MECHANICAL AND ELECTRICAL RESPONSES OF UNSTRIATED MUSCLE IN SODIUM-FREE SOLUTIONS

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OVERTON (1902) demonstrated that frog muscles became inexcitable when they were immersed in isotonic solutions containing less than 10 per cent. of the normal sodium chloride concentration. He also showed that chloride ions were not essential constituent of Ringer's solution, since excitability was maintained in solutions of sodium nitrate, bromide, sulphate, phosphate, bicarbonate, benzoate, etc. Lithium was the only ion that could be substituted for sodium. Overton was unable to repeat his experiment with a frog's sciatic nerve, which maintained its excitability for long periods of time in salt-free solutions. This has been considered as due to retention of salt in the interstitial spaces of the nerve trunk. Thus Kato (1936) found that application of isotonic dextrose to single medullated fibres of the frog caused a rapid but reversible loss of excitability, and a similar result was obtained by Erlanger and Blair (1938) on the sensory roots of the bull frog. For other references see Hodgkin (1951).

Singh and Singh (1943) and Singh (1944) found that the stomach muscle of the frog, *Rana tigrina*, had the remarkable property, that it remained irritable in hypotonic (0·112 *M*) solution of sucrose for several hours. In the present research, these experiments have been extended to determine the role of sodium in excitability and action potentials; a preliminary report has been published elsewhere (Singh, 1957; Singh and Bhatt, 1957).

EXPERIMENTAL

In these experiments transverse pieces of the stomach muscle of the frog, *Rana tigrina*, were used. Some experiments were also performed on similar pieces of dog's stomach muscle, and on tænia coli of the guniea pig. The muscle was stimulated electrically with alternating current, 12 volts, 50 cycles, and direct current, 12 volts, by the method of Winton (1937). It was also stimulated with acetylcholine (1 in 100,000) and adrenaline (1 in million).

Sodium was estimated by the method of Weinbach (1935), as described in "Practical Physiological Chemistry" by Hawk, Oser and Summerson

1949), and potassium by the method of Looney and Dyer (1942) similarly lescribed. Preliminary digestion of the muscles was made with 50 per cent. ıitric acid as described by McDowall, Munro and Zayat (1955).

In the sucrose solution, using the Cossor Oscillograph, Model 1049, he action potentials were recorded by thrusting two fine copper wires into he muscle at two points; they were recorded when the muscle showed pontaneous contractions, the mechanical response being also recorded at he same time.

The muscle was immeresed in about 100 c.c. of sucrose and other sodium-ree solutions. This was changed every 15, or 30 or 60 minutes to remove odium from the muscle, as thoroughly as possible. After immersion for , or 4 or 6 hours, when contracting strongly, the muscle was removed and nalysed for sodium and potassium. The muscle usually weighed from ·5 to 1 gm. and the maxiumm tension it develops when stimulated lectrically is about 5 to 10 gm.

Results

Effect of sucrose.—In full tonic sucrose (0·224 *M*) the frog's stomach ıuscle loses all excitability, and may pass into a tonic contraction, presum-bly due to imbalance of ions without and within the muscle fibres. If about) per cent. of the sodium chloride of the saline is repalced with sucrose, ıen the mechanical response diminishes within a minute and becomes steady ı 10 to 25 minutes. This shows that there is no diffusion barrier against ›dium, such as might be due to a connective tissue sheath around the ıuscle fibres. Drugs, such as adrenaline, acetylcholine produce their effect most immediately, showing that they have no particular difficulty in :aching the surface of the fibres.

If the osmotic pressure of the sucrose solution is reduced by half, so that s strength is 0·112 *M*, then the muscle behaves differently. When the uscle is immersed in hypertonic sucrose solution, a phasic contraction produced at first. This is followed by a tonic contraction (Fig. 1), and e muscle does not respond to any stimulation. This period of depressed citability usually lasts for about 2 hours at 25–30° C., and longer at lower mperatures. The response then increases in magnitude and may be several nes bigger than that which occurs normally in saline. The muscle ›ntracts rhythmically at 25–30° C. in the hypotonic solution (Figs. 1, 2). ıe response gradually declines, till in 24 hours it is about 20 per cent. of ›rmal (Fig. 3). The response to alternating current in saline is bigger than at to direct current, but after some time in sucrose, reverse is the case, so that later on, it may not respond to alternating current, but continues to respond to direct current. Acetylcholine causes contraction, and adrenaline relaxation.

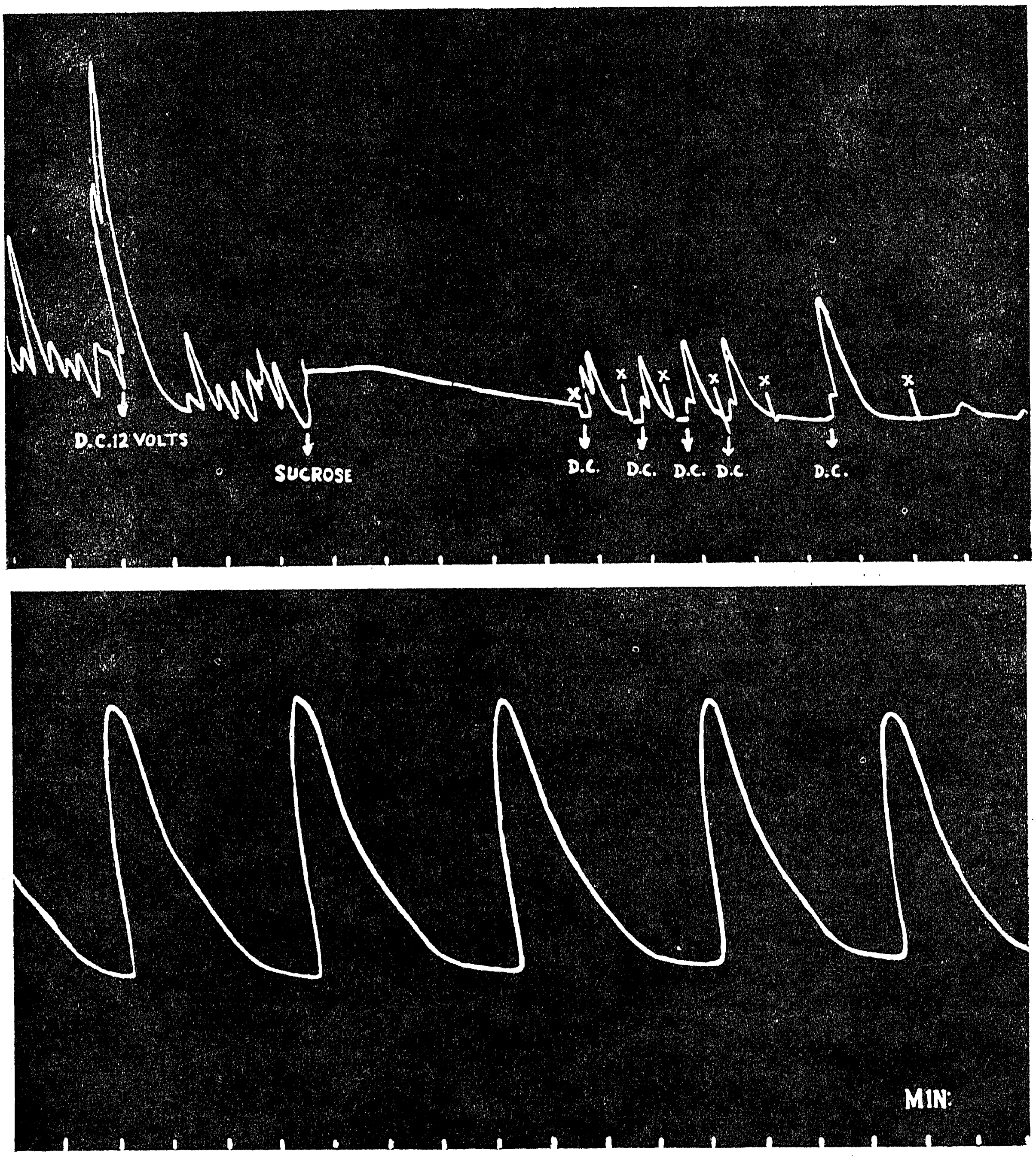

FIG. 1. Frog's stomach muscle. It was at first immersed in saline, then in hypotonic sucrose (0·112 *M*). At points marked D.C., it was stimulated with direct current, 12 volts for 10 sec. At X, the drum was stopped. Lower tracing shows rhythmic contractions in sucrose solution at the end of 3 hours. The sucrose solution was renewed every 15 minutes.

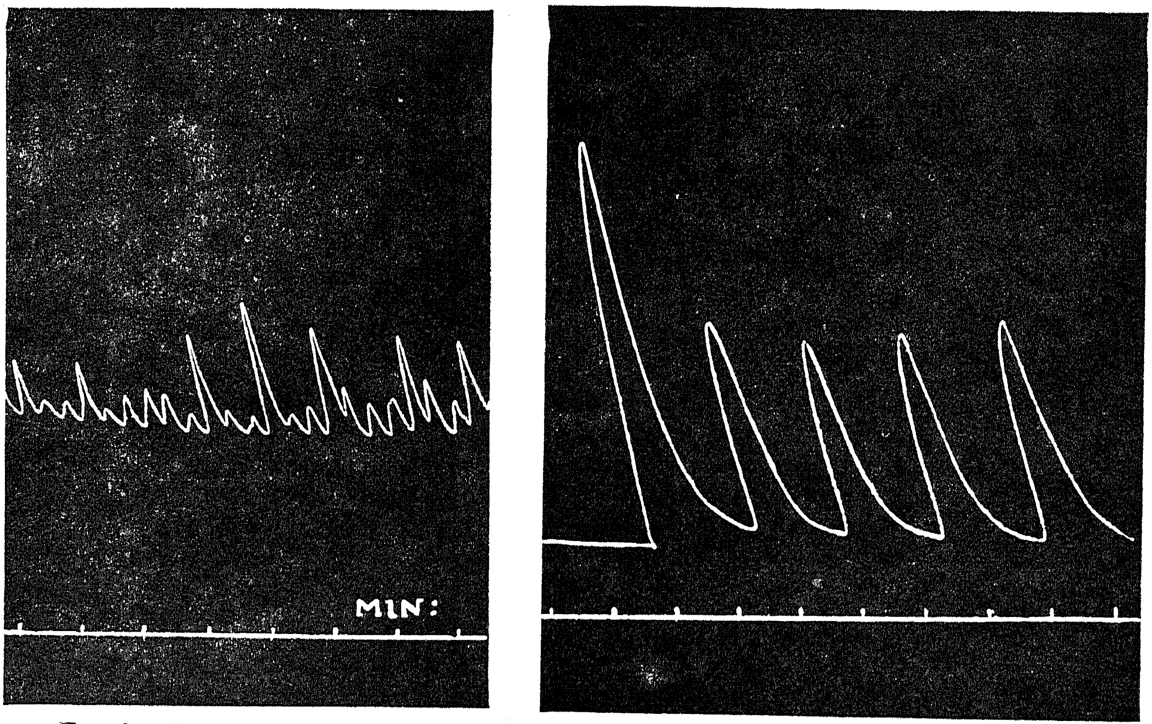

FIG. 2. Frog's stomach muscle. 1st tracing shows rhythmic contraction in saline. 2nd tracing shows rhythmic contractions 3 hours after hypotonic sucrose (0·112 *M*).

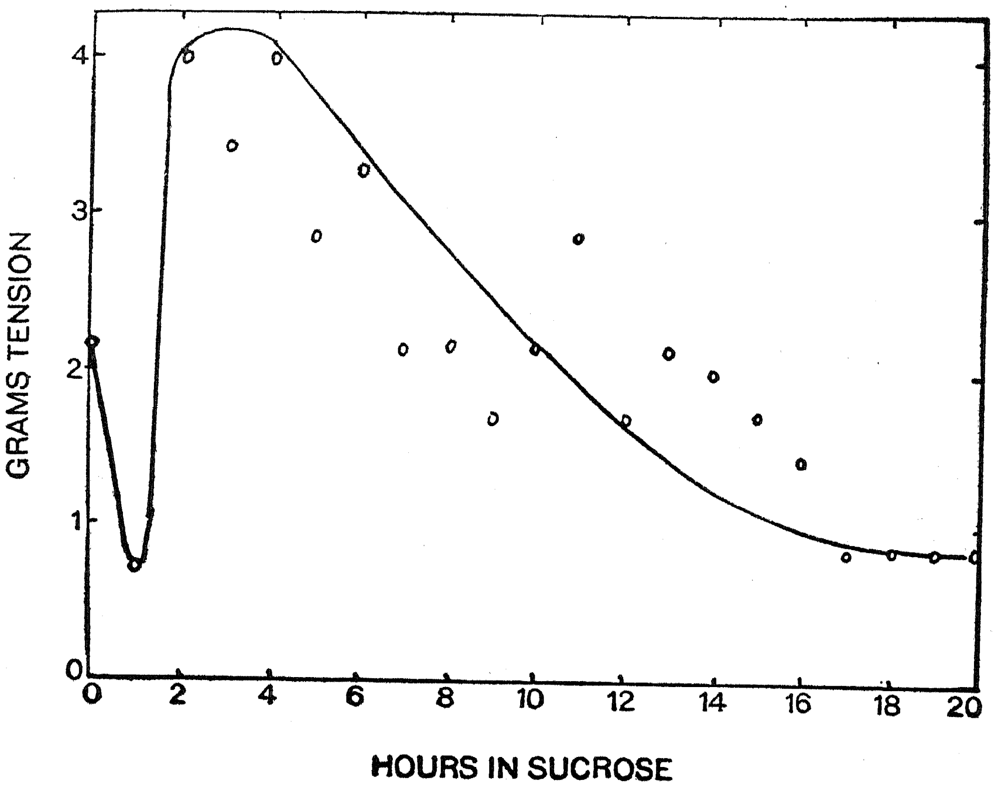

FIG. 3. Frog's stomach muscle. Effect of hypotonic (0·112 *M*) sucrose.

Spontaneous contractions are a companied by irregular electrical oscillations, suggesting asynchronous contraction of fibres (Fig. 4). When the muscle is contracting strongly, chemical analysis shows that it does not contain any significant amount of sodium. No precipitate is obtained with uranyl zinc acetate.

When the muscle is contracting strongly, or afterwards, reintroduction of sodium chloride causes immediate depression of excitability. The spontaneous contractions are abolished for about 2 hours, before they return to normal (Fig. 5). Thus the muscle at first acclimatises to sucrose. When it has thus acclimatised, sodium is found to be toxic. Similarly, it acclimatises to sodium.

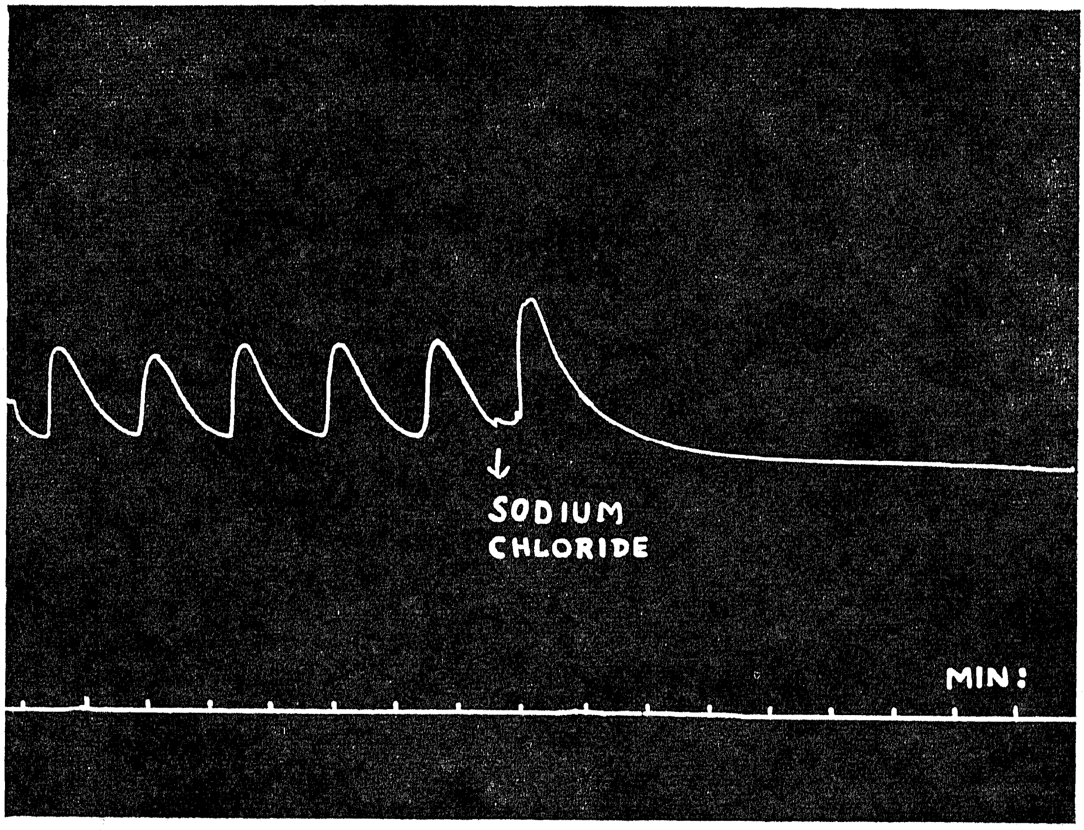

FIG. 5. Frog's stomach muscle. The muscle is contracting rhythmically in hypotonic (0·112 M) sucrose. Immersion in 0·112 M sodium chloride produces immediate depression of the mechanical response.

The measure of excitability of the muscle in these experiments is the height of spontaneous contractions, as the variation in the mechanical response due to electrical stimulation might be due to changes in conductivity of the solution containing varying quantities of electrolyte. In these experiments, the silver or silver chloride electrodes were placed at a distance of 1 to 2 inches above and below the muscle in the muscle chamber and the muscle contracted when stimulated electrically even though the intervening sucrose solution was practically non-conducting. The sciatic nerve from the frog *Rana tigrina* can be similarly stimulated, though the electrodes may be placed in the sucrose solution laterally at a distance.

The dog's stomach muscle becomes inexcitable in hypotonic sucrose, but responds to electrical and chemical stimulation for about 12 hours if the temperature is kept from 18 to 20° C. The tænia coli of the guinea pig become inexcitable in about 10 min. The sartorius and gastrœnemins muscle from the frog *Rana tigrina* become inexcitable in an hour's time, but the sciatic nerve responds for over 12 hours when teased into smaller bundles. The heart from the same frog responds for about an hour both electrically and mechanically (Singh, Sehra and Singh, 1945 *a*, *b*). There is, however, great variability in its reaction to sucrose; some frog's hearts become inexcitable within 10 minutes in sucrose solution. The neuro-muscular junction however is more susceptible to sodium lack than either the nerve or muscle (Singh and Singh, 1948).

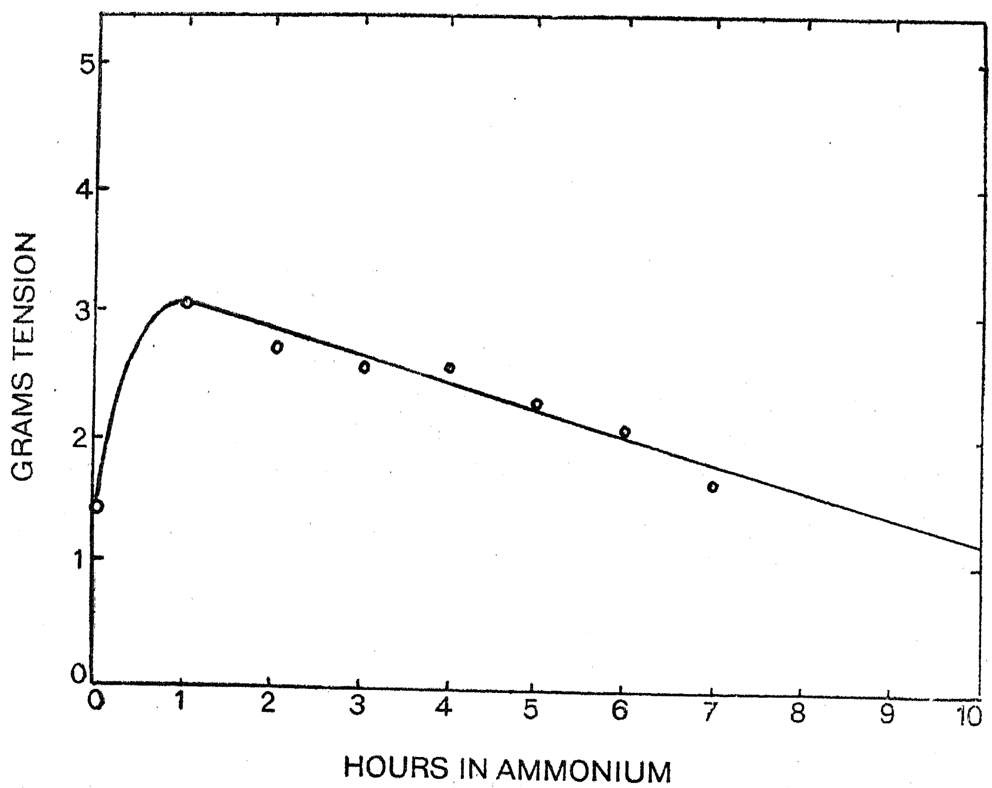

FIG. 6. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic ammonium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

Effect of other ions.—The frog's stomach muscle is remarkable in the fact that it gives apparently normal mechanical responses if the sodium of the saline is completely replaced with other ions, such as potassium, ammonium, calcium, magnesium and strontium (Singh and Singh, 1947). In ammonium, magnesium, calcium and strontium the responses are bigger than in saline (Figs. 6, 7, 8, 9), but in potassium chloride they are depressed, though the muscle responds for about 12 hours (Fig. 10). The sodium

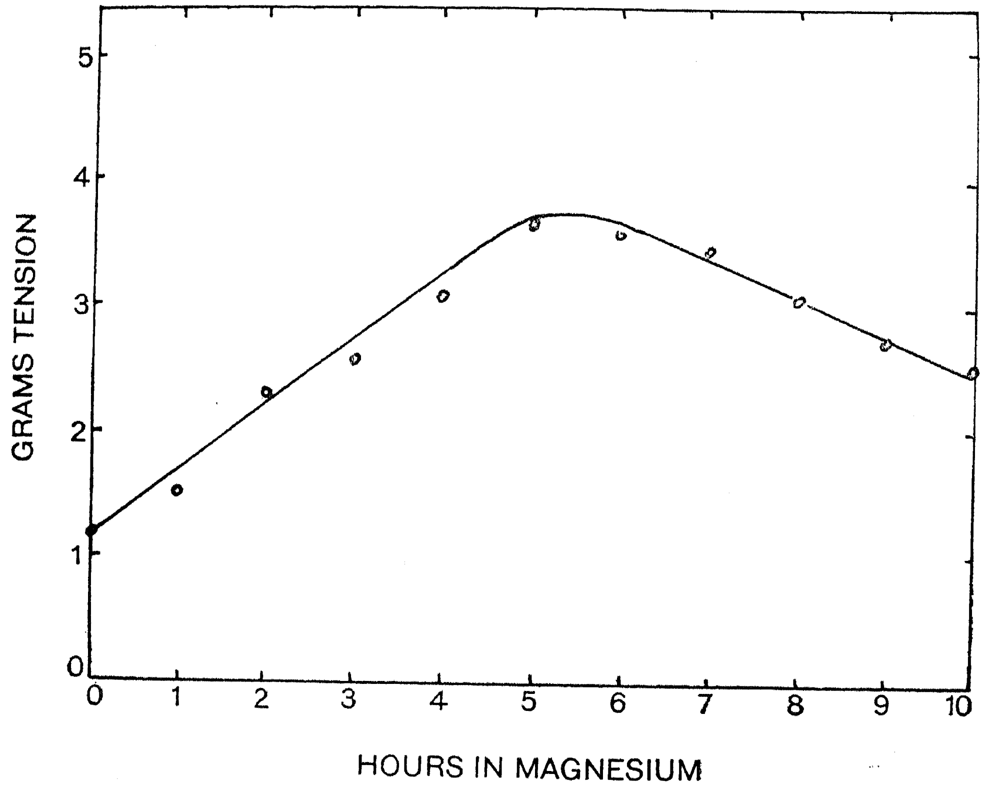

FIG. 7. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic magnesium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

content of these muscles is such that it does not give a precipitate with uranyl zinc acetate; it is thus negligible. The muscle shows spontaneous contractions in ammonium, calcium, magnesium and strontium chlorides (Fig. 11), but not in potassium chloride.

After the muscle has acclimatised to sucrose it becomes quite sensitive to small concentrations of calcium which are normally present in the saline. These small concentrations (0·003 *M* $CaCl_2$) produce a tonic contraction. Sodium chloride usually produces a twitch, but it may also produce a tonic contraction. Potassium chloride acts likewise.

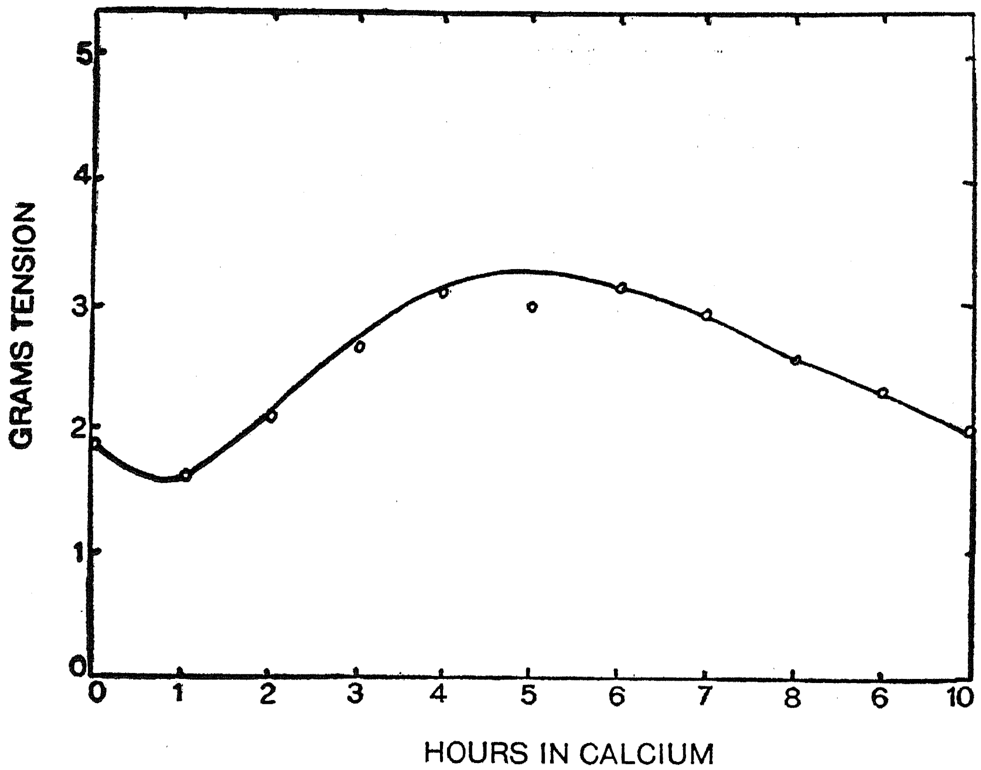

FIG. 8. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic calcium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

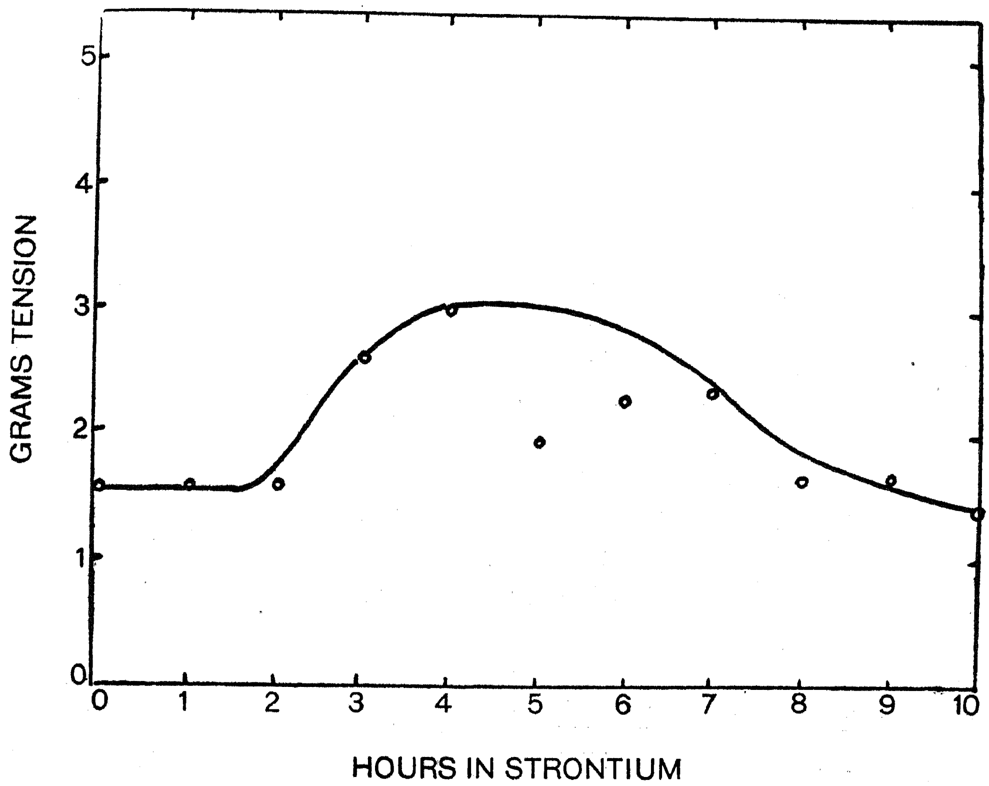

FIG. 9. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic strontium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

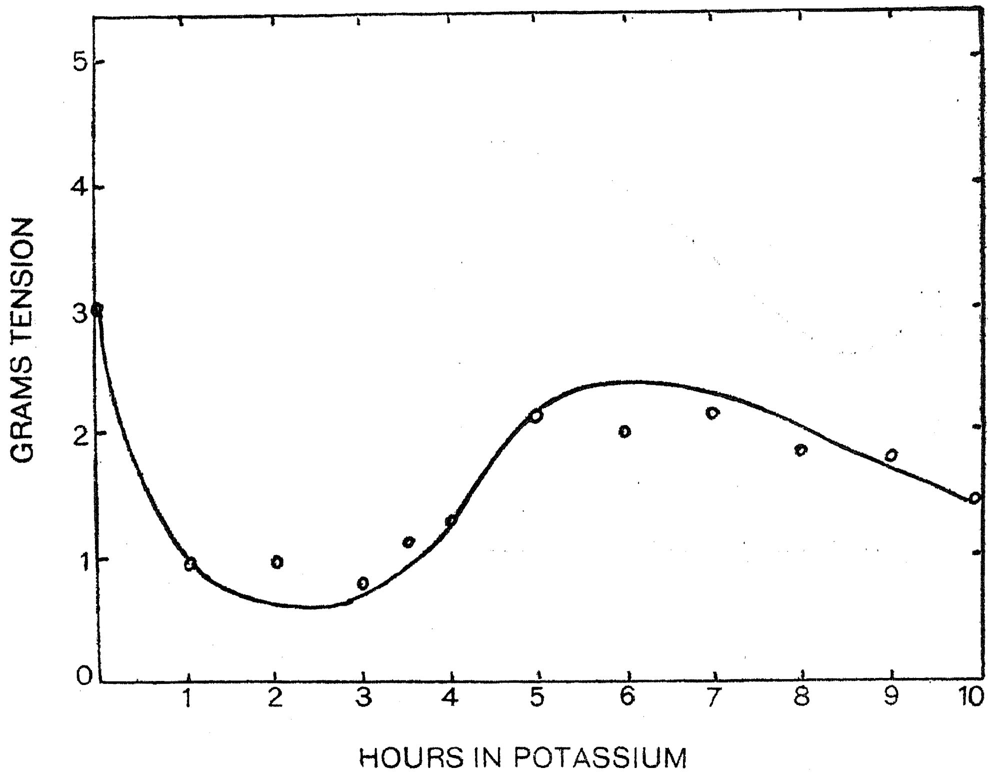

FIG. 10. Frog's stomach muscle. The effect of saline, the sodium of which had been replaced with potassium, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

When immersed in sucrose, the behaviour of frog's stomach muscle from different animals shows variations; muscles from some animals become inexcitable in 4 to 5 hours, whilst others remain excitable for over 24 hours. This variability is partly due presumably to loss of potassium, as the irritability of the muscles is maintained for longer periods, if a little potassium is added to the sucrose solution, the optimum concentration being 0·0036 *M* KCl. Even much smaller concentrations of potassium chloride, such as one-fifth of the above amount keep the muscles in a good excitable condition for several hours. Small concentrations of calcium chloride, normally present in the saline, do not exert any beneficial effect. On the contrary the duration during which the muscle remains excitable may become shorter. Calcium in the normal saline is therefore required to antagonise sodium. The gradual diminution of the response in sucrose as shown in Fig. 3, is partly due presumably to the loss of potassium, as shown by preliminary potassium estimations in the muscle; a full account of these estimations will be presented in a subsequent paper.

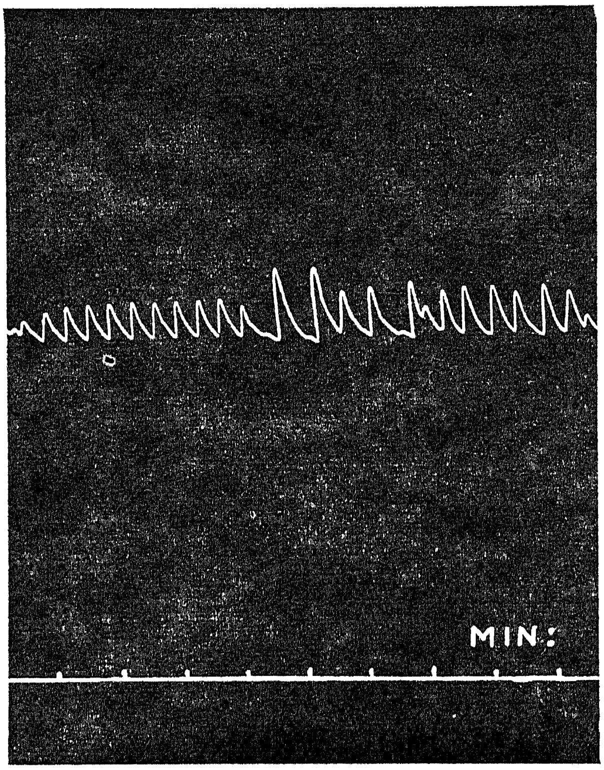

FIG. 11. Frog's stomach muscle. Spontaneous contraction in saline, the sodium chloride of which had been replaced with iso-osmotic strontium chloride, after 10 hours immersion and frequent renewal of the solution.

DISCUSSION

These experiments leave no doubt as to the dispensability of sodium for excitability and action potentials. The quantity of sodium present in the muscle soaked in sodium-free solutions must be utterly negligible. It may be argued, however, that the muscle responds owing to retention of sodium behind some connective tissue sheath which prevents its diffusion outwards. That this is not likely is shown by the fact that there appears to be no such diffusion barriers for sodium and other substances.

The crucial experiment which excludes the above possibility, is the one which demonstrates the depressant action of sodium after the muscle has acclimatised to sucrose (Fig. 4). As a matter of fact, more thoroughly the soduim is washed out, such as by changing the surrounding sucrose solution every 15 minutes, the better the muscle behaves. If the excitability of the muscle was due to retention of sodium in the interspaces between the fibres, such a depressant action of sodium could not occur. When the muscle has acclimatised to surcose, sodium becomes toxic.

The clue to the action of sodium in excitability is provided by the action of other ions. The frog's stomach muscle is unique in another way, that it responds in the presence of large concentrations of other ions such as ammonium, potassium, magnesium, calcium and strontium, which usually render other tissues rapidly inexcitable. This shows that the membrane of frog's stomach muscle fibres is comparatively resistant, and the action of sodium is to increase this resistance. In the membrane of frog's muscle, there must be some other factor present which allows sodium to be dispensed with. In tissues which become inexcitable in the absence of sodium, this factor must be deficient. The variability in the response of various tissues, or seasonal variations, are then probably due to the variable action of this second factor. The membrane of frog's stomach muscle is therefore comparatively resistant, and hence the muscle can be deprived of sodium, and still maintain its excitability.

Since the nature of excitability and the electrical response must have a common basis in all biological tissues, it appears to us that the role required of sodium in the hypothesis of Hodgkin and Katz (1945) is very unlikely.

When the muscle is immersed in full tonic sucrose solution there is a great imbalance of ions within and without the muscle fibres, hence the muscle becomes inexcitable. The recovery of excitability in half-tonic sucrose solution is due to the fact, that in a hypertonic solution, the entrance of water into the muscle fibres would reduce the concentration of ions within the fibres, and thus partly remove the ionic imbalance within and without the fibres. This view is supported by the fact, that if there is excess of potassium outside the fibres, its depressant effect is increased if the saline is rendered hypotonic, and decreased, if the saline is rendered hypertonic (Singh, 1939). Increase in tonicity of the saline would increase the concentration of potassium inside the fibres (Gokhale and Singh, 1945) and thus offset the increase of such concentration outside the fibres. Hypotonic solution would have an opposite effect. This view is supported by the fact that the depressant effect of increase in osmotic pressure of the saline is antagonised by increase in concentration of potassium outside the fibres (Singh, 1945).

SUMMARY AND CONCLUSIONS

1. If frog's stomach muscle is immersed in hypotonic sucrose solution, it maintains its excitability for about 24 hours, after a preliminary depres-

sion. The responses in the sucrose solutions may be several times bigger than in saline.

2. The spontaneous contractions of the muscle in sucrose solution are accompanied by action potentials.

3. When the muscle is contracting strongly in the sucrose solution, the sodium content (of the muscle) is negligible thus showing that sodium is not necessary for the production of mechanical and electrical changes.

4. When the muscle has acclimatised to sucrose, sodium has a depressant action, showing that the mechanical and electrical responses of the muscle are not due to any retention of sodium in the interspaces between the fibres.

5. The muscle also responds as in sucrose solution, if all the sodium of the saline is replaced with ammonium, potassium, calcium, strontium and magnesium. This suggests that the membrane of this tissue is comparatively resistant and hence this muscle can be deprived of sodium, and still maintain its excitability.

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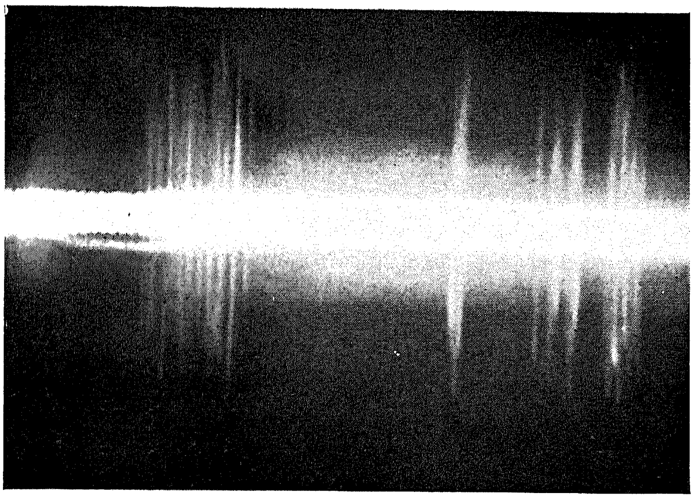

FIG. 4. Frog's stomach muscle. Action potentials due to spontaneous contractions after 3 hours immersion in hypotonic ($0 \cdot 112\ M$) sucrose.