*J. Physiol.* (1963), **166**, *pp.* 29–58
*With* 14 *text-figures*
*Printed in Great Britain*



# EFFECTS OF CHANGES IN THE EXTERNAL SODIUM AND CALCIUM CONCENTRATIONS ON SPONTANEOUS ELECTRICAL ACTIVITY IN SMOOTH MUSCLE OF GUINEA-PIG TAENIA COLI

BY EDITH BÜLBRING AND H. KURIYAMA
*From the Department of Pharmacology, University of Oxford*

(*Received* 13 *April* 1962)

The main differences between the electrical properties of the smooth muscle cells of taenia coli and those of skeletal muscle cells are as follows: In the taenia the membrane potential is lower; the spike amplitude is variable; the maximum rate of rise and fall of the spike is lower, and the cells are capable of generating spontaneous spikes as well as propagating excitation (Bülbring, 1954, 1955, 1957; Holman, 1958; Bülbring, Burnstock & Holman, 1958; Kuriyama, 1963). Holman (1957, 1958) observed that spike generation continued indefinitely in solutions containing 5 mM-$Na^+$ (replacement with sucrose and choline) but was abolished in 2 mM-$Na^+$. The spike height was normal in concentrations as low as 20 mM-$Na^+$. Using the sucrose-gap method similar observations were made after partial or complete substitution of $Na^+$ with lithium or hydrazine by Burnstock & Straub (1958), Axelsson, Bueding & Bülbring (1959), and Axelsson (1961). Nevertheless, in general Holman accepted the ionic hypothesis put forward by Hodgkin (1951) as the basis of electrical activity in this muscle.

In other smooth muscle tissues similar observations have been described. In $Na^+$-free or $Na^+$-deficient solution uterine myometrium could generate spikes (Daniel & Singh, 1958*a*, *b*; Kuriyama, 1961). In frog stomach muscle Kolodny & van der Kloot (1961) recorded spike discharges in sodium-free solution and also in ion-free sucrose solution of half the normal osmotic pressure (0·12 M), using micro-electrodes and confirming the results of Singh & Acharya (1957) obtained with macro-electrodes.

The essential role of the calcium ion for the generation of the spontaneous discharges has, however, been emphasized by all investigators, excepting Kolodny & van der Kloot (1961). In taenia coli calcium-free solution causes a depolarization of the membrane by 10–20 mV (Burnstock & Straub, 1958; Holman, 1958; Axelsson, 1961). The effect of calcium deficiency on electrical activity, however, varies in different experiments.

Usually the spike frequency is transiently increased and subsequently stopped, but occasionally spike discharge ceases without the initial acceleration (Axelsson, 1961; Bülbring, Kuriyama & Twarog, 1962).

In excess calcium the membrane potential and spike amplitude is increased while the spike frequency is decreased. Yet again in some experiments, during the progress of hyperpolarization of the membrane, the spike frequency is transiently accelerated (Bülbring *et al.* 1962). In uterine smooth muscle similar confusing results on the effect of varying the calcium concentrations on the membrane activity have been observed (Coutinho & Csapo. 1959; Goto & Csapo, 1959; Hurwitz, Tinsley & Battle, 1960; Kuriyama & Csapo, 1961) and also in cardiac muscle (Weidmann, 1955, 1956; Cranefield & Hoffman, 1958).

From the observations reported in the preceding paper (Kuriyama, 1963) it was concluded that calcium may act by diminishing the extent to which the high sodium conductance affects the membrane potential of taenia coli.

The influence of sodium and calcium on membrane activity was investigated in the experiments to be described in this paper. It was found that the rate of rise and fall, but not the amplitude of the action potential, were related to the external sodium concentration. On the other hand, both the rate of rise and fall, and the amplitude of the spike were a function of the external calcium concentration.

Some of the results have been communicated at the First International Pharmacological Meeting in Stockholm (Bülbring & Kuriyama, 1961) at the Symposium on Vascular Smooth Muscle in Washington (Bülbring, 1962) and at a meeting of the Physiological Society (Bülbring *et al.* 1962).

## METHODS

The smooth muscle of the taenia coli of the guinea-pig was used as described previously (Bülbring, 1954, 1955, 1957; Kuriyama, 1963).

The normal Krebs's solution used in all experiments contained (mM): Na 137·4, K 5·9, Mg 1·2, Ca 2·5, Cl 134, $H_2PO_4$ 1·2, $HCO_3$ 15·5, glucose 11·5; and was aerated with 97% $O_2$+3% $CO_2$. Sodium excess solution was prepared by adding sodium ethanesulphonate (Goodford & Ing, 1959) or sodium toluene-*p*-sulphonate, thereby avoiding other changes of ionic composition. The sodium-free or sodium-deficient solutions were generally prepared with Tris chloride. We used Sigma 7–9, which is a preparation of Tris (hydroxymethyl) amino methane (Sigma Chemical Company, U.S.A.) and which was converted to Tris chloride by titration with HCl to pH 7·3. Other substitutes used were sucrose, choline, tetraethylammonium (TEA) and lithium. The $NaHCO_3$ was replaced with $KHCO_3$, KCl and $KH_2PO_4$ being omitted. The pH was adjusted to 7·3. Chloride-deficient solution was prepared by replacing all the sodium chloride with sodium ethanesulphonate, and KCl with $KHCO_3$. However, the solution still contained 6 mM chloride as $CaCl_2$ and $MgCl_2$.

Atropine sulphate and cocaine hydrochloride were used and the concentrations are given as weight per volume.

## RESULTS

### *Normal electrical activity*

*The local potentials (or slow waves, or generator potentials)*

As described previously (Bülbring *et al.* 1958; Holman, 1958), the spontaneous discharge consisted of local potentials or slow waves as well as spike potentials. It is at present not always possible to distinguish clearly between a slow local depolarization of pace-maker type in the impaled cell and a graded potential due to electrotonic spread of activity from active neighbouring cells. The former type, when clearly distinguishable, has been considered here.

The amplitude of the local potential varied from 5 to 20 mV (mean value 13 mV $\pm$ 0·6, $n = 75$) and the rate of rise was 0·05 $\pm$ 0·006 V/sec, the half-duration was 300–1200 msec (mean value 450 $\pm$ 50 msec). If the local potential was of long duration it often triggered two and occasionally three spikes. The size of these spikes was not the same, sometimes the first, sometimes a subsequent spike being larger. The magnitude of the local potential had also no relation to the magnitude of the spike. Local potentials of similar size often triggered action potentials of different size. Many spikes appeared without any relation to the local potential, confirming the observation (Bülbring *et al.* 1958) that in the cells of taenia coli both pace-maker activity and propagated activity may be observed side by side in the same cell.

Atropine ($10^{-4}$) and cocaine ($10^{-5}$) had no effect on the local potential.

*The action potentials*

Changes in frequency of spike discharge were usually associated with changes in membrane potential. During the slow fluctuations of the membrane potential a polarization up to 70 mV was sometimes observed. Such periods lasted from 10 to 100 sec and were accompanied by a decrease in the frequency or cessation of spike discharge (Fig. 1*a*). Sometimes the discharge ceased without the slow polarization of the membrane. Figure 1*b*, *c*, *d* shows three possible mechanisms of block. In (*b*) a sequence of pace-maker potentials is interrupted as the slow pre-potential fails to initiate a spike and only produces a small wave. In (*c*) a series of propagated spikes is interrupted, owing to block of conduction. In (*d*) the spike discharge is blocked by a sudden hyperpolarization reminiscent of the inhibitory polarization observed in the slow stretch receptors of the crayfish by Eyzaguirre & Kuffler (1955).

The parameters of the membrane activity in Krebs's solution at 35 °C are shown in Table 1. The mean value of the membrane potential was

measured at the time of maximum polarization between spikes in the spontaneously active membrane.

The amplitude of the spikes was irregular and an overshoot was not always observed. The simple uncomplicated spikes had a greater amplitude than the complicated shapes and usually the propagated spike was bigger than the locally initiated action potential.

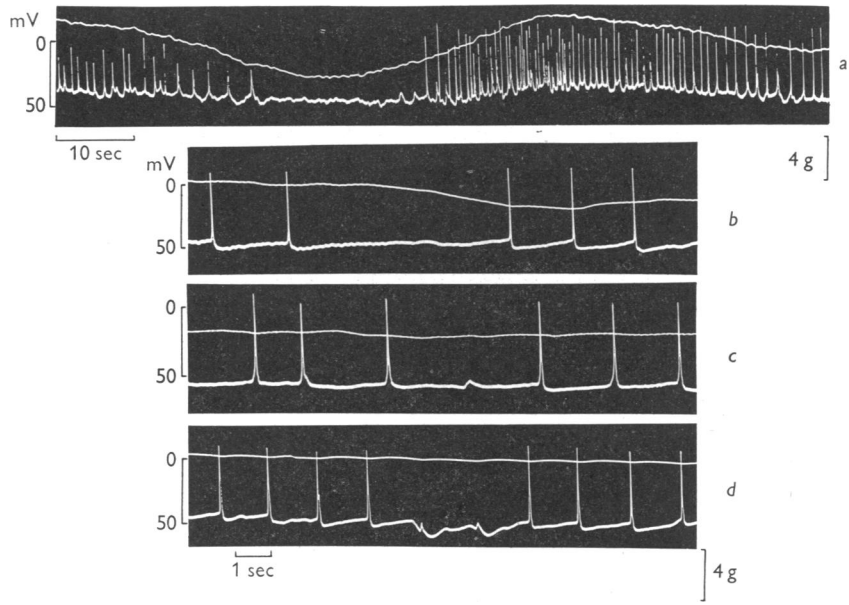


Fig. 1. Patterns of spontaneous activity in taenia coli. Records of isometric tension and membrane potential. *a* shows block of spike discharge associated with a rise in membrane potential. *b*, *c* and *d* show different types of block, described in the text.

The duration of the action potential measured at 50 % of its amplitude (half-duration) was similar to that recorded by Holman (1958). However, the average maximum rates of rise and fall were lower than those found by Holman because of a less rigorous selection of penetrations. The two rates were almost the same, but sometimes the maximum rate of fall was faster than the maximum rate of rise (cf. Table 1). This may be one of the most characteristic differences between the membrane of smooth muscle and the skeletal muscle membrane.

Two kinds of positive after-potentials were observed with uncomplicated, simple action potentials. One was a brief hyperpolarization of several millivolts (mean $6{\cdot}0 \pm 0{\cdot}4$ mV ($n = 12$)) and the peak was reached in 50–100 msec (half-duration $69 \pm 0{\cdot}8$ msec) as shown in Fig. 6*a*. The other

was a 5–10 mV positive after-potential (mean $8{\cdot}2 \pm 0{\cdot}6$ mV, $n = 42$) reaching its peak in $1{\cdot}0 \pm 0{\cdot}06$ sec (see Fig. 1*b*), seen in pace-maker cells.

*Electrical activity in the presence of abnormal external ion concentrations*

The effect of each abnormal ionic environment was tested in at least three different preparations and repeated 2 or 3 times in different order. The values of the mean membrane potential and of the spike parameters measured after 15–30 min exposure to the different ionic environment are given in Tables 1 and 2.

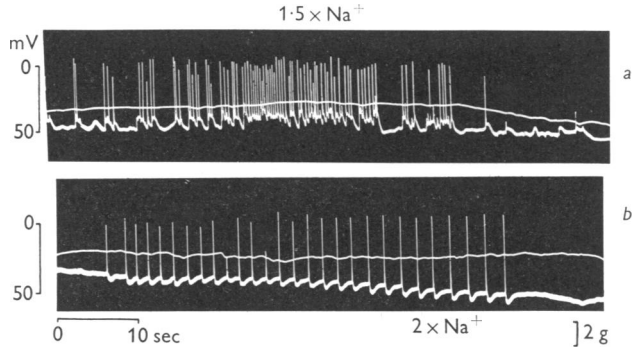


Fig. 2. The effect of $Na^+$ excess. Record *a* was taken after 12 min exposure to a solution containing 204 mM-$Na^+$; *b* after 40 min in 274 mM-$Na^+$.

*Effect of sodium excess*

1·5 *times and twice the normal* $Na^+$ *concentration.* In a solution containing 204 mM-$Na^+$ the membrane became transiently depolarized by 5–8 mV and then partially repolarized. The frequency of the spike discharge increased during the depolarization. The duration of the local potentials became gradually longer, lasting 0·8–2·5 sec at the 50 % level, and their amplitude increased up to 20 mV even though the membrane was already depolarized. Multiple spikes were triggered by these large local potentials. A typical record is shown in Fig. 2*a*. Gradually the bursts of discharge appeared only periodically, the pauses between the bursts becoming progressively longer. Eventually the mean frequency was decreased by excess sodium, and after 45–60 min became very low; finally activity stopped.

In a solution containing 275 mM-$Na^+$ the depolarization of the membrane was greater and the excitatory phase was shorter than in 204 mM-$Na^+$. Though periodical multiple spike discharge on prolonged generator potentials appeared, the magnitude of the local potentials was not further increased. More often, as shown in Fig. 2*b*, the membrane was depolarized, the spike discharge became regular and less rapid and each spike was followed by a large positive after-potential.

The rates of rise and fall, and the spike amplitude increased initially in excess sodium, but after prolonged exposure they gradually decreased. In 204 mM-$Na^+$ an overshoot up to 20 mV was often observed, in 275 mM-$Na^+$ the maximum overshoot was 30 mV.

When the solution containing excess $Na^+$ was replaced by normal Krebs's solution at a time when electrical activity was still high, the spike frequency was decreased for a short period (2–3 min) and local potentials became very small. Complete recovery took place within 15–20 min.

TABLE 1. Effects of varying the external Na concentration

| | Membrane potential (mV) | Action potential (mV) | Half-duration (msec) | Maximum rate of rise (V/sec) | Maximum rate of fall (V/sec) |
|---|---|---|---|---|---|
| Standard Krebs's solution ($n$ = 240) | 55 ± 0·5 | 62 ± 0·6 | 7·5 ± 0·1 | 7·6 ± 0·2 | 7·4 ± 0·3 |
| **Na excess** | | | | | |
| Control | | | | | |
| Na 137 mM ($n$ = 60) | 58 ± 0·4 | 63 ± 0·4 | 7·3 ± 0·2 | 7·0 ± 0·3 | 7·2 ± 0·2 |
| Na 204 mM ($n$ = 30) | 54 ± 0·5 | 67 ± 0·7 | 9·0 ± 0·1 | 9·6 ± 0·2 | 10·4 ± 0·4 |
| Na 275 mM ($n$ = 30) | 51 ± 0·5 | 70 ± 0·6 | 31 ± 0·2 | 3·3 ± 0·1 | 4·5 ± 0·2 |
| **Na deficiency** | | | | | |
| Control | | | | | |
| Na 137 mM ($n$ = 180) | 53 ± 0·6 | 61 ± 0·5 | 7·5 ± 0·1 | 8·2 ± 0·2 | 8·1 ± 0·3 |
| Na 68 mM Li ($n$ = 30) | 51 ± 0·4 | 54 ± 0·3 | 30·1 ± 0·4 | 3·3 ± 0·5 | 2·6 ± 0·4 |
| Tris ($n$ = 30) | 55 ± 0·2 | 62 ± 0·2 | 8·1 ± 0·2 | 7·9 ± 0·3 | 6·4 ± 0·2 |
| Na 14 mM Li ($n$ = 30) | 40 ± 0·5 | 43 ± 0·2 | 43 ± 0·3 | 2·1 ± 0·3 | 1·9 ± 0·4 |
| Tris ($n$ = 30) | 50 ± 0·1 | 56 ± 0·1 | 14 ± 0·1 | 5·1 ± 0·1 | 4·2 ± 0·1 |
| Na 7 mM Tris ($n$ = 30) | 49 ± 0·4 | 52 ± 0·5 | 19·2 ± 0·3 | 4·6 ± 0·2 | 1·9 ± 0·2 |
| Na 0 mM Li ($n$ = 30) | 36 ± 0·6 | 32 ± 0·3 | 57 ± 0·4 | 0·8 ± 0·3 | 0·7 ± 0·2 |
| Tris ($n$ = 30) | 46 ± 0·1 | 52 ± 0·1 | 28 ± 0·4 | 3·6 ± 0·06 | 1·8 ± 0·09 |

*$Na^+$ excess with additional change in $Cl^-$ concentration.* The replacement of chloride by a larger, and probably less permeant anion (Kuriyama, 1963), ethanesulphonate (Goodford & Ing, 1959) changed the effect of excess sodium. A solution containing 204 mM-$Na^+$ and 6 mM-$Cl^-$ caused a greater depolarization than in normal $Cl^-$ and the membrane remained depolarized throughout the exposure (see Table 2). The development of large and prolonged local potentials which was invariably seen in 204 mM-$Na^+$ and normal $Cl^-$ was not observed; instead, the local potentials became very small in a $Cl^-$-deficient solution.

Excess $Na^+$ as well as $Cl^-$, i.e. a solution containing 204 mM-NaCl, caused only a slight depolarization. But $Cl^-$ excess prevented the increase of the spike amplitude otherwise observed in the presence of high external sodium concentration (see Table 2).

*Excess $Na^+$ with additional change in $K^+$ concentration.* Excess $Na^+$ intensified the initial spike acceleration due to the removal of $K^+$ and reduced the eventual hyperpolarization produced by $K^+$-free solution.

TABLE 2. Effects of varying the external concentration of Na and other ions simultaneously

| | | Membrane potential | | | Action potential | | | Spike frequency (% of control) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $Na^+$ 0 | 137 mM | 204 mM | 0 | 137 mM | 204 mM | 0 | 137 mM | 204 mM |
| $Cl^-$ | 6 mM | 47 ± 0·2 (30) | 52 ± 0·3 (30) | 49 ± 0·3 (30) | 53 ± 0·5 (30) | 51 ± 0·5 (30) | 61 ± 0·2 (30) | 19 | 36 | 74 |
| | 134 mM | 46 ± 0·1 (30) | 55 ± 0·5 (240) | 54 ± 0·5 (30) | 52 ± 0·1 (30) | 62 ± 0·6 (240) | 67 ± 0·7 (30) | 121 | 100 | 76 |
| | 210 mM | 45 ± 0·3 (30) | 48 ± 0·7 (30) | 51 ± 0·4 (30) | 50 ± 0·7 (30) | 57 ± 0·4 (30) | 58 ± 0·5 (30) | 68 | 78 | 65 |
| $K^+$ | 0 mM | 56 ± 0·3 (20) | 62 ± 0·7 (30) | 53 ± 0·3 (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6 mM | 46 ± 0·1 (30) | 55 ± 0·5 (240) | 54 ± 0·5 (30) | 52 ± 0·1 (30) | 62 ± 0·6 (240) | 67 ± 0·7 (30) | 107 | 100 | 76 |
| | 12 mM | 45 ± 0·4 (30) | 47 ± 0·2 (30) | 48 ± 0·3 (30) | 51 ± 0·2 (30) | 53 ± 0·3 (30) | 59 ± 0·2 (30) | 120 | 136 | 95 |
| | 24 mM | 36 ± 0·2 (30) | 42 ± 0·1 (30) | 36 ± 0·2 (30) | 0 | 37 ± 0·1 (30) | 43 ± 0·08 (30) | 0 | 188 | 142 |
| $Ca^{2+}$ | 0 mM | 43 ± 0·2 (30) | 49 ± 0·3 (30) | 34 ± 0·2 (30) | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1·25 mM | — | 51 ± 0·4 (20) | — | — | 52 ± 0·4 (20) | — | — | — | — |
| | 2·5 mM | 46 ± 0·1 (30) | 55 ± 0·5 (240) | 54 ± 0·5 (30) | 52 ± 0·1 (30) | 62 ± 0·6 (240) | 67 ± 0·7 (30) | 121 | 100 | 76 |
| | 5 mM | — | 58 ± 0·4 (20) | — | — | 65 ± 0·3 (20) | — | — | — | — |
| | 7·5 mM | 54 ± 0·3 (30) | 64 ± 0·1 (30) | 63 ± 0·2 (30) | 56 ± 0·2 (30) | 69 ± 0·2 (30) | 74 ± 0·1 (30) | 21 | 62 | 32 |
| | 15 mM | — | 66 ± 0·4 (20) | — | — | 77 ± 0·3 (30) | — | — | — | — |

Numbers in brackets indicate number of observations.

On the other hand, a solution containing excess $Na^+$ and excess $K^+$ depolarized the membrane more than excess $K^+$ alone (see Table 2). Moreover, a solution containing 204 mM-$Na^+$ and 24 mM-$K^+$ caused in the course of 20–30 min a depolarization block of spike discharge. Such a block was not observed in the presence of 24 mM-$K^+$ and normal $Na^+$, and it occurred at a lesser degree of depolarization than the block produced by higher external $K^+$ concentrations.

*Hyperosmotic solution.* In order to test how far the effects of high external $Na^+$ concentrations could be attributed to the sodium ion, a hyperosmotic solution was prepared containing normal $Na^+$ and $Cl^-$ concentrations. This had the following composition (mM): $Na^+$ 137, $Cl^-$ 137, ethanesulphonate$^-$ 64, $Tris^+$ 64, other ions unchanged. In such a solution the membrane potential and spike amplitude were not affected. The spike frequency, however, increased transiently. The local potentials increased in amplitude (by 2–5 mV), but did not persist long enough to trigger multiple spike discharges (Fig. 9*b*) as they did in excess sodium (Fig. 9*a*). They sometimes generated two or three spikes as in normal Krebs's solution, but never more.

When the muscle was soaked in hyperosmotic solution containing low sodium but high chloride (68 mM-$Na^+$, 207 mM-$Cl^-$ and 137 mM $Tris^+$) the magnitude of the local potential was not increased.

*Effect of sodium deficiency*

*Low sodium* (68 mM, 14 mM *and* 7 mM-*$Na^+$*). Reduction of the external $Na^+$ concentration slightly increased the membrane potential at first. Membrane activity became slower and more regular. When 50 % of the $Na^+$ was replaced with Tris the membrane potential remained high, but when the external $Na^+$ concentration was decreased to one tenth the initial hyperpolarization was followed by a slight fall in membrane potential. There was no change in spike height, and the overshoot decreased only from an average of 7 to 5 mV, even when the muscle had been soaked in a solution containing only 14 mM-$Na^+$ for at least 30 min. However, the maximum rates of rise and fall of the action potential decreased gradually (see Table 1).

The effects produced by $Na^+$ deficiency depended on the substitute used. Thus the membrane potential and the rates of rise and fall of the action potential were lower in lithium than in Tris solution. Lithium also caused a greater prolongation of the duration of the action potential. The tension response diminished progressively in solutions containing lithium (cf. Axelsson, 1961), but it did not diminish in the presence of Tris.

When the muscle was soaked in a solution containing 7 mM-$Na^+$ (+130 mM $Tris^+$), the spontaneous discharge ceased after 45–60 min.

However, electrical stimulation continued to elicit spikes for more than 150 min (Twarog, unpublished). The size of the local potential was decreased but the spike was often transiently increased, though the duration was prolonged and the rates of rise and fall decreased. Ultimately spike discharge ceased abruptly without a preceding diminution in overshoot.

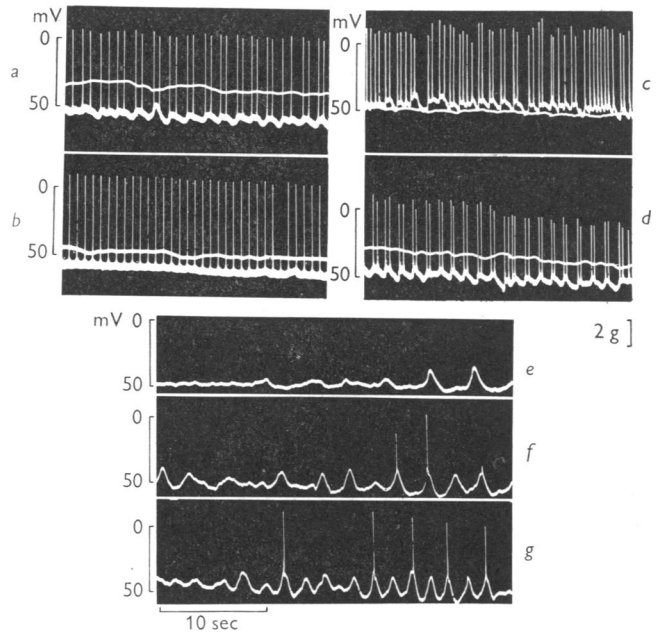


Fig. 3. The effect of complete absence of $Na^+$ (replacement with Tris). *a*, control; *b*, after 16 min in $Na^+$-free solution. Note regular discharge and absence of local potentials. *c*, 11 min and *d* 16 min after return to normal Krebs's solution. The continuous record (*e*, *f*, *g*) is taken from another experiment, showing the recovery from $Na^+$ deficiency, 15 min after return to normal Krebs's solution. For description see text.

*Sodium-free solution.* When the muscle was exposed to sodium-free (Tris chloride 137 mM) solution, all spontaneous activity of the membrane ceased after 2–5 min. But then, after a pause of 5–10 min, spontaneous activity returned (Fig. 3*a*, *b*). The typical changes were as follows: (i) The membrane potential rose during the first few minutes and then fell slowly over a period of 30 min, but the depolarization did not exceed 15 mV. (ii) The size of the action potentials became regular and an overshoot was recorded for at least 20–30 min in the absence of sodium. (iii) The rate of rise and fall decreased and the duration of the action potentials increased slowly while the spike height remained constant. (iv) The local

potential was abolished, the membrane potential being stable between the spikes.

After about 45 min exposure to zero $Na^+$ the membrane became depolarized, spike height decreased, and finally the spike discharge ceased. During the terminal stage periodical oscillatory potentials appeared. When the substitute for $Na^+$ was $Tris^+$, the tension development was not decreased as long as spikes persisted and it followed the changes in spike frequency.

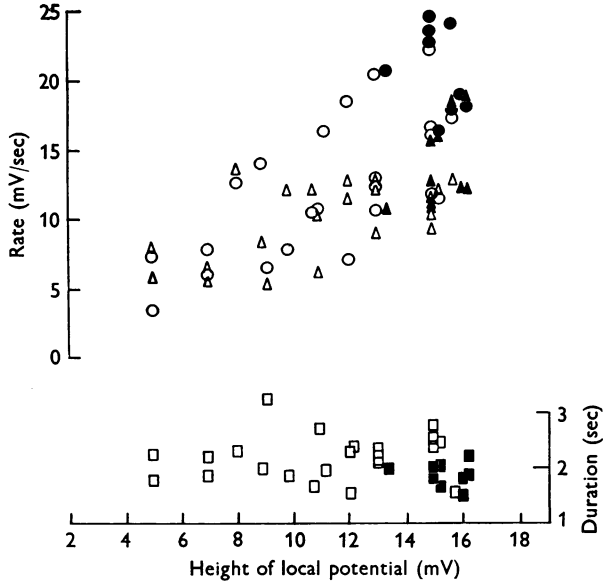


Fig. 4. Relation between the height of the local potentials (abscissa) and the rates of rise (○) and fall (△), and the duration (□) (ordinates). Black symbols indicate that a spike was generated by the local potential, open symbols indicate absence of spike.

On washing out with Krebs's solution, the membrane became hyperpolarized and the tension suddenly fell. During recovery (Fig. 3*e*, *f*, *g*) small fluctuations appeared which gradually developed to local potentials. When these local potentials reached firing level, spikes were generated. During the recovery from exposure to $Na^+$-free solution the relation between the rates of rise and fall and the height of the local potential was particularly clearly seen.

In Fig. 4 the amplitude of the local potential is plotted against the rates of rise and fall, and its duration. The figure shows that the amplitude was directly proportional to the rate of rise but not to the duration. Furthermore, the triggering of an action potential depended on the rate of rise and the amplitude of the local potential, not on its duration. The

threshold for triggering of a spike was higher during recovery from $Na^+$ depletion than before treatment.

Subsequently the spike frequency became very fast and for some time the pattern of activity resembled that observed in sodium excess (Fig. 3*c*), the local potentials triggering multiple discharges. Recovery was complete within 15–20 min.

When sodium was entirely replaced with lithium instead of Tris, the membrane was more depolarized and the spike frequency faster. The size of the action potentials became regular, but the overshoot diminished. The tension declined markedly and continuously, even though the spike frequency was increased (cf. Axelsson, 1961). Spike activity stopped after a shorter period than in Tris solution.

In tetraethyl ammonium chloride solution (all NaCl replaced with TEACl) the membrane was suddenly depolarized and depolarization block of spikes occurred. However, also in TEA a short period of slight hyperpolarization during which the spike height was increased preceded the depolarization. When the muscle was kept in TEA with atropine $10^{-5}$, membrane activity continued for 30–60 min, as it did in choline solution containing atropine (Holman, 1958).

The addition of atropine ($10^{-6}$ to $10^{-4}$) had no effect on the course of events when sodium was substituted by Tris. Atropine had also no effect on the reappearance of the local potential when Tris solution was changed to Krebs's solution. Recovery took place as described before.

*Effect of varying the external calcium concentration*

The calcium concentrations in the Krebs's solution were varied from 0·25 to 15 mM. Figure 5 shows the membrane potential, the spike amplitude, the rates of rise and fall, and the half duration of the spike as a function of the external $Ca^{2+}$ concentration (*a*), to be compared with that of the external $Na^+$ concentration (*b*). All the mean values were measured 20–30 min after placing the muscle in the test solution, and in different experiments the order of exposure to different $Ca^{2+}$ concentrations was changed.

The membrane potential was increased by excess calcium and decreased by calcium deficiency. However, the spike frequency did not always change in parallel with the membrane potential, but depended on the membrane activity before exposure to the test solution, i.e. on the condition of the tissue in response to the degree it was stretched (Bülbring *et al.* 1962). If the preparation was very active spontaneously, excess calcium slowed the spike frequency, but if the spike frequency was low in Krebs's solution excess calcium transiently accelerated it even though the membrane was hyperpolarized (Bülbring *et al.* 1962). The final effect

was always a decrease of spike frequency. The mean decrease measured after 30 min exposure in three experiments was from 0·85 impulses/sec in 2·5 mM calcium, to 0·7/sec in 5 mM, 0·5 /sec in 10 mM and 0·4/sec in 15 mM calcium.

In the absence of $Ca^{2+}$ the membrane was always depolarized. Once more, two different effects on electrical activity were observed: spontaneous discharge was either transiently increased (up to 10 min) and then all spike activity ceased (3 out of 6 experiments), confirming Holman (1958), or the membrane activity was gradually decreased without an initial acceleration (cf. Axelsson, 1961; Bülbring *et al.* 1962).

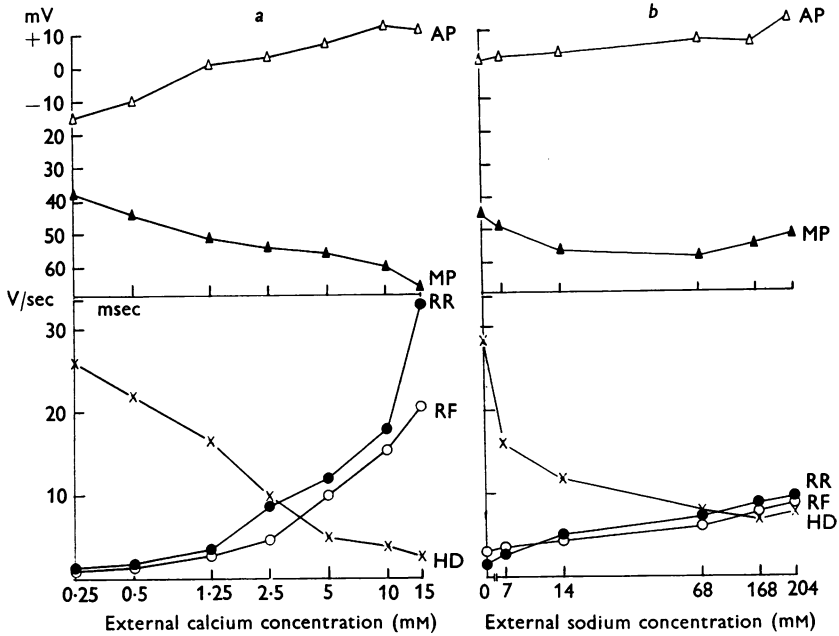


Fig. 5. The relation between *a* the external calcium concentration, *b* the external sodium concentration (abscissa) and the membrane potential ▲ (MP), action potential △ (AP), rate of rise ● (RR), rate of fall ○ (RF) and half-duration × (HD) of the action potential (ordinate).

The amplitude and the rates of rise and fall of the action potential were increased by excess calcium. Calcium deficiency had the opposite effect, as shown in Fig. 5. The spike parameters are given in Table 2.

The shape of the local potential was completely changed by excess calcium, as illustrated in Fig. 6. In this preparation the rate of fall of the action potential was already fast in Krebs's solution and had a tendency towards a positive after-potential, temporarily interrupting the course of the local potential but never wiping it out (Fig. 6*a'*). In the presence of 10 mM-$Ca^{2+}$, as the membrane became hyperpolarized, the falling

phase of the local potential was gradually suppressed (Fig. 6*b'*) until it was abolished (*c'*). The shape of the local potential resembled a 'pre-potential' of pace-maker type initiating an action potential (second spike in *c'*), which wiped out the local potential. This pattern of activity is also seen in Fig. 9*d*.

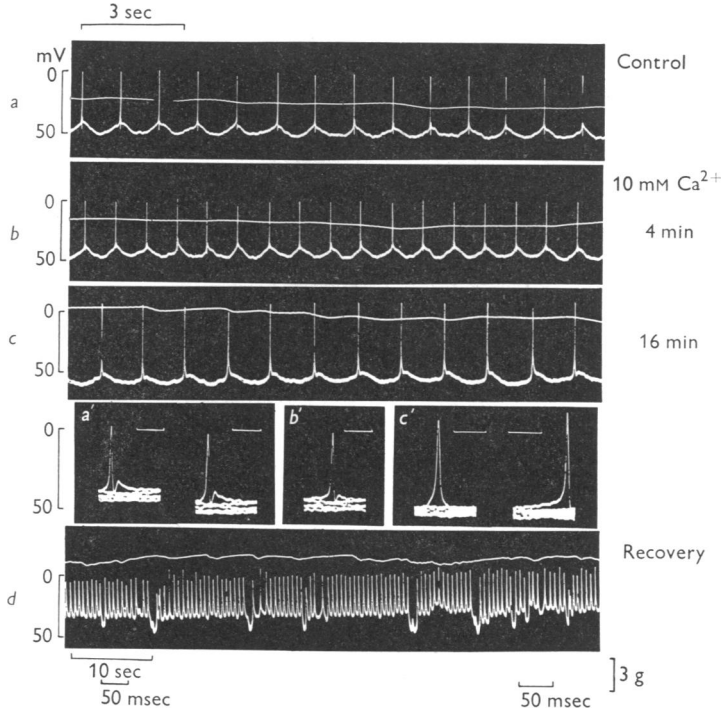


Fig. 6. The effect of increasing the external calcium concentration on single cell activity. *a*, control; *b*, after 4 min; *c* after 16 min exposure to 10 mM-$Ca^{2+}$. *a'*, *b'*, *c'* are corresponding records on a fast time base taken immediately after *a*, *b*, *c*. *d* 5 min after returning to normal Krebs's solution.

When, after long exposure to excess calcium (10 mM), the muscle was again placed in Krebs's solution, the membrane rapidly depolarized (from a mean value of 60 to 41 mV) and the mean spike frequency increased to 2·8 times that in 10 mM-$Ca^{2+}$. The mean amplitude of the action potential decreased from 72 to 43 mV and then gradually returned to the normal size seen before treatment. During the stage of increased membrane activity slow fluctuations of the membrane potential (full cycle 2–3 min over a range of 15 mV), and also brief hyperpolarizations as shown in Fig. 6*d*, were observed. Such a brief hyperpolarization suppressed the spike generation and also blocked the propagation of excitation, as shown by the abortive spikes which sometimes occurred.

*The effect of calcium in excess $K^+$ (18 mM) and in $K^+$ deficiency (0·3 mM)*

The following experiments were carried out to see if the proportional relationship between the spike amplitude and calcium concentration was a direct consequence of the change in external calcium concentration or an indirect consequence of the change in membrane potential. Low $K^+$ (0·3 mM) was used to produce a high membrane potential (mean 62 mV) and high $K^+$ (18 mM) to produce a low membrane potential (mean 42 mV) (Fig. 7). It was found that different $Ca^{2+}$ concentrations changed the

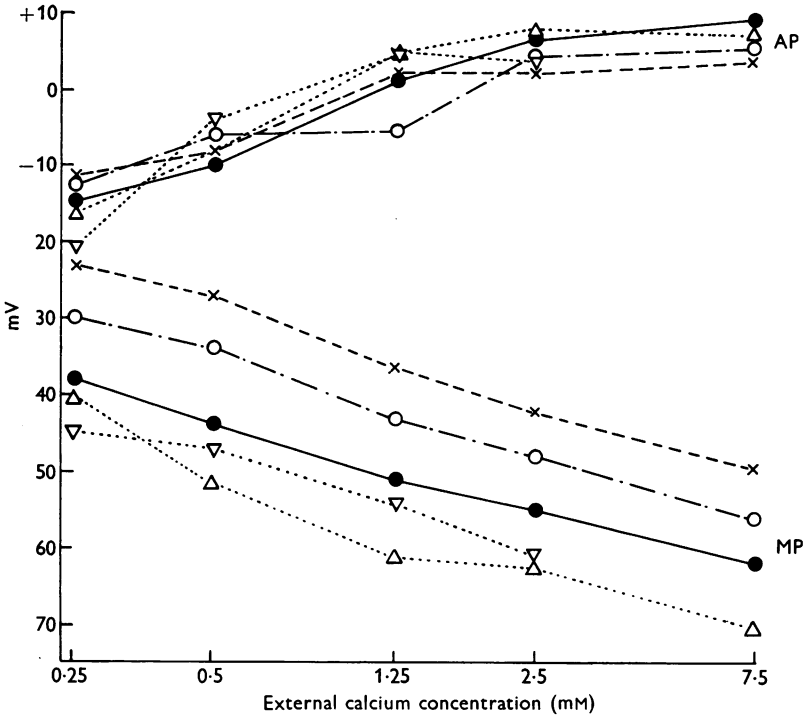


Fig. 7. The relation between external calcium concentration (abscissa) and membrane potential and action potential (ordinate). ●, remaining ions normal; ○, in the absence of $Na^+$; ×, in excess $K^+$ (18 mM); △, in $K^+$ deficiency (0·3 mM); ▽, in low $K^+$ (0·3 mM) and low $Cl^-$ (2·5 mM).

membrane potential in high and in low $K^+$ parallel with the changes seen in normal solution. The spike amplitude was increased by excess $Ca^{2+}$ and reduced by low $Ca^{2+}$, but though the membrane potentials differed by 20 mV in the two different external $K^+$ concentrations the overshoot, i.e. the absolute potential at the peak of the spike, was almost the same in every solution.

The maximum rates of rise and fall of the spikes were reduced by excess potassium as well as by potassium deficiency even at normal $Ca^{2+}$ concentrations. $Ca^{2+}$ deficiency lowered them further in both $K^+$ deficiency and $K^+$ excess (Fig. 8). $Ca^{2+}$ excess accelerated the rates of rise and fall in $K^+$ deficiency, but never to the extent observed in normal solution. Moreover, whereas in normal solution the two rates were accelerated almost in parallel, in $K^+$ deficiency the rate of fall was accelerated much less. The effect of excess $Ca^{2+}$ was very small in the presence of excess $K^+$.

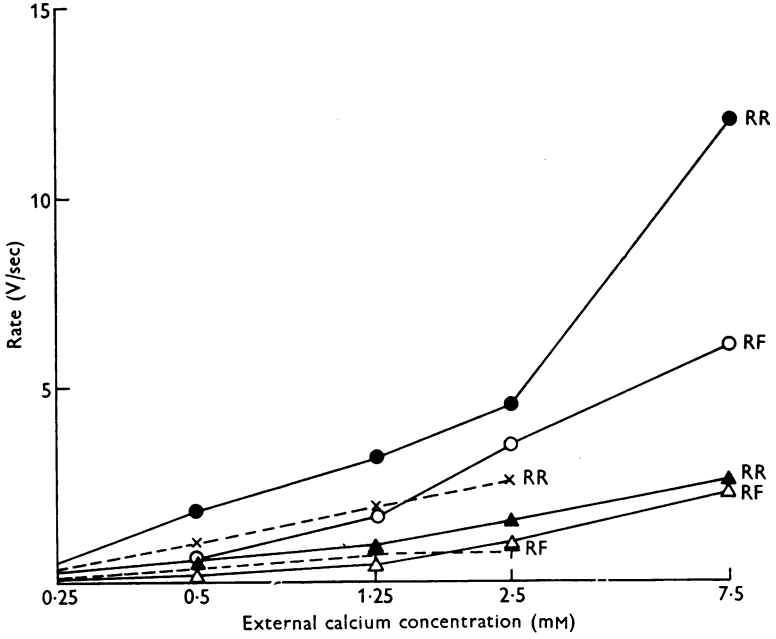


Fig. 8. Relation between the external calcium concentration (abscissa) and the rates of rise (RR) and fall (FR) of the action potential (ordinate). ● and ○, in the presence of low $K^+$ (0·3 mM); ▲ and △, in the presence of excess $K^+$ (18 mM); × and +, in the presence of low $K^+$ (0·3 mM) and low $Cl^-$ (2·5 mM) (see text).

*The effect of calcium in different external $Na^+$ concentrations*

*Excess $Na^+$ and excess $Ca^{2+}$.* The striking effect of excess $Na^+$ on electrical activity was prevented by the simultaneous presence of excess $Ca^{2+}$. Whereas in 204 mM-$Na^+$ (Fig. 9*a*) irregular bursts of spikes occurred on large prolonged local potentials, the simultaneous presence of 7·5 mM-$Ca^{2+}$ (Fig. 9*d*) hyperpolarized the membrane and regularized activity. The spike amplitude became more uniform and was greatly increased (cf. Table 2). The largest action potentials ever recorded were seen in this condition. The spikes wiped out the local potential and the positive after-potential

often reached 10 mV or more. On passing off it continued as a slow depolarization leading up to the next action potential. At first occasional spikes were blocked, then the silent periods became longer and after about 30 min spontaneous activity ceased.

*Zero $Ca^{2+}$ and excess $Na^{+}$.* A solution containing 204 mM-$Na^{+}$ and no $Ca^{2+}$ caused greater depolarization than calcium deficiency alone. During the progress of membrane depolarization the shape of the spikes changed very soon to oscillations (Fig. 9*c*) and depolarization block occurred within 3–4 min.

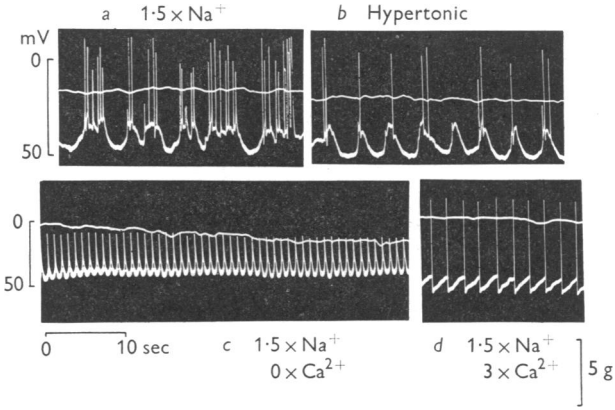


Fig. 9. Comparison of the effects of *a*, excess $Na^{+}$ (204 mM); *b*, hyperosmotic solution containing the normal $Na^{+}$ concentration (137 mM); *c*, excess $Na^{+}$ (204 mM) in the absence of $Ca^{2+}$; *d*, excess $Na^{+}$ (204 mM) in the presence of excess $Ca^{2+}$ (7·5 mM). For description see text.

*The effect of $Ca^{2+}$ in $Na^{+}$-deficient and $Na^{+}$-free solutions.* Changes of membrane potential, spike height and the time for cessation of spontaneous discharges were observed in various concentrations of calcium in sodium-free solution (2·5 mM, 1·3 mM, 0·6 mM, 0·3 mM and 0·25 mM). When the calcium concentration was decreased, the membrane became depolarized. The time for which spontaneous activity continued in the absence of $Na^{+}$ also depended on the external $Ca^{2+}$ concentration. Activity stopped in one experiment 32 min after the muscle was bathed in sodium-free solution containing 2·5 mM calcium. However, in a solution containing 1·3 mM calcium, the spontaneous discharge ceased after 28 min, in 0·6 mM calcium after 15 min and in 0·3 mM calcium after 8 min. The decrease in the size of action potential as the calcium concentrations were lowered is shown in Fig. 10.

From Fig. 7, in which the membrane potential and spike amplitude are plotted as a function of the external calcium concentration, it can be seen that the relationship was the same in the presence and absence

of $Na^+$. Though the membrane potential was 10 mV lower in zero $Na^+$, excess $Ca^{2+}$ increased and $Ca^{2+}$ deficiency decreased the overshoot to the same extent.

Excess $Ca^{2+}$ produced the same change in the shape of the action potential in the absence of $Na^+$ as it did in normal or in excess $Na^+$ (see Fig. 9), i.e. the spike amplitude was increased and a pronounced positive after-potential appeared. In sodium-free and calcium-free solution the muscle activity stopped almost immediately.

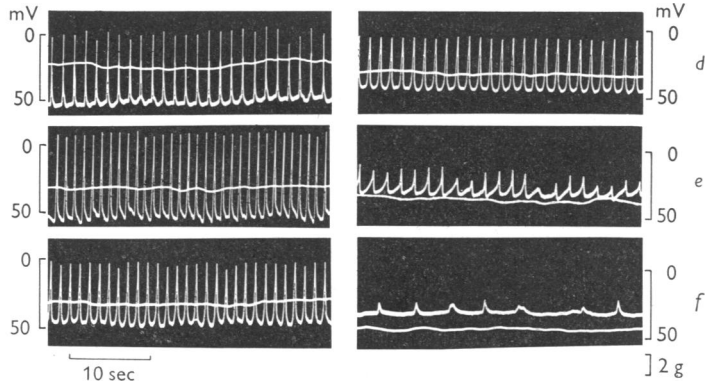


Fig. 10. The influence of the external $Ca^{2+}$ concentration in the absence of $Na^+$. *a*, control; *b–f*, were recorded 10–15 min after exposure to the $Na^+$-free solutions containing *b*, 2·5; *c*, 1·3; *d*, 0·6; *e*, 0·3 and *f*, 0·25 mM $Ca^{2+}$.

The maximum rates of rise and fall of the action potential in normal, low and zero $Na^+$ are shown in Fig. 11 as a function of the external calcium concentration. Over the range from 0·25 to 2·5 mM $Ca^{2+}$ the changes in a sodium-deficient solution (6·8 mM-Na) were similar to those in normal solution. But the effect of excess calcium was very much less marked in sodium-deficient and sodium-free solutions than in normal Krebs's solution. Thus when the external calcium concentration was increased above 2·5 mM very little acceleration of the rates of rise and fall were seen in sodium deficiency and in the absence of sodium.

*The importance of $Ca^{2+}$ in the absence of $Na^+$.* Figure 12 shows records from an experiment in which the muscle was soaked in a solution containing only Tris chloride (150 mM) and glucose (11·5 mM). The membrane activity quickly declined and ceased after 3–5 min (Fig. 12*a*). Several minutes later it started again, but the spike amplitude was small and soon activity stopped (Fig. 12*b*). After 20 min 2·5 mM calcium was added. The membrane activity recovered after a latent period of 10 min and the spike discharge then continued for about 15 min (*c*). When the $Ca^{2+}$ concentration was doubled (5·0 mM) the frequency and magnitude of the spike discharges increased, but an overshoot potential was not observed

in this condition. When the $Ca^{2+}$ concentration was increased 3 times (7·5 mM) overshoot occurred (*d*).

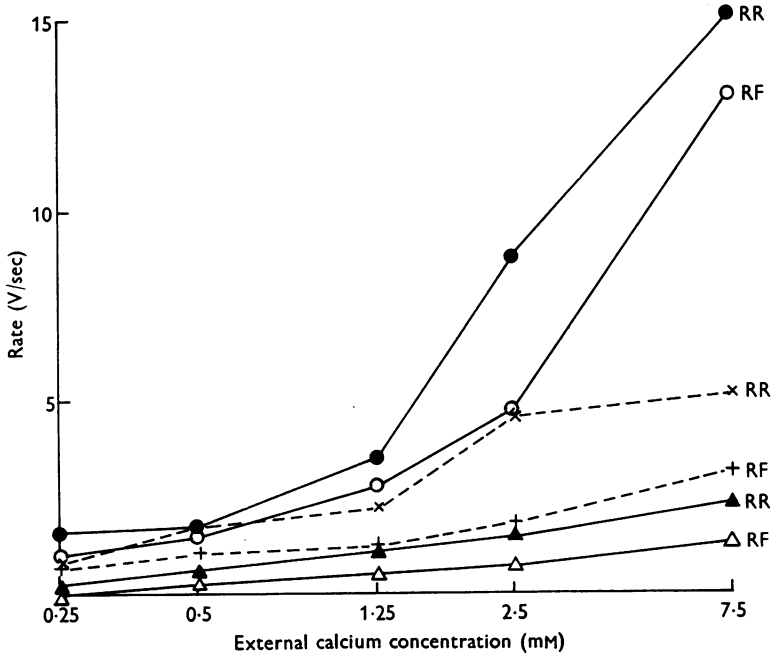


Fig. 11. Relation between the external calcium concentration and rates of rise (RR) and fall (RF) of the spike in the presence of 137 mM-Na ● and ○, 6·8 mM-$Na^+$ × and +, 0 mM-$Na^+$ ▲ and △.

*Effect of calcium in the glucose-free solution*

Axelsson & Bülbring (1961), using the sucrose-gap method, observed that glucose-free solution gradually decreased the membrane potential but, in spite of an increased spike frequency, the tension gradually declined. In the present experiments, in which intracellular recording was used, it was found that after the muscle was soaked in glucose-free solution for 30 min, the membrane potential fell from a mean of 54 mV to a mean of 49 mV. The spike frequency was increased from 0·96 to 1·36/sec and the maximum rate of rise of the spike was decreased from 8·2 to 3·1 V/sec. Subsequently the spikes changed to oscillatory discharges, but activity stopped only after 3 hr or more, the period varying over a wide range in different preparations. Figure 13 illustrates the effect of excess calcium in glucose-free solution. The experiment was done in the following way: control records were taken (*a*). The preparation was then exposed throughout to glucose-free solution containing, for alternate periods, the normal $Ca^{2+}$ concentration (2·5 mM) and excess $Ca^{2+}$ (7·5 mM). After 30 min ex-

posure to glucose-free solution containing normal $Ca^{2+}$ (Fig. 13*b*) the addition of excess calcium (7·5 mM) restored the membrane potential from 46 to 56 mV (Fig. 8*c*), slowed the spike frequency from 1·8 to 0·72/sec and increased the maximum rate of rise from 3·7 to 7·8 V/sec. When

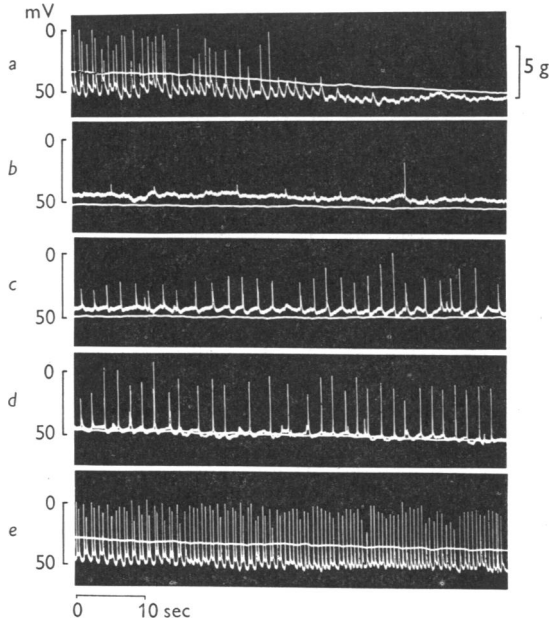


Fig. 12. The effect of various amounts of $Ca^{2+}$ added to a solution containing no other ions except Tris chloride (150 mM) and glucose (11·5 mM). *a*, control; *b*, 12 min after exposure to abnormal medium; *c*, 8 min after adding the normal $Ca^{2+}$ concentration (2·5 mM); *d*, 5 min after adding 3 times the normal $Ca^{2+}$ concentration (7·5 mM); *e*, 20 min after return to normal Krebs's solution.

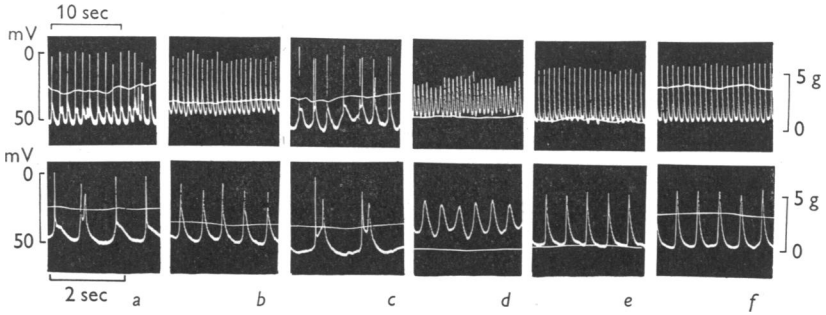


Fig. 13. The effect of excess calcium in the absence of glucose. Top slow, bottom fast time base. *a*, control. Records *b–f* in the absence of glucose: *b*, for 30 min; *c*, for 60 min; *d*, for 65 min; *e*, 150 min; *f*, 180 min in glucose-free solution. Records *b*, *d*, *e* in the presence of 2·5 mM-$Ca^{2+}$; *c* and *f* in the presence of excess $Ca^{2+}$, 7·5 mM. For description see text.

the muscle was once more exposed to glucose-free solution containing the normal $Ca^{2+}$ concentration (2·5 mM) the membrane depolarized at first (Fig. 13*d*) by about 20 mV with slow fluctuations and increased spike frequency. Figure 13*e* was recorded after 150 min exposure to glucose-free solution. The membrane potential was low (mean potential 41 mV) and the spike frequency was fast (1·6/sec). At this stage excess calcium had much less effect than at the beginning of the experiment. The membrane potential rose only to 46 mV, the spike frequency fell to 1·4/sec and the maximum rate of rise increased from 1·2 to 2·7 V/sec. It should be noted, however, that even at this time excess $Ca^{2+}$ still caused a marked increase of the tension response.

In other experiments the muscle was soaked with glucose-free solution containing excess calcium throughout the experiment and it was found that initially the effect of the excess calcium on the membrane activity was dominant, i.e. it prevented the effect of lack of glucose. However, after 60 min the effect of excess calcium gradually disappeared, the membrane became depolarized, the spike frequency increased and the membrane activity was changed to that usually observed in glucose-free solution. However, the tension was not abolished. The presence of excess calcium maintained the tension response in the absence of glucose for 150 min. Longer periods were not investigated.

*The effect of magnesium in relation to the action of calcium*

Experiments were carried out in order to investigate whether magnesium could substitute for calcium. It was found that an increase of the $Mg^{2+}$ concentration to six times normal (7·2 mM) scarcely altered the effect produced by the absence of calcium. The change of electrical activity was the same as that observed in calcium-free solution and only the time required for complete cessation of activity was prolonged.

In another series of experiments the effects of $Mg^{2+}$ in the presence of the normal $Ca^{2+}$ concentration was studied. It was found that in high external $Mg^{2+}$ concentrations the local potential increased and the delayed repolarization of the local potential sometimes led to a plateau triggering multiple spikes.

All observations on the effect of different $Mg^{2+}$ concentrations on membrane potential and electrical activity in the presence of normal, reduced and zero $Ca^{2+}$ are given in Table 3. They were made after 20–30 min exposure to the test solution.

TABLE 3. The effect of magnesium in relation to the action of calcium

| | Membrane potential (mV ± S.E.) $n = 30$ | Action potential (mV ± S.E.) $n = 30$ | Half duration of the spike (msec ± S.E.) $n = 30$ | Rate of rise (V/sec ± S.E.) $n = 30$ | Rate of fall (V/sec ± S.E.) $n = 30$ |
|---|---|---|---|---|---|
| Ca 0<br>Mg 1·2 mM | 38 ± 0·38<br>(35–42) | 0 | 0 | 0 | 0 |
| Ca 0<br>Mg 7·2 mM | 39 ± 0·42<br>(36–46) | 36 ± 0·43<br>(31–50) | 42·2 ± 0·41<br>(30–56) | 1·3 ± 0·54<br>(1·1–2·5) | 0·5 ± 0·37<br>(0·2–1·4) |
| Ca 1·3 mM<br>Mg 1·2 mM | 51 ± 0·31<br>(48–56) | 52 ± 0·28<br>(34–65) | 16·6 ± 0·31<br>(13–23) | 3·5 ± 0·27<br>(2·3–5·6) | 2·8 ± 0·41<br>(1·0–5·1) |
| Ca 1·3 mM<br>Mg 3·6 mM | 49 ± 0·47<br>(42–52) | 52 ± 0·33<br>(38–63) | 22·8 ± 0·36<br>(18–36) | 3·5 ± 0·41<br>(2·4–6·1) | 2·2 ± 0·39<br>(0·8–4·2) |
| Ca 2·5 mM<br>Mg 0 | 50 ± 0·34<br>(47–55) | 53·1 ± 0·27<br>(41–68) | 19 ± 0·36<br>(9–28) | 3·5 ± 0·31<br>(2·8–6·3) | 2·3 ± 0·33<br>(1·3–4·1) |
| Ca 2·5 mM<br>Mg 1·2 mM | 53 ± 0·36<br>(45–61) | 57 ± 0·28<br>(43–67) | 12·2 ± 0·31<br>(7–16) | 6·3 ± 0·24<br>(5·1–10·5) | 4·5 ± 0·35<br>(3·1–7·5) |
| Ca 2·5 mM<br>Mg 3·6 mM | 57 ± 0·37<br>(51–63) | 60·8 ± 0·24<br>(45–71) | 12·6 ± 0·24<br>(8–15) | 5·4 ± 0·18<br>(4·3–8·6) | 4·3 ± 0·28<br>(2·8–6·1) |
| Ca 2·5 mM<br>Mg 7·2 mM | 56 ± 0·41<br>(49–65) | 59·4 ± 0·31<br>(42–68) | 13·2 ± 0·26<br>(9–16) | 4·8 ± 0·22<br>(3·8–9·1) | 4·1 ± 0·18<br>(3·1–6·3) |

Numbers in brackets indicate range of measured values.

### *Effect of barium*

Three different concentrations of $BaCl_2$ were used ($5 \times 10^{-6}$ ($2{\cdot}4 \times 10^{-5}$ M), $10^{-5}$ ($4{\cdot}8 \times 10^{-5}$ M) and $5 \times 10^{-5}$ ($2{\cdot}4 \times 10^{-4}$ M)). Each transiently increased the membrane potential, which then gradually decreased. After 30 min the membrane was depolarized 5 mV in $5 \times 10^{-6}$ $BaCl_2$, 6 mV in $10^{-5}$ $BaCl_2$ and 12 mV in $5 \times 10^{-5}$ $BaCl_2$. The spike duration was prolonged chiefly because the rate of fall was slowed. For example, in one experiment this was slowed from 3·5 V/sec in Krebs's solution to 0·8 V/sec in the $5 \times 10^{-6}$ $BaCl_2$, 0·5 V/sec in the $10^{-5}$ $BaCl_2$ and 0·3 V/sec in the $5 \times 10^{-5}$ $BaCl_2$. The rate of rise was not affected to the same extent, e.g. it was slowed from 5·8 to 2·4 V/sec in $5 \times 10^{-6}$ $BaCl_2$, to 1·8 V/sec in $10^{-5}$ $BaCl_2$ and to 1·4 V/sec in $5 \times 10^{-5}$ $BaCl_2$. The decrease in spike amplitude proceeded in parallel with the prolongation of the spike duration (Fig. 14) and often a negative after-potential developed which sometimes reached the same amplitude as the spike (Fig. 14*c'*). There was, however, no repetitive spike discharge on the plateau.

## DISCUSSION

The main facts described in this paper are that in sodium-deficient or in sodium-free solution the muscle generates spikes, and that the size and the overshoot of the action potential is not dependent on the external $Na^+$ concentration between 0 and 137 mM-$Na^+$. On the other hand, when

the muscle is kept in excess sodium (204 mM to 274 mM-$Na^+$) the overshoot potential is increased. In normal solution the ratio between the outside and inside sodium concentration in the squid giant nerve fibres is $[Na]_o/Na]_i = 9$ (Hodgkin, 1951); in frog sartorius it is 7 (Keynes, 1951); but in guinea-pig taenia coli the average is only 2 and never exceeds 4 (Goodford & Hermansen, 1961). Moreover, the sodium exchange is very rapid (Goodford & Hermansen, 1961; Durbin & Monson,1961). Thus when the muscle is soaked in sodium-free solution the intracellular sodium which is not bound should leak out rapidly. Indeed, when the external

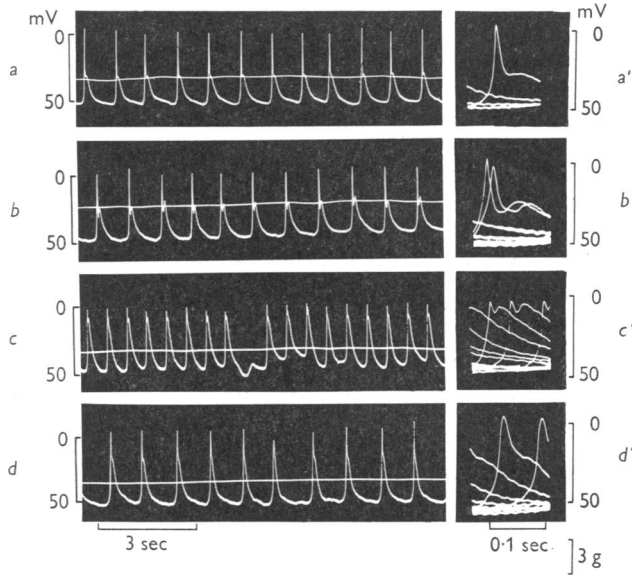


Fig. 14. The effect of barium on membrane activity. *a*, *a'*, control; *b*, *b'*, after 10 min; *c*, *c'* after 30 min exposure to $10^{-5}$ $BaCl_2$; *d*, *d'*, 30 min after returning to normal Krebs's solution.

sodium is reduced the internal $Na^+$ concentration does not remain constant but declines in parallel (Goodford, 1962). In the absence of sodium in the external medium, only 3·7 mM-$Na^+$ is found in the tissue after 15 min and 1·5 mM-$Na^+$ after 30 min (see also Bozler, Calvin & Watson, 1958). In fact, during the first few minutes of exposure to Na-free solution, activity ceases transiently but then spontaneous discharge recovers completely and continues for about 30 min without significant change in spike height.

Obviously, our observations are only compatible with the present hypothesis of membrane excitation if sufficient sodium is retained in the tissue to maintain spike activity. We do not know whether the very

low amount of sodium remaining is sufficient for the maintenance of a high sodium concentration immediately outside the cell. Of course, the substitute, Tris or lithium or choline, may take the place of the sodium ion for the spike mechanism. But we cannot be sure whether the abolition of spike discharges 45–60 min after NaCl is replaced with Tris chloride is due to the absence of sodium or to disturbances of the metabolism in the tissue (Abood, Koketsu, Barbat & Dobb, 1961). It is in any case remarkable to find continued electrical and mechanical activity in a tissue the intracellular ionic composition of which must be changed so drastically.

In contrast to the independence of the spike amplitude, the rate of rise and fall are, to some extent, a function of the external $Na^+$ concentration. Hodgkin & Katz (1949) showed that the rate of rise of the action potential in the giant axon of the squid is directly proportional to the external sodium concentration, and suggested that it is determined by the rate of sodium entry. In the squid giant nerve fibre the maximum rate of rise is 630 V/sec and the maximum rate of fall 380 V/sec. However, in the taenia coli the maximum rates of rise and fall are almost the same 7·6 and 7·4 V/sec (18 and 15 V/sec according to Holman, 1958) and often the rate of fall is even faster than the rate of rise. Furthermore, both rates are related to the external sodium concentration, while in the giant nerve fibre only the maximum rate of rise is directly proportional.

Holman (1958) speculated that the sodium carrier mechanism might be poorly developed in taenia coli. In a few observations she found that the rates of rise and fall of the action potential were reduced in the absence of calcium while they were increased in the presence of 4 times the normal $Ca^{2+}$ concentration. This may mean that normally the carrier is largely inactivated, and the action of $Ca^{2+}$ could then be explained by an increased availability of $Na^+$ carrier. In the present work the effect of different calcium concentrations from 0 to 15 mM has therefore been studied, and it was found that excess $Ca^{2+}$ consistently increased the rate of rise of the action potential. This finding contrasts with that in other excitable tissues in which excess $Ca^{2+}$ decreases the rate of rise, i.e. Purkinje fibres (Weidmann, 1955), skeletal muscle (Ishiko & Sato, 1957), myelinated nerve fibre (Frankenhäuser, 1957) and squid axon (Frankenhäuser & Hodgkin, 1957). One difference between these tissues and taenia coli is that in smooth muscle the membrane potential is much lower. In fact, when in the other tissues the membrane potential was lowered, by using the voltage clamp, excess calcium increased the rates of rise of the spike as it does in taenia coli. Frankenhäuser & Hodgkin (1957) interpreted their results by assuming 'that changes in calcium concentration and changes in membrane potential have similar effects on the systems which allow sodium and potassium to move through the membrane during the spike'.

They found that a fivefold increase in calcium was equivalent to a hyperpolarization of 10–15 mV. They also suggested that the role of calcium at the membrane may be a blockage of holes through which sodium moves. A poor fixation of calcium at the smooth muscle membrane of taenia coli could therefore limit the number of sites at which the Na permeability, which is already high in the resting state, can be further increased during the spike. Hence the marked acceleration of the rate of rise in the presence of excess $Ca^{2+}$, and the absence of this effect when the external $Na^+$ concentration is very low.

It is not possible at present to apply the 'voltage clamp' to the smooth muscle membrane. The effect of calcium on the membrane either depolarized by excess $K^+$ or hyperpolarized by $K^+$ deficiency was, however, studied. It was found that, though the initial membrane potentials differed by 20 mV, the relation between spike amplitude and external calcium concentration remained the same. However, while excess $Ca^{2+}$ increased the rate of rise in low $K^+$, it scarcely affected the rate of fall, as the net efflux of $K^+$ was probably low. The reduction of $Cl^-$ as well as $K^+$ did not alter the effect of calcium.

The importance of calcium for electrical activity in the absence of sodium has been stressed by Bozler (1960), Kuriyama (1961) and Axelsson (1961), though Kolodny & van der Kloot (1961) recorded activity for many hours in dilute sucrose solution in the absence of calcium. In our experiments we found that, if calcium was absent in a $Na^+$-free solution containing only Tris chloride and glucose, spike activity quickly stopped completely. It could be restarted by the addition of $Ca^{2+}$, whereupon it continued for about 30 min.

There are two possible explanations for this action of calcium. First, the extracellular calcium may prevent the leakage of sodium through the resting membrane, so that triggering of the spike is possible, especially with the extremely small amount of sodium remaining in the tissue. Secondly, the calcium ions themselves may carry the charge and generate the action potential in the absence of sodium. Such a mechanism has been considered for crustacean muscle (Fatt & Ginsborg, 1958). It may well be that the rapidly exchangeable calcium fraction believed by Schatzmann (1961) to be adsorbed at the cell membrane is involved in electrical activity, not only for the control of sodium movement in normal solution but also capable of replacing sodium when this is removed from the external medium.

*The local potential or slow wave or generator potential*

Nothing is known about the mechanism which brings about the local potentials. Some may be due to electrotonic spread from active neigh-

bouring cells but usually, as already pointed out by Holman (1958), the duration is much longer and the maximum rates of rise and fall are a hundred times less than those of the action potential. The local potential may be analogous to the myo-myo-junction potential of uterine muscle described by Goto, Kuriyama & Abe (1960, 1961) or to the neuromuscular junction potential recorded in the vas deferens by Burnstock & Holman (1961). However, atropine abolishes the effect of the nervous transmitter acetylcholine while it has no effect on the local potential.

Sodium excess increases the amplitude and the duration of the local potentials, while they are abolished in the absence of sodium. During recovery, after treatment with sodium-free solution, the local potentials develop again, gradually growing and initiating spikes when they have reached the threshold level. This suggests the possibility that the local potential may be brought about by a similar mechanism as the pre-potential or pacemaker potential in heart muscle (Trautwein, 1961). It may well be that an intrinsic, sodium-dependent mechanism is chiefly responsible for the automaticity.

In this connexion another observation is of importance. In excess calcium, regardless of whether sodium is present or not, the action potential is followed by a marked hyperpolarization which wipes out the local potential and, on passing off, continues as a slow depolarization leading to the next spike. In this way excess calcium produces a characteristic pattern of activity. Particularly in the presence of excess sodium the irregular activity, consisting of large local potentials and bursts of variable spikes, is transformed by excess calcium into a very regular, pace-maker type of activity.

In the taenia coli glucose-free solution causes depolarization and accelerates spontaneous spike discharge (Axelsson & Bülbring, 1961). In the present work it was found that both the spike amplitude and the maximum rate of rise were decreased in the absence of glucose. The administration of excess calcium increased both to nearly normal values during the initial stage of glucose depletion, but failed to restore the normal pattern of activity after several hours exposure to glucose-free solution. It may be that the metabolic processes necessary for stabilizing the membrane require the presence of calcium, or that a decrease in metabolic energy results in the release of bound calcium from the cell membrane and thus induces an increased interchange of sodium and potassium ions. During the first hour of glucose depletion such a process appears to be reversible by the addition of excess calcium.

Another action of excess calcium was seen on the tension development. The continuous decline of the tension in the absence of glucose was prevented by excess calcium. Moreover, when the tension had already

fallen after prolonged exposure to glucose-free solution with the normal calcium concentration, a threefold increase of the external calcium restored the tension. It did so at a time when the calcium effect on membrane activity could no longer be obtained. It may be that in glucose-free solution the calcium permeability of the cell membrane is increased and when the extracellular calcium concentration is raised it may reach the contractile protein more easily. It may also be that bound calcium is lost in glucose deficiency and excess calcium is able to restore the link between membrane activity and contraction.

The influence of magnesium on the electrical properties of the squid axon has been shown to be similar to that of calcium though weaker (Frankenhäuser & Hodgkin, 1957). However, in the taenia coli, six times the normal concentrations of magnesium could not replace calcium. In the presence of the normal $Ca^{2+}$ concentration, the local potential was increased by excess magnesium and often triggered multiple spikes.

Barium prolongs the slow depolarization of the membrane in cat intestinal muscle (Bortoff, 1961), and prolongs the negative after-potential in rat and guinea-pig ureter (Bennett, Burnstock, Holman & Walker, 1962). In the taenia coli, barium depolarized the membrane and delayed the repolarization of the spike. This produced a plateau phase like that seen in heart muscle. It appeared that the barium ion acted in the opposite direction to the calcium ion, barium delaying and calcium accelerating repolarization.

There is at present no evidence which forces us to abandon the sodium hypothesis for the spontaneous spike mechanism in normal solution. Moreover, the local potentials are clearly sodium-dependent. If they are a consequence of a high membrane permeability to sodium they would be expected to be large in excess sodium and absent in zero sodium, which is what is observed. Furthermore, as calcium is known to control sodium permeability, excess sodium would be expected to make the membrane potential even more unstable if calcium is absent. In fact, the membrane potential oscillates in this condition. A poor fixation of calcium at the membrane would explain the fact that the membrane potential, the amplitude and the rate of rise of the spike are a function of the external calcium concentration. The spike mechanism would then be qualitatively similar to that in other excitable tissues, but calcium, by controlling the sodium permeability of the resting membrane, would determine the extent to which the Na permeability is increased in the active membrane.

SUMMARY

1. The membrane activity and tension in the presence of normal and abnormal ionic concentrations were recorded in the isolated taenia coli of the guinea-pig. In Krebs's solution the mean membrane potential was 55 mV and the mean parameters of the action potential were: spike amplitude 62 mV, half-duration 7·5 msec, maximum rate of rise and fall 7·6 and 7·4 V/sec, respectively. The mean parameters of the local potential were: amplitude 13 mV, half-duration 0·45 sec, rate of rise and fall 0·05 and 0·02 V/sec, respectively. Positive after-potentials of 5–10 mV, one type with a mean duration of 70 msec, the other of 1 sec, were observed.

2. In the presence of excess $Na^+$ (204 mM; 274 mM) the membrane was only slightly depolarized. In a solution containing 204 mM-$Na^+$, 137 mM-$Cl^-$, 67 mM-$C_2H_5SO_3^-$ the overshoot of the action potentials increased up to 20 mV. Spike discharge was accelerated and multiple spikes appeared on large local potentials; after 20–30 min activity stopped. In a solution containing 274 mM-$Na^+$, 137 mM-$Cl^-$ and 137 mM-$C_2H_5SO_3^-$ the overshoot increased up to a maximum of 30 mV.

3. Total absence of $Na^+$ in the external solution (replacement with $Tris^+$) transiently hyperpolarized the membrane and stopped spontaneous discharge for a few minutes. Activity then returned and continued for 30–60 min. Spikes of normal amplitude, but reduced rates of rise and fall, were discharged spontaneously from a steady, slightly reduced potential level. Local potentials were entirely abolished.

4. In the absence of $Na^+$ the time during which spontaneous activity continued as well as the spike amplitude were a function of the external calcium concentration. When, in the absence of $Na^+$ and $Ca^{2+}$ all activity had ceased, the addition of calcium restored spontaneous discharge of action potentials for 30 min.

5. The membrane potential, the spike amplitude and the rates of rise and fall of the spike are decreased in calcium deficient solution (0·25 to 1·25 mM) and increased in excess calcium (2·5 to 15 mM).

6. In excess $Na^+$ the absence of $Ca^{2+}$ caused the membrane potential to oscillate. But excess $Ca^{2+}$ stabilized the potential and altered the pattern of activity to resemble that of pace-maker cells in which the positive after-potential following each spike gave way to a slow depolarization initiating the next spike.

7. In the absence of $Na^+$ the membrane potential and spike amplitude were also a function of the external $Ca^{2+}$ concentration, but its effect on the rates of rise and fall was abolished.

8. The effect of glucose depletion is antagonized by excess calcium (7·5 mM). During the early stages it repolarizes the membrane, slows the

spike frequency and increases the amplitude and rate of rise. After prolonged exposure to glucose-free solution excess calcium no longer restores the membrane activity but still restores the tension development.

9. Magnesium does not substitute for calcium.

10. Barium decreases the membrane potential, the amplitude and the maximum rate of rise of the spike. A prolonged negative after-potential of the spike gradually develops into a plateau.

11. The results observed on membrane activity are consistent with the view that in the smooth muscle there is a poor fixation of calcium at the membrane. This may be the reason for a high Na conductance producing a low membrane potential and also for a limited number of sites at which the Na conductance can be further increased during membrane activity.

We wish to thank the Wellcome Trust for a grant to one of us (H.K.), the Medical Research Council and the Smith, Kline and French Research Institute for grants towards the cost of apparatus. We are grateful to the United States Air Force, European Office, Air Research and Development Command, for the support of our research.

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