not be produced if the action of ions outside is inhibitory as in frog stomach.

Ordinarily the action of ions outside antagonises the action of ions inside, and this results in (1) diminution of the primary tension; (2) decrease of the rate of relaxation. If the action of ions outside is very powerful, then the primary contraction produced by alternating current may be entirely neutralised and a tonic contraction produced by the current from the outset, instead of after sometime. If this happens then the primary tension will increase with decrease in the rate of relaxation. This is actually found to be the case. Thus barium and cyanide are the two most powerful stimulants, and in their case, not only the rate of relaxation decreases, but the primary tension produced by the current also may increase (Figs. 1, 2).

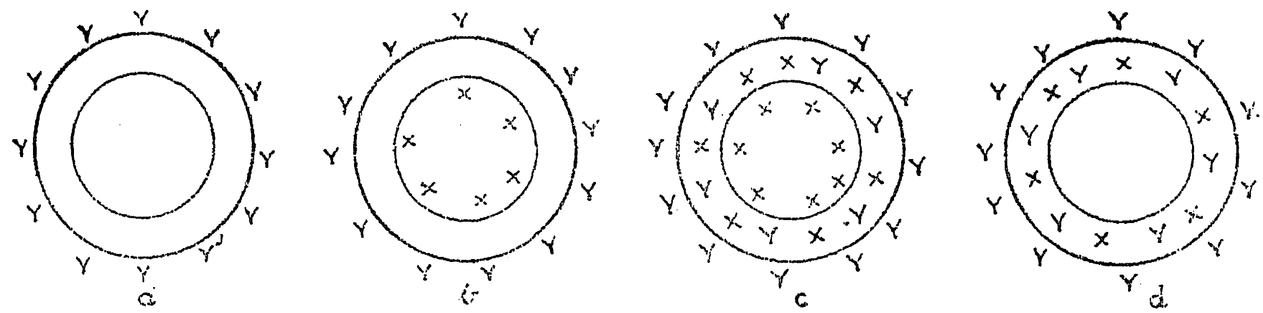
A.C. off-contracture.—In *Mytilus* muscle the primary tension may completely subside during the passage of alternating current. On cessation of the current a contraction is produced, having the properties of the potassium contraction; the relaxation may be rapid. This shows that it is produced by ions outside the fibres. This phenomenon is produced by ions that increase the permeability of the muscle. The explanation appears to be that during stimulation, ions leak into the outer zone and cause subsequent stimulation on cessation of the current (Fig. 3).

But it also happens that there is no decline of tension during the passage of the current and the off-contracture is still produced, but its relaxation is very slow. This then cannot be solely due to leakage of ions from the inner zone, but can be explained as due to leakage of ions into the outer zone both from the inner zone as well as the exterior, as this is more marked if stimulating anions are added to the saline (Figs 2, 4).

Slow relaxation of plain muscle.—This is due to the entrance of ions in the outer zone during stimulation (Fig. 4). This view is supported by the fact that it is produced by all substances that increase the permeability, and



3



4

FIG. 3. Schematic representation of the A.C. off-contracture: (*a*) Resting State. (*b*) Increased concentration of ions inside the fibres produced by A.C. stimulation. (*c*) Leakage of ions to the exterior. (*d*) The A.C. off-contracture.

FIG. 4. Schematic representation of the A.C. off-contracture: (*a*) Resting muscle. (*b*) A.C. stimulation. (*c*) Leakage of ions. (*d*) The A.C. off-contracture.

inhibited by calcium which decreases the permeability. The entrance of ions in the outer zone produces two things: (1) They may cause stimulation after cessation of the stimulus as occurs with veratrine; (2) or just increase the viscosity and decrease the rate of relaxation. Continued frequent stimulation causes contracture and increases the viscosity, as well as the sodium content, as is found both in striated and in unstriated muscles. Veratrine decreases the electrical resistance of nerve (Guttman and Cole, 1942).

Contraction produced by stretch and release (Singh, 1938 *b*, 1943 *d*).—As this contraction has properties of the sodium chloride contraction, it is produced by sodium or chloride. It appears that sudden change of length alters the permeability and sodium chloride enters the outer zone and causes excitation. The muscle appears to have greatest permeability at a certain length, and sudden approach to this length from either side causes contraction; the muscle has an optimum length for the potassium contraction.

This leads to an important law which may be enunciated as follows.—The action of any factor which increases the sensitivity of the muscle to excitation by ions without the fibres, when suddenly intensified, evokes a contraction, the properties of which are the same as those of a contraction produced by excitation by ions without; the converse of this is not true.

Mechanical and other effects.—Anything that injures the outer membrane will make it more permeable and thus cause contraction, hence the production of mechanical and injury contractions or inhibitions. Chemicals may injure the membrane; hence substances like ether may cause contraction. Lack of oxygen will produce similar effects, as energy will be required to maintain comparative impermeability. Hence a temporary increase in excitability to potassium might be expected (Singh, 1938 *b*) or a temporary increase in tonus (Magnus, 1904; Mickuliez-Rodecki and Luez, 1924).

Correlation of the Theories of Ionic Concentration and Surface Action

Difference causing Contraction and that of Clark.—Drugs are active in such minute concentrations that their action could not be due to a difference in concentration within and without the fibres. But just as stretch or release sensitises the muscle to ions outside, so it is possible that some of the drugs produced their effects not *per se*, but through the agency of other ions by sensitising the muscle to these ions, the mechanism of this sensitisation being an increase of permeability (Singh, 1943 *g*).

Correlation of the Colloid Theory (see Heilbrunn, 1937) *and the permeability theory.*—These two theories are not mutually exclusive. Thus ions probably enter the muscle during stimulation or liberated in active form under certain conditions, and may either cause contraction or increase the viscosity, the latter must be due to action of these ions on the colloids.

Adaptation

If excitation is associated with the entrance of ions into the outer zone, then inhibition must be associated with an opposite change. The action of calcium on *Mytilus* muscle is suggestive. In its absence the muscle gains weight and base; in its absence the swelling is reversed. It is probably the latter action which makes the ions leave the outer zone.

Adaptation is probably produced by liberation of calcium ions in the outer zone, excluding other ions from it, thus diminishing R_o. The decrease in R_o will diminish tone, and increase the excitability to A.C., but if the concentration of calcium increases further, then R_o will increase, causing diminution in excitability to A.C. as well.

Two agencies diminish tonic contraction in plain muscle: (*a*) calcium; (*b*) increase in osmotic pressure. The former decreases R_o and the latter increases R_i, the result being diminution of the ratio R_o/R_i in both instances.

Inhibition

The fact that potassium causes inhibition or excitation in the same muscle, shows that the factor determining inhibition or excitation resides, not in the stimulus, but in the cell. To produce inhibition, however, ions have to enter the outer zone so that for inhibition an increase of permeability would be necessary, and this would be followed by a decrease, produced by inhibition *per se*.

Spontaneous or Rhythmic Contractions

These occur when the excitability of the muscle is intermediate between that which induces continuous tension, and that which induces no tension, that is, between great excitability and inexcitability. If excitability to excitation from without is dependent upon permeability or affinity of muscle colloids for ions, then for spontaneous contractions an intermediate permeability or affinity is necessary, as shown by the following observations:—

(1) Heart muscle contains sodium intermediate in amount between that contained in striated muscle and that in unstriated muscle. Isolated striated muscle is relaxed, cardiac muscle is in spontaneous contractions, and unstriated muscle is in tone.

(2) *Mytilus* muscle is more permeable to potassium than to sodium, with the result that if sodium chloride produces spontaneous contractions, potassium produces continuous tension.

(3) The muscle is more permeable to thiocyanate than to bromide, and more to the latter than to chloride: thiocyanate gives continuous tension, bromide may give spontaneous contractions, and chloride may not excite the muscle.

(4) The muscle is more permeable to potassium than to ammonium, and more to latter than to sodium. There might be no tension with sodium, spontaneous contractions with ammonium, and continuous tension with potassium.

(5) There might be no tension in *Mytilus* saline containing 0·02 *M* $CaCl_2$, spontaneous contractions with 0·01 *M* $CaCl_2$ and continuous tension in the absence of calcium; calcium decreases permeability to sodium.

(6) If no contractions are produced in the presence of 0·01 *M* $CaCl_2$, spontaneous contractions may be produced in the absence of calcium.

(7) Excess of potassium might produce spontaneous contractions in 0·02 *M* $CaCl_2$ and continuous tension with 0·01 *M* $CaCl_2$; calcium decreases premeability to potassium.

(8) The muscle is less permeable to ions with decreasing pH. Caffeine might produce no tension at pH 6, spontaneous contractions at pH 7, and continuous tension at pH 7·8.

(9) Barium like potassium might give spontaneous contractions in 0·01 *M* $CaCl_2$, and continuous tension in the absence of calcium decreases permeability to barium.

Spontaneous contractions in *Mytilus* muscle can be explained on the basis of liberation of calcium by ions (Singh, 1938 *f*, 1944 *a*). Ions enter the outer zone, and thus cause excitation. Their presence liberates calcium so that the ions then leave the outer zone. This in turn causes calcium to recombine, so that ions again enter the outer zone, and so the cycle is repeated.

The reason for an intermediate permeability for these contractions is then understood. If the permeability is less, the muscle will be inexcitable; if the permeability is great, then the adaptation factor will be neutralised or overcome so that continuous tension will result.

The above phenomena are compared to partial defence of a place. If the defence is strong, then the attacking forces will not be able to penetrate defence positions. If the defence is rather weak then the attacking forces will penetrate, but will be repelled by counter-attack each time. If the defence is very weak, then the attacking troops will overcome the defence entirely.

Summary

Excitatory phenomena in unstriated muscle can be explained if it is assumed that the muscle consists of two zones, outer and inner, and excitation be due to difference in concentration of ions in these two zones. Moderate increase in permeability would diminish the excitability to alternating current and increase that to potassium; great increase would diminish the excitability to both. An increase in the permeability of the outer membrane by physiological action, injury, asphyxia would cause excitation or inhibition. Spontaneous contractions are caused by increase in permeability, not great enough to cause continuous tension. Substances to which the muscle is moderately permeable, such as sodium and barium, produce continuous tension as they are unable to enter the inner zone. Substances to which the muscle is more permeable such as ammonium or potassium produce only a temporary contraction.

REFERENCES

Carleton, H. M. .. *Schalers essentials of Histology*, London, 1943.

Clark, A. J. .. *Mode of Action of Drugs on Cells*, London, 1935.

Fenn, W. O. .. *Physiol. Rev.*, 1936, **16**, 450.

Gokhale, S. K., and Singh, I. .. Unpubiished resuitf.

Guttman, R., and Cole, K. S. .. *Proc. Soc. Exp. Biol. Med.* Quoted from Gerrard, R. W., *Ann. Rev. Physiol.*, 1942, **4**, 329.

Heilbrunn, L. V. .. *Outline of General Physiology*, London, 1937.

Levin, A., and Wyman, J. .. *Proc. Roy. Soc.*, B, 1927, **101**, 218.

Magnus .. *Arch. Gesamint. Physiol.*, 1904, **102**, 123. Quoted from Garry, R. C., *J. Physiol.*, 1928, **66**, 235.

Mickuliez-Rodecki, F. V., and Luez, W. *Pflugers Arch.*, 1934, **203**, 570. Quoted from Prasad, J., *J. Physiole.*, 1935, **85**, 239.

Osterhout, W. J. V. .. *Physiol. Rev.*, 1936, **16**, 216.

Rao, M. S., and Singh, I. .. *J. Physiol.*, 1940, **98**, 12.

Roskin .. *Z. f. Zellforschung*, 1925, **11**, 766. Quoted from Evans, C. L., *Physiol. Rev.*, 1926, **6**, 358.

Singh, I., and Mrs. Singh, I. .. *Proc. Ind. Acad. Sci.*, 1943, **18**.

Singh, I. .. *J. Physiol.*, 1936, **89**, 10 P.

——— .. *Ibid.*, 1937, **89**, 8 P.

——— .. *Ibid.*, 1938*a*, **91**, 398.

——— .. *Ibid.*, 1938*b*, **92**, 62.

——— .. *Ibid.*, 1938*c*, **92**, 232.

——— .. *Ibid.*, 1938*d*, **92**, 241.

——— .. *Ibid.*, 1938*e*, **94**, 1.

——— .. *Ibid.*, 1938*f*, **94**, 322.

——— .. *Ibid.*, 1939*a*, **96**, 1.

Singh, I. .. *Ibid.*, 1939*b*, **96**, 367.
——— .. *Ibid.*, 1940, **98**, 155.
——— .. *Ind. J. Med. Res.*, 1942*a*, **30**, 449.
——— .. *Ibid.*, 1942*b*, **36**, 29.
——— .. *Proc. Ind. Acad. Sci.*, 1943*a*, **17**, 13.
——— .. *Ibid.*, 1943*b*, **17**, 20.
——— .. *Curr., Sci.*, 1943*c*, **12**, 56.
——— .. *Ibid.*, 1943*d*, **12**, 57.
——— .. *Ibid.*, 1943*e*, **12**, 83.
——— .. *Proc. Ind. Acad. Sci.*, 1943*f*, **17**, 143.
——— .. *Ibid.*, 1943*g*, **17**, 149.
——— .. *Ibid.*, 1943*h*, **18**.
——— .. *Nature*, 1943*i*, **132**, 152.
——— .. *Proc. Ind. Acad. Sci.*, 1944*a*, **18**.
——— .. *Ibid.*, 1944*b*, **19**, 91.
——— .. *Curr. Sci.*, 1944*c*.
Straub, W. .. *Pfluger's Arch.*, 1903, **98**, 233.
——— .. *Ibid.*, 1907, **119**, 127.

1208-44—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore,

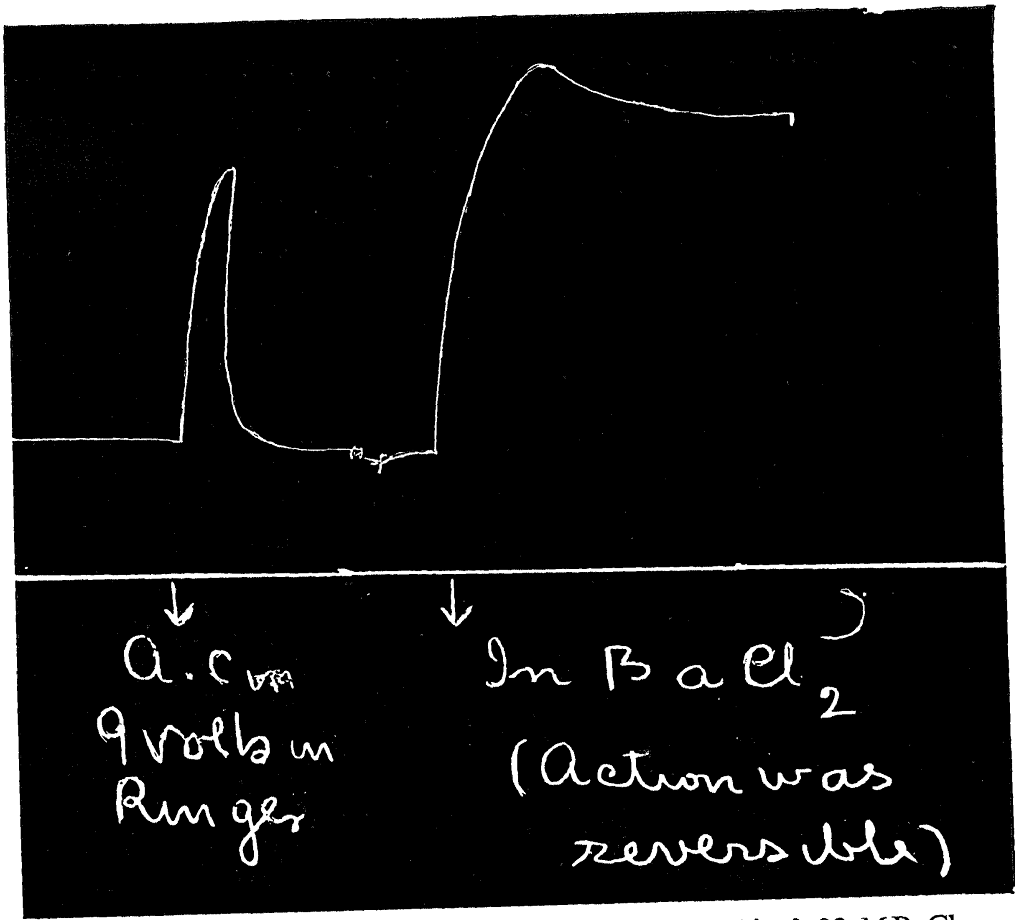


FIG. 1. *Mytilus* muscle. Stimulation with A.C. 9 V in 0·02 *M* $BaCl_2$

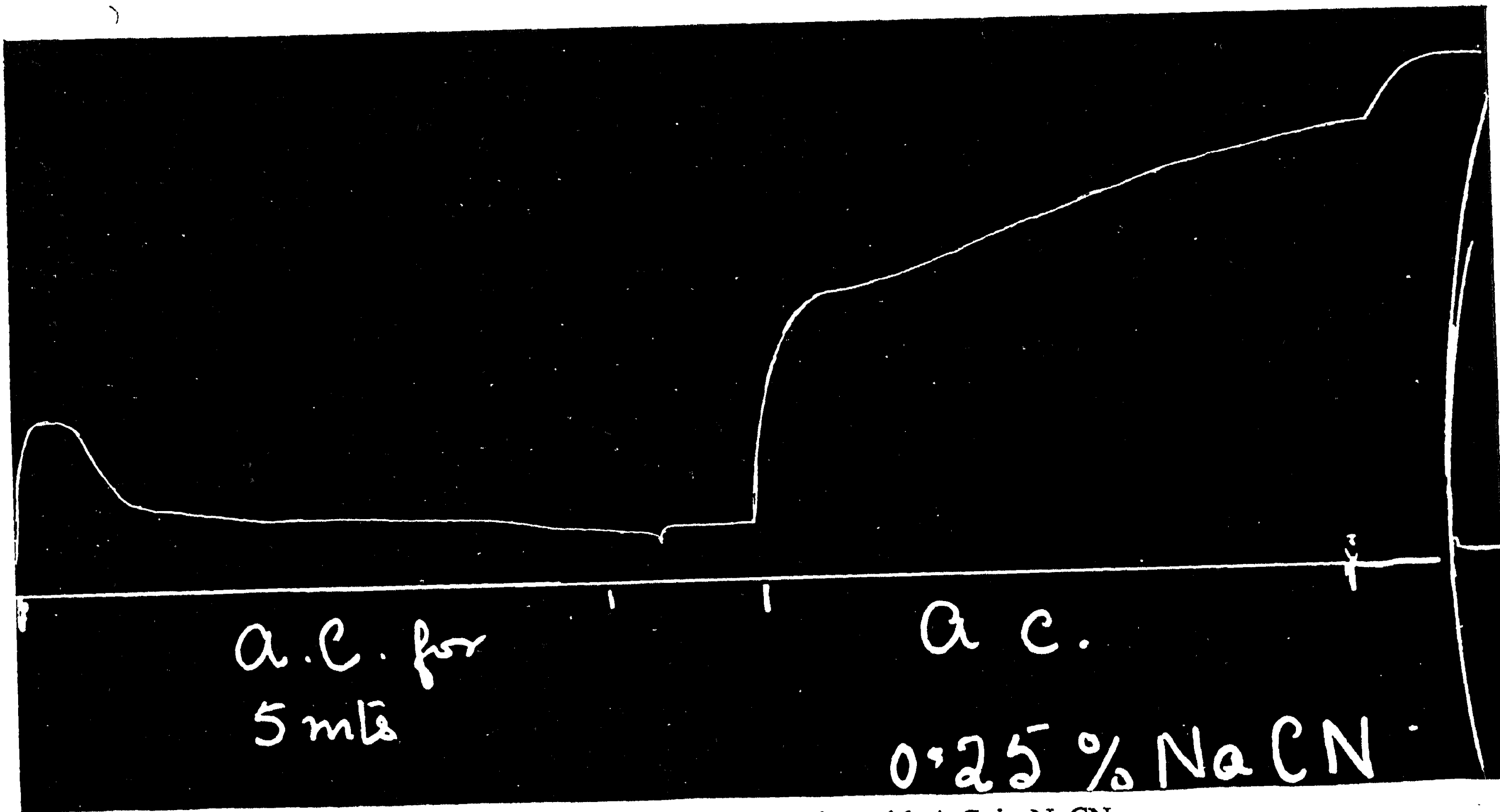


FIG. 2. *Mytilus* muscle. Stimulation with A.C. in NaCN