IONIC CHANGES IN UNSTRIATED MUSCLE IMMERSED IN SODIUM-FREE SOLUTIONS

By Inderjit Singh, F.A.Sc. and A. K. Acharya

(From the Department of Physiology, Medical College, Agra)

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Singh and Singh (1943), Singh (1944, 1957) and Singh and Bhatt (1957 *a*, *b*), 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 over 24 hours. They found that the muscle at first acclimatises to sucrose solution, and the contractions in sucrose are several times bigger than in saline and are accompanied by action potentials. When the muscle has acclimatised to sucrose, introduction of sodium chloride suppresses the spontaneous contractions till the muscle has acclimatised again to sodium. This shows that the spontaneous contractions could not be due to the retention of sodium in the interspaces between the fibres. This muscle has another remarkable property that it remains irritable in sodium-free solutions of other ions, such as ammonium, potassium, calcium, strontium and magnesium chlorides.

In the present research these experiments have been extended and the sodium and potassium content of the muscle and experimental solutions have been determined under various experimental conditions.

Material and Methods

Transverse pieces of the stomach muscle of the frog, *Rana tigrina*, were used. They were suspended in saline (Singh, 1939) in the muscle chamber, and then contractions recorded. The saline was then replaced with hypotonic sucrose solution (0·112 *M*), or other sodium-free solutions. These were renewed every 15 minutes. At the end of 4 hours, when the spontaneous contractions were several times bigger than in saline, the muscle was removed for analysis.

The muscle was gently blotted between ash-free filter-papers and weighed. It was dried by heating at 105° C. for 24 hours. It was then digested in 50 per cent. nitric acid as described by McDowall, Munro and Zayat (1955). Sodium was estimated by EEL's flame photometer.

These experiments were performed at room temperature, 30° C.

Results

Sodium in the sucrose solution.—Even the purest sucrose (AR, B.D.H.) contains traces of sodium but no potassium, as shown by the flame photometer; the colour of the flame is no more yellow, but still a slight deflection of the galvanometer occurs when tested for sodium. The hypotonic solution of sucrose, 0·112 *M*, used in these experiments, contained 0·0035 m.Eq. of sodium per litre. That this trace of sodium in the external medium is of no importance for excitability, is shown by the fact that in the summer, the heart and the sartorius muscle from the same animal loose their excitability completely within 10 minutes of immersion in the sucrose solution. In the winter season, they last longer, remaining excitable for about an hour. The frog's stomach muscle, however, behaves better in summer than in winter.

When the muscle is immersed in sucrose solution, sodium begins to diffuse out. The outward diffusion of sodium from the muscle into the sucrose solution was tested by immersing the muscle in 100 ml. of oxygenated solution and analysing the solution at the end of every 15 minutes, the sucrose solution being renewed each time. At the end of 2½ hours, very little sodium comes out of the muscle, and at this time the spontaneous contractions begin (Table I). Thus when the muscle has acclimatised to sucrose, the more the sodium is removed from the interspaces, the better it contracts. The contractions in the sucrose solution, therefore, could not be due to retention of any sodium in the interspaces.

TABLE I

Frog's stomach muscle. The amount of sodium leaking out of the muscle into 100 *ml. of hypotonic,* 0·112 M *sucrose*

Time of immersion in sucrose (minutes)	m.Eq. of sodium per litre of sucrose solution
0	112
15	22·2
30	4·7
45	1·2
60	0·8
90	0·4
120	0·08
150	0·0035
180	0·0035

The above conclusion is also substantiated by the fact, that re-immersion of the muscle in sodium chloride solution, completely suppresses the spontaneous contractions. This also happens, if the sodium chloride contains a little calcium chloride, normally present in the saline (Fig. 1). Suppression of the spontaneous contractions is still produced if the sodium chloride solution besides containing calcium, also contains a little potassium normally present in the saline.

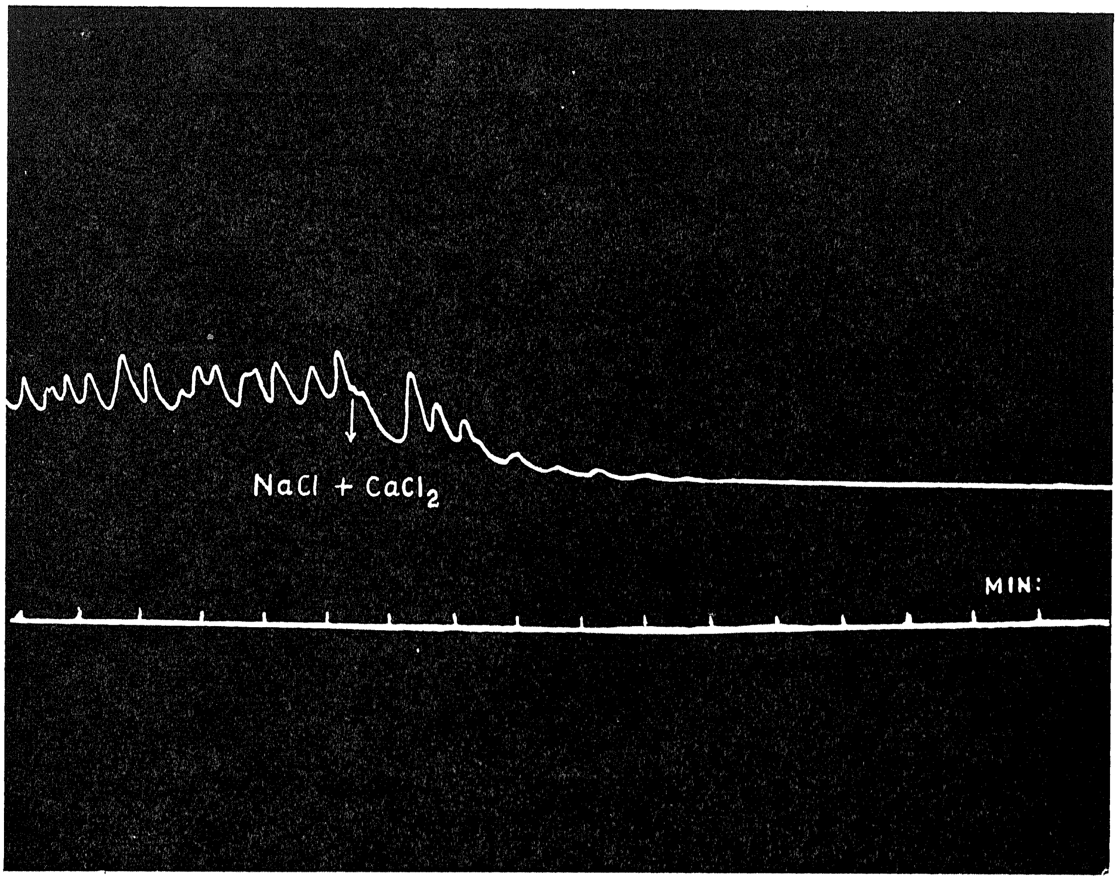

FIG. 1. Frog's stomach muscle. The muscle is contracting rhythmically in hypotonic (0·112 *M*) sucrose. Immersion in 0·108 *M* sodium chloride with 0·00412 *M* calcium chloride produces immediate depression of tone and the spontaneous contractions.

Sodium and potassium in the muscle.—The sodium and potassium content of 12 muscles immersed in hypotonic sucrose, 0·112 *M*, are shown in Table II. The mean sodium concentration in the muscle is 0·0018 m.Eq. per ml. of water in the muscle. The mean potassium concentration is 0·03 m.Eq. The total sodium and potassium is equal to 0·0318 m.Eq. per ml.

The potassium content of unsoaked muscle is about 0·06 m.Eq. per gram of wet muscle (Gokhale and Singh, 1945). The potassium content of muscle, soaked in sucrose solution, is therefore, about half this amount, owing to the hypotonicity of the solution.

The mechanical response of the muscle gradually declines in sucrose solution. This is presumably due to leakage of potassium. The sodium

TABLE II

Sodium and potassium content of frog's stomach muscle immersed in 0·112 M sucrose solution for 4 hours, exhibiting vigorous spontaneous contractions; these are intracellular ions

Serial No.	m.Eq.			
	Na		K	
	Per gram of wet muscle	Per ml. of muscle water	Per gram of wet muscle	Per ml. of muscle water
1	0·0015	0·0017	0·028	0·033
2	0·0015	0·0008	0·025	0·030
3	0·0019	0·0024	0·025	0·031
4	0·0019	0·0025	0·026	0·035
5	0·0010	0·0012	0·027	0·033
6	0·0010	0·0012	0·024	0·036
7	0·0020	0·0024	0·028	0·034
8	0·0015	0·0016	0·017	0·020
9	0·0017	0·0020	0·020	0·020
10	0·0015	0·0019	0·020	0·025
11	0·0012	0·0015	0·022	0·027
12	0·0019	0·0021	0·029	0·032
Mean ..	0·0016	0·0018	0·024	0·030
S.D. ..	±0·0003	±0·0005	±0·003	±0·005

TABLE III

Sodium and potassium content of frog's stomach muscle immersed in 0·112 M sucrose solution at different intervals; these are intracellular ions

Sl. No.	m.Eq. per gram of wet muscle					
	After 1 hour		After 5 hours		After 9 hours	
	Na	K	Na	K	Na	K
1	0·0045	0·038	0·0017	0·021	0·0012	0·016
2	0·0034	0·022	0·0016	0·010	0·0013	0·008
3	0·0030	0·036	0·0023	0·030	0·0017	0·020
Mean ..	0·0036	0·032	0·0019	0·020	0·0014	0·015
S.D. ..	±0·0006	±0·007	±0·0004	±0·008	±0·0003	±0·005

and potassium contents of the muscle soaked in various periods in the hypotonic sucrose solution are shown in Table III.

Discussion

These experiments show that it is the intracellular potassium rather than the extracellular sodium that is mainly responsible for excitability. Various other experiments point to the same conclusion. Thus the phasic response of the muscle is related to its intracellular potassium. It increases with increase of the osmotic pressure of the medium to 1·4 times normal (Singh) 1939), which increases the intracellular concentration of potassium by about 35 p.c. (Gokhale and Singh, 1945). The muscle is excited if the osmotic pressure of the medium is increased suddenly to 1·4 times normal (Singh, 1942). Similarly the muscle is excited by relative increase in the intracellular concentration of ions caused by withdrawal of ions from the medium by immersing the muscle in sucrose solution. The muscle is also excited if there is absolute or relative increase in the concentration of extracellular ions. Thus excess of potassium outside causes contraction.

An interesting feature is that the intracellular potassium can be partly replaced with ammonium (Gokhale and Singh, 1945), and if after immersion in saline containing ammonium, the muscle is re-immersed in ammonium-free saline, a contraction is caused (Singh, 1939). Thus a difference in concentration of ammonium on two sides of the muscle membrane stimulates the muscle.

Tonic contraction of the muscle is presumably due to the action of intracellular ions on the actomyosin system. Increase in the intracellular concentration of potassium increases the phasic response as well as one kind of tone, but decreases another kind of tone (Singh, 1939). This suggests that in the muscle, there are two systems of polypeptide chains, one responsible for the phasic and one kind of tonic response, and the other for the second kind of tonic response. These two systems can be differentially destroyed; thus sudden stretching destroys one kind of phasic response (Singh and Singh, 1951 *a*, Singh, 1952) or the second kind of tonic response (Singh and Singh, 1949, 1950 *a*). One of these systems relaxes actively, and the other passively (Singh and Singh, 1950 *b*, 1951 *b*).

The frog's stomach muscle, immersed in sucrose solution, can be stimulated if the electrodes are placed at a distance of about 1–2 inches on either side of the muscle. The intervening solution is practically non-conducting, so that it might appear that the contractile system of the muscle is stimulated by the longitudinal electrical field (Singh, 1944; Singh and Bhatt, 1957 *b*). It is tempting to assume that this experiment provides the missing link between the excitability and the contractile systems of the muscle and supports the "Window field" theory (Szent Gyorgyi, 1953). This would be if the muscle responds to electric current only. But the muscle contracts spontaneously, and reacts to adrenaline and acetylcholine. In fact, it behaves in every way like a normal muscle, though, there are no ions in the external medium. This shows that its excitatory system is functioning normally. The muscle is undoubtedly stimulated by the longitudinal electrical field (0·8 cm./volt), but the action is on the membrane and not on the contractile mechanism.

The frog's stomach muscle also responds if the sodium of the saline is partly or completely replaced with potassium (Singh and Singh, 1947; Singh and Bhatt, 1957); this abolishes the sodium and potassium concentration gradients across the muscle membrane. It would again be tempting to assume that the contractile system of the muscle is stimulated by the longitudinal electrical field, if it responded only to electrical current. But since it also responds to acetylcholine, we presume that the electrical field acts on the membrane and not on the contractile mechanism; we are, therefore, unable to agree with Csapo (1954).

SUMMARY AND CONCLUSIONS

1. Frog's stomach muscle responds when immersed in hypotonic sucrose solution. The more thoroughly, sodium is eliminated from the external medium, the better it responds.

2. After the muscle has acclimatised to sucrose solution, re-immersion in solution of sodium chloride, or sodium chloride containing a little calcium or potassium abolishes the spontaneous contractions.

3. The sodium and potassium concentration in the muscle after immersion in sucrose for 4 hours becomes 0·0018 m.Eq. and 0·03 m.Eq. respectively per ml. of water in the muscle.

4. The mechanical response of the muscle in sucrose solution varies as the intracellular potassium.

5. These experiments suggest that intracellular potassium, and not extracellular sodium, that is mainly responsible for excitability of the muscle.

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