J. Physiol. (1967), **192**, *pp.* 145–157
With 3 *text-figures*
Printed in Great Britain

THE CALCIUM CONTENT OF THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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(*Received* 17 *March* 1967)

SUMMARY

1. The *in vitro* calcium content of the smooth muscle of the guinea-pig taenia coli was 3·0 m-mole Ca/kg wet wt. when phosphate was omitted from the bathing medium, and was almost independent of pH changes in the range 6·7–7·6.

2. The calcium content was not changed when 1 mM phosphate was included in the medium, if the pH was 6·7 or 7·0. However, when the pH was 7·6, the calcium content increased by 1·5 m-mole Ca/kg wet wt. in the presence of phosphate.

3. The calcium content rose by 1·1 m-mole Ca/kg wet wt. when NaCl in the bathing medium was replaced by isotonic sucrose, and rose by 0·7 m-mole Ca/kg wet wt. when $MgCl_2$ in the bathing medium was replaced. These increases may reflect a competition between Ca^{2+} and other cations for fixed negative sites in the tissue.

4. The initial rapid phase of ^{42}K exchange corresponded to an 'extracellular ^{42}K-space' of 470 ml./kg fresh wt. in normal solution, rising to 560 ml./kg. fresh wt. in low-sodium solution and to 760 ml./kg fresh wt. in calcium-free low-sodium solution. In this last medium the extracellular [^{14}C]sorbitol space was only 390 ml./kg fresh wt., so that there was a large excess of rapidly-exchanging potassium which may have been competing at fixed negative sites.

INTRODUCTION

It is now generally agreed that sodium and calcium ions may compete for negative charges at the cardiac muscle cell surface (Wilbrandt & Koller, 1948; Niedergerke & Lüttgau, 1957), and a similar effect has been observed in skeletal muscle (Schaechtelin, 1961) although it is not so well defined. The role of potassium has been emphasized in smooth muscle, however, and there is evidence that K^+, Na^+, and Ca^{2+} may all compete

at superficial anionic sites in the guinea-pig taenia coli (Goodford, 1965*a*; Goodford, 1966). This may indicate that the negative regions in smooth muscle are relatively unselective in their choice of cations, or that there are different types of anionic site in this tissue. The competition might, for example, be predominantly between Na^+ and Ca^{2+} in one region, and predominantly between Na^+ and K^+ elsewhere.

An attempt has now been made to establish, firstly, whether changes of $[H^+]_o$ $[Na^+]_o$ or $[Mg^{2+}]_o$ influence the muscle calcium content in the direction predicted by the competition hypothesis, and secondly whether changes of $[Ca^{2+}]_o$ influence the muscle potassium distribution in the predicted manner.

METHODS

Solutions. Modified Krebs solutions (Table 1) were prepared from isotonic stock solutions (Krebs, 1950), and were equilibrated with mixtures of oxygen and carbon dioxide. Radioactive materials were supplied by the Radiochemical Centre, Amersham. ^{42}K was always checked for purity by half-life measurements after experiments had been completed.

TABLE 1. The composition of some physiological saline solutions in mM

Solution	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	Phosphate	Sucrose	Glucose	CO_2 (%)
A	137	5·9	1·25	2·5	135	15	—	—	11	3
B	137	5·9	1·25	2·5	134	15	1	—	11	3
C	137	5·9	1·25	2·5	144	5·9	—	—	11	1
D	—	5·9	1·25	2·5	7	5·9	—	274	11	1
E	2·4	5·9	1·25	—	4·9	5·9	—	276	11	1
F	2·4	5·9	1·25	2·5	9·9	5·9	—	269	11	1

Procedure. White guinea-pigs weighing between 300 and 450 g were stunned, bled, and pieces of taenia coli 25 mm long were rapidly dissected, weighed to give the fresh weight, and immersed in oxygenated solution at room temperature. After tying threads to each muscle they were suspended with a load of 2 g in the appropriate solution at 35° C, and left for 2 hr to equilibrate. They were then transferred to chemically identical solutions which contained radioactive tracer and were finally removed, weighed to give the wet weight, and analysed.

Calcium was determined after ashing with 0·2 ml. HNO_3 (sp.gr. 1·5) and 0·2 ml. $HClO_4$ (sp.gr. 1·7). Potassium, ^{42}K, and ^{14}C were measured in tissues ashed with 1 ml. H_2O_2 (30 %. 100 vol.), made alkaline with LiOH or ammonia. Samples and controls were dissolved in a mixture of 950 ml. water, 42 ml. HNO_3 and 1 ml. H_3PO_4 for K and Ca determinations by flame photometry. ^{14}C was measured by liquid scintillation counting (Bauer, Goodford & Hüter, 1965), and ^{42}K in a scintillation counter.

Statistical methods. Results have been expressed as mean values with the estimated standard error of the mean, and the number of observations in brackets, thus: $1·1 \pm 0·1$ (19). Where more appropriate the number of degrees of freedom have been printed in the brackets in *italics*. Means have been compared on a probability scale so that smaller values of P correspond to increasingly significant differences.

RESULTS

The influence of pH. Pieces of taenia were equilibrated in four different organ-baths each containing 600 ml. of the same modified Krebs solution (*A*, Table 1). The chemical composition of the solutions was therefore identical, but the pH ranged from 6·7 to 7·6, because different mixtures of oxygen and carbon dioxide were passed through each bath, and the only buffer present was HCO_3^-/CO_2. The large bath volume ensured that the solutions had sufficient buffer capacity, so that the muscle samples did not themselves influence the pH of the bathing medium. There was a slight trend for the calcium content of the taenia to rise at high pH (Fig. 1, open circles), when the hydrogen ion concentration of the solution was

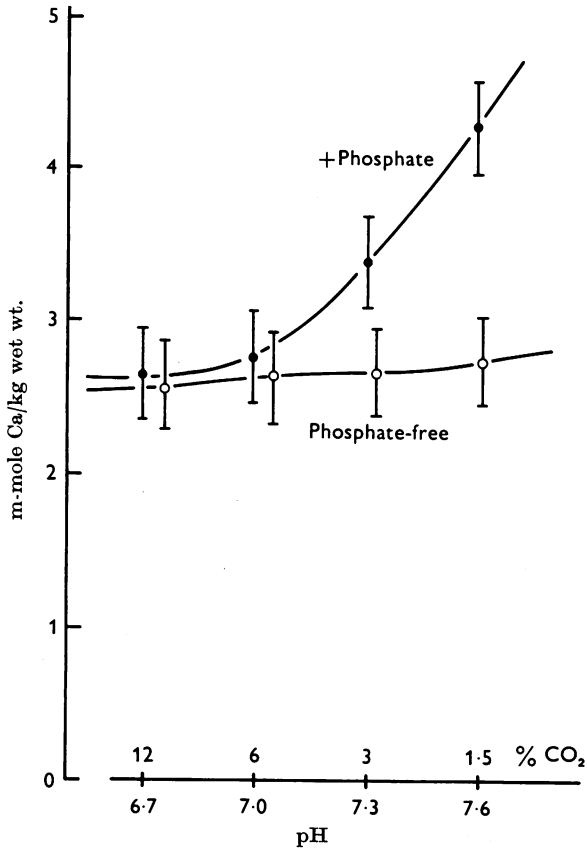

Fig. 1. The *in vitro* calcium content of the taenia coli, after 2 hr immersion in normal solution at 35° C. Open circles: solution *A* without phosphate. Filled circles: solution *B* with 1 mM phosphate. Abscissae: the % of CO_2 in the oxygenation gas, and the resulting solution pH. Ordinate: total tissue calcium, which rose in slightly alkaline solutions if they contained phosphate.

relatively low, but the effect was statistically insignificant, and it was tentatively concluded that any negative site which attracted an appreciable weight of calcium ions did not have a pK in the range 6·7–7·6.

Similar experiments were carried out with solution B (Table 1), which also contained 1·2 mM of phosphate. In contrast to the previous observations, it was then found that the calcium content of the taenia increased when the proportion of carbon dioxide in the gas mixture was low, and the pH was high (Fig. 1, filled circles). However, different guinea-pigs were used for this second series of observations, and it was possible that a difference between animals (Bauer *et al.* 1965), rather than the effect of phosphate, was being measured. Further observations were therefore made using paired muscles, one in solution A and one in B, but both gassed with the same mixture of 3 % CO_2 in oxygen which is normal for this type of medium. The calcium content of the taenia was $3{\cdot}0 \pm 0{\cdot}1$ (6) m-mole Ca/kg wet wt. in the phosphate-free solution, and $3{\cdot}8 \pm 0{\cdot}1$ (6) m-mole Ca/kg wet wt. when phosphate was present, and there was no overlap between the two groups. It was concluded that the calcium content of the taenia might be higher, under normal conditions *in vitro*, if phosphate ions were present in the bathing medium.

These observations may go some way towards explaining the different calcium contents of the taenia coli which have already been measured *in vitro*. Thus Bauer *et al.* (1965) used phosphate-free solutions and found 2·8 m-mole Ca/kg fresh wt., almost all of which exchanged with ^{45}Ca. Schatzmann (1961) used a solution containing phosphate and found more than 4 m-mole Ca/kg, but about 1·7 of this could not be leached out of the tissue. The *extractable* calcium in his experiments was not significantly different from the *exchangeable* calcium in those of Bauer *et al.*, and Schatzmann's measurements would correspond to a pH slightly above 7·4 in Fig. 1. Chujyo & Holland (1963) also used a solution which contained phosphate, but they had less bicarbonate than Schatzmann, so that their bathing medium would be more acid than his, and this may be why their observations agreed with those of Bauer *et al.* (1965).

The second pK of orthophosphoric acid lies in the range 6·7–7·6 (Newman & Newman, 1958), and the increased calcium content in slightly alkaline solutions containing phosphate could be due to the microprecipitation of $CaHPO_4$ in the tissue (Goodford, 1965*b*), since this salt is less soluble than $Ca(H_2PO_4)_2$, which would be the stable form of calcium phosphate in acid media. Indeed, the inexchangeable unextractable tissue calcium, which sometimes forms in the presence of phosphate, may represent an extreme example of cation–anion interaction in the taenia, and the effects of pH changes could be described as a competition between H^+ and Ca^{2+} ions.

Preliminary experiments with the sucrose-gap (E. Bülbring, personal

communication) have shown that adding or removing 1 mM phosphate from the bathing medium does not have any detectable effect upon the electrophysiological or mechanical activity of the taenia and, since the calcium content of the muscle may be more variable in the presence of phosphate, the following experiments were all carried out in phosphate-free solutions. In these the total tissue calcium does not rise appreciably until the pH approaches 7·8, which is the extreme limit of the normal physiological range.

Sodium-free solution. Bauer *et al.* (1965) measured the calcium content of the taenia in normal solution (*C*, Table 1), and after transfer to a similar sodium-free medium prepared with LiCl instead of NaCl. The muscle contracted and the tissue calcium content increased slightly after this change, but the increase was not maintained and much larger effects have been observed in heart muscle (Niedergerke, 1963). The calcium content of the taenia has now been compared between normal solution (*C*, Table 1) and sodium-free solution *D* when sucrose was substituted for NaCl and an appreciable and statistically significant ($0{\cdot}001 > P$) increase of $+1{\cdot}1 \pm 0{\cdot}1$ (19) m-mole Ca/kg fresh wt. was observed in each of a series of paired experiments. This could be due to the uptake of calcium ions at sites previously occupied by Na^+, and the smaller increase seen when LiCl was substituted may have been due to a partial affinity of Li^+ for the anionic sites in smooth muscle. The observations may therefore indicate that these sites are not specific for sodium and calcium ions, to the complete exclusion of all others.

Magnesium-free solutions. The solutions used in the previous experiments all contained Mg^{2+} ions, which might have been competing for anionic charges in the tissue. Indeed the small size and double positive charge of the magnesium ion should, according to Fajan's rule, make it a particularly effective competitor. Sodium-free solutions analogous to *D* (Table 1) were therefore prepared in which sucrose stock solution was substituted for $MgCl_2$, and the muscle calcium content per kg fresh wt. was higher in twenty out of twenty-one paired trials when Mg^{2+} ions were omitted, although the observations were scattered and were not normally distributed.

Pieces of taenia were also equilibrated with normal solutions (Type *C*, Table 1) to which no magnesium had been added, or only 1·25 mM, or 5·0 mM-Mg^{2+}. The calcium content was $3{\cdot}0 \pm 0{\cdot}1$ (8) m-mole Ca/kg wet wt. when bathed in the usual magnesium concentration of 1·25 mM, and this was close to the mean value of Bauer *et al.* (1965) in the same solution. However, the muscle calcium content rose to $3{\cdot}7 \pm 0{\cdot}1$ (8) m-mole Ca/kg wet wt. when magnesium was omitted, showing a statistically significant ($0{\cdot}005 > P > 0{\cdot}001$) increase of $0{\cdot}7 \pm 0{\cdot}2$ (*14*) m-mole Ca/kg wet wt., and it was concluded that the small amount of magnesium normally present

in Krebs solution was sufficient to depress the tissue calcium content. There was, moreover, a further but statistically insignificant fall of $0{\cdot}4 \pm 0{\cdot}3$ (22) m-mole Ca/kg fresh wt. when the solution concentration of magnesium was raised to 5·0 mM, and in this last medium the muscle calcium content was $1{\cdot}2 \pm 0{\cdot}5$ (22) m-mole Ca/kg fresh wt. less than in the nominally 'magnesium-free' solution. In every experiment, therefore, the tissue calcium content declined when the solution magnesium concentration was increased.

Muscle weight. The presence or absence of 1 mM phosphate had no significant effect on the wet weight of the taenia coli, but changes were observed in association with alterations of the gas mixture and pH of normal solutions (Table 2, i). There was an even more marked loss of weight in nominally 'sodium-free' solutions prepared with sucrose instead of sodium chloride (Table 2, ii), and this loss might be due to sodium or other ions leaving the cells at a faster rate than sucrose could enter, which should lead to a simple osmotic shrinkage. A loss of weight was normally observed

TABLE 2. Total tissue weight (g) at the end of an experiment, per kilogram fresh weight immediately after dissection

Expt.	Solution	Gas mixture O_2 %/CO_2 %	Wet wt. (g) per kg fresh wt.
i	*A*. Phosphate-free	99/1	963 ± 12 (8)
	A. Phosphate-free	95/5	1023 ± 19 (8)
	B. +1 mM phosphate	99/1	963 ± 12 (8)
	B. +1 mM phosphate	95/5	1017 ± 11 (8)
ii	*D*. Na-free. Ca-free	99/1	820 ± 17 (7)
	D. Na-free. 2·5 mM-Ca	99/1	770 ± 16 (7)
	D. Na-free. 10 mM-Ca	99/1	700 ± 21 (7)
iii	*C*. —	99/1	955 ± 5 (80)
	C. Mg-free	99/1	932 ± 5 (80)

in 'sodium-free' media but the results were not consistently reproducible, and the effect of altered calcium concentration shown in Table 2, ii, is exceptionally well defined for these conditions.

It is not easy to establish a steady-state in 'sodium-free' solutions (Goodford, 1966), and the rate at which equilibrium is approached may depend on the concentration of calcium and other solutes in the medium. The effects shown in Table 2, ii, may reflect the different rates at which osmotic equilibrium is being reached, and they should not be regarded as steady-state values. Manery (1954) has emphasized that 'osmotic equilibrium is probably never attained but always approached', and the non-equilibrium situation may well account for the lack of reproducibility of the wet weights which has sometimes been observed in 'sodium-free' media. Another factor may be the presence of variable traces of sodium which are always found in nominally 'sodium-free' solutions at the end

of an experiment, and more phenomena may also be involved (Bozler, 1962).

The absence of magnesium from an otherwise normal solution caused a small but significant fall in the wet weight of the taenia (Table 2, iii), and the extracellular [^{14}C]sorbitol space fell at the same time from 409 ± 8 (77) g/kg fresh wt. to 389 ± 8 (77) g/k fresh wt. There was no significant change in the calculated cell volume, and these small changes do not account for the raised calcium content which was observed when magnesium was omitted.

^{42}K uptake. Figure 2 shows the uptake of radioactive ^{42}K by the taenia coli in normal solution (C. Table 1). The amount of tracer in the muscle is expressed as:

$$\frac{\text{concn. of } ^{42}\text{K in muscle at time } t}{\text{concn. of } ^{42}\text{K in solution}} = \frac{\text{c/s.kg fresh wt.}}{\text{c/s.ml.}} = \text{ml./kg fresh wt.}$$

and is plotted on semi-logarithmic axes which do not differ appreciably from linear co-ordinates at this early stage of tracer exchange. The observations between 0·5 and 7 min lie close to a straight line, and they are therefore compatible with a single exponential uptake corresponding, perhaps, to the slow entry of tracer potassium into the cells. The best fitting straight line through the observations has an intercept at $t = 0$ of 362 ± 26 (*60*) ml./kg fresh wt. but it is generally agreed (Harris & Burn, 1949; Huxley, 1960; Weatherall, 1962; Goodford, 1966) that this underestimates the amount of rapidly-exchanging potassium in the tissue. Huxley's analysis shows that 490 ml./kg fresh wt. would be a better estimate, if the initial uptake of ^{42}K were an exponential process with a half-time of 30 sec (Goodford & Hermansen, 1961; Freeman-Narrod & Goodford, 1962; Goodford, 1966) and the present observations therefore agree tolerably with an earlier estimate of 510 ml./kg fresh wt. based on the same assumptions (Goodford, 1966), although in both cases the result depends critically upon the precise value of the initial rate of uptake (e.g. the half-time of 30 sec) which is not easily measured. It may, however, be calculated by assuming that diffusion is the main process for the invasion of the extracellular space by tracer (Goodford & Hermansen, 1961), and the smooth uptake curve in Fig. 2 was drawn on this basis. It is the sum of a large number of exponential terms but, since it becomes virtually linear after 2 min when plotted on semi-logarithmic axes, only one exponential is significant at longer times. The amount of rapidly-exchanging potassium is therefore measured by the total sum of the many terms which contribute to the initial exchange and is 470 ml./kg fresh wt., which does not differ appreciably from the result of 490 ml./kg fresh wt. already calculated by Huxley's method.

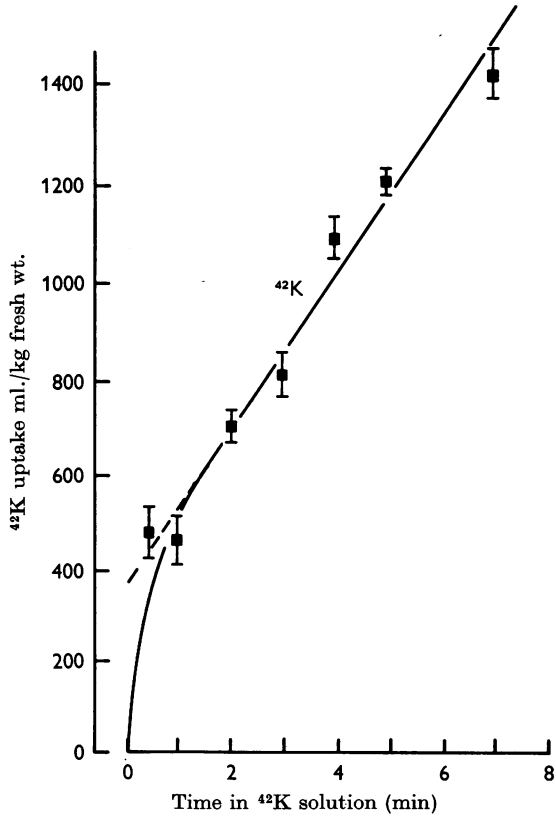

Fig. 2. The exchange of radioactive ^{42}K in the taenia coli *in vitro*, after 2 hr immersion in normal solution (*C*) at 35° C. Abscissa; time after application of tracer (min). Logarithmic ordinate; tracer uptake/kg fresh wt. (see text). The logarithmic ordinate does not differ appreciably from linearity at this early stage of exchange. The smooth uptake curve is a diffusion equation, which does not differ sensibly from a single exponential after the first 2 min (see text).

Calcium-free solutions. The effect of omitting calcium from the medium was studied in low-sodium solutions (*E* and *F*, Table 1), in which potassium ions might occupy a greater than normal proportion of superficial anionic sites. So called 'sodium-free' media were not used, however, because they always contained a low concentration of sodium which might vary by as much as 10-fold at the end of an experiment (i.e. from 0·1 to 0·01 mM-Na).

The uptake of [^{14}C]sorbitol reached a steady value of 390 ± 20 (12) ml./kg fresh wt. when calcium was omitted from the bathing medium (*E*). This was taken as a measure of the extracellular space (Goodford & Leach, 1966) and, at the concentration of potassium in the medium, would contain 2·3 m-mole K/kg fresh wt. which may be compared with the amount of rapidly exchanging potassium in the tissue. ^{42}K uptake was therefore

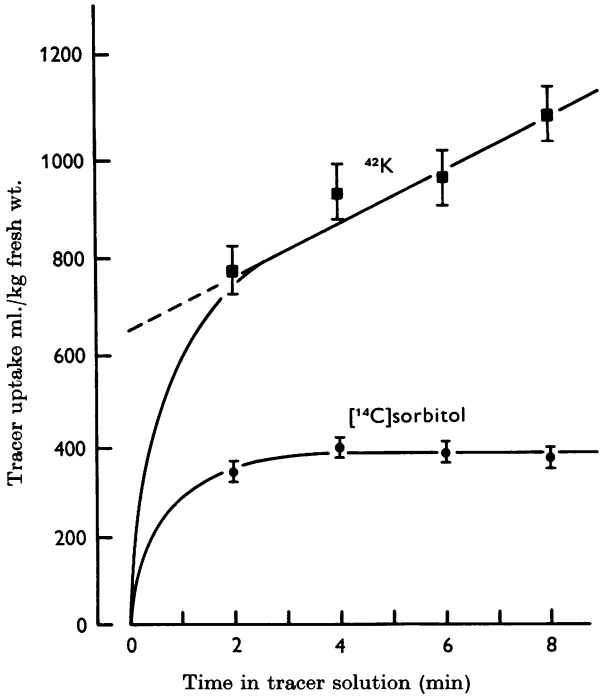

Fig. 3. Radioactive tracer exchange after 2 hr immersion in low-sodium calcium-free solution (*E*) at 35° C. Abscissa and logarithmic ordinate as in Fig. 2. Upper points: ^{42}K exchange. Lower points: the uptake of [^{14}C]sorbitol, which was used as an extracellular marker. Note that the first rapid phase of ^{42}K exchange is appreciably larger than the [^{14}C]sorbitol space, in this solution.

observed in the same muscle samples (Fig. 3), and the rapidly-exchanging potassium in fact corresponded to 760 ml./kg fresh wt. on the basis of diffusion theory, or 780 ml./kg fresh wt. by Huxley's exponential analysis. These estimates were comparable with the wet weight of the tissue which was 790 ± 14 (16) g/kg fresh wt. in solution *E*, and were equivalent to some 4·6 m-mole K/kg fresh wt. of rapidly-exchanging potassium of which only half was accounted for by the [^{14}C]sorbitol space. In the virtual absence of sodium and calcium which normally occupy a significant proportion of the superficial anionic sites (Goodford, 1966), the excess rapidly-exchanging potassium could be a counter-cation, and would amount to 2·3 m-mole fresh wt.

The extracellular [^{14}C]sorbitol space was 370 ± 20 (12) ml./kg fresh wt. in solution *F*, which contained 2·5 mM-$CaCl_2$ but otherwise resembled *E*. The rapidly exchanging potassium then corresponded to 560 ml./kg fresh wt. by either method of calculation, and the excess rapidly-exchanging potassium therefore fell from 2·3 to 1·1 m-mole K/kg fresh wt. when the

calcium concentration was increased from 0 to 2·5 mM-Ca. There was a similar drop from 2·6 to 1·3 m-mole K/kg fresh wt., when the experiments were repeated in solutions to which no magnesium had been added, and on the present interpretation these reductions could be due to a competition between Ca^{2+} and K^+ ions for some of the fixed superficial negative charge in the tissue.

DISCUSSION

The present observations are compatible with Wilbrandt & Koller's (1948) postulate that Na^+ and Ca^{2+} ions compete at anionic regions of the cell surface. However, it would appear that Na^+, K^+, Li^+, Ca^{2+} and Mg^{2+} can all compete to a greater or lesser extent in the smooth muscle of the guinea-pig taenia coli (Goodford, 1965*a*, 1966). There may be just one uniform region of competition in this tissue, or several regions each of which favours a certain group of cations, or an almost continuous distribution of anionic sites of varied character such as might be expected from the known composition of cell membranes. Gorter & Grendel (1925) suggested that these were composed of two monomolecular lipid layers, and their model is still the accepted basis of membrane structure. At its simplest the membrane is regarded as an electrically insulating film, formed by the hydrocarbon part of the lipid, and on each surface of this insulation there is a layer of negative charges due to the polar groups. Each molecule in such a condensed layer would occupy rather more than 20 $Å^2$ of the surface on one side of the membrane, and this area would have one negative charge if the molecule were univalent. Since varying types of lipid molecules may be present, it is reasonable to assume that they have differing chemical affinities and that under normal conditions the cell surface may *not* be covered with a uniform mixture of counter-cations. When, however, only one cation is present in the bathing solution, it would presumably predominate as a counter-cation, and might tend to saturate the negative sites on the cell surface. Under such conditions counter-cation determinations have indicated that there may be rather less than 4 m-equiv/kg fresh wt. of superficial fixed anion in the taenia coli (Goodford, 1966), and this is compatible with the present results in low-sodium calcium-free solution (*E*), and with Schatzmann's (1961) observations.

The ratio of cell volume to cell surface area is $1·5 \times 10^{-4}$ cm in the taenia coli, and the cell volume is roughly 500 ml./kg fresh wt. (Goodford & Hermansen, 1961), from which one may calculate that 1 kg of muscle contains about $3·3 \times 10^6$ cm^2 of cell membrane. There would be an average concentration of 9×10^{-10} equiv/cm^2 spread over the surface of the cells if the amount of fixed anion involved in the competition were 3×10^{-3}

equiv/kg fresh wt., or one fixed anionic charge to every 19 Å^2 of cell surface. It must be largely coincidental that the known molecular cross-section of a condensed lipid film (20 Å) and the surface density of negative charge calculated from the excess rapidly exchanging potassium in the taenia coli (19 Å), agree so very closely. Nevertheless, it indicates that, on the hypothesis under discussion, an appreciable proportion of the cell surface may be covered with fixed anionic charges and their counter-cations, of which two may be specially favoured under normal conditions: calcium because of its double positive charge and tendency to form complexes, and sodium which is present in excess. These factors alone may, in fact, account for the emphasis which has been placed upon Ca^{2+} and Na^+ as counter-cations in heart muscle, although the apparent differences between tissues may also be due to the use of different experimental methods.

Very little free Ca^{2+} ion is present in cell water, but Ashley, Caldwell, Lowe, Richards & Schirmer (1965) have shown that a total sarcoplasmic concentration of 0·2–0·5 mM calcium is needed for the contraction of skeletal muscle, almost all of which calcium may interact with actomyosin in the approximate ratio 1–2 mole Ca/1 mole myosin (Weber & Herz, 1963). The available evidence in smooth muscle shows, however, that only one tenth as much actomyosin is present (Needham & Williams, 1963) as in skeletal fibres, and if these proportions are maintained in the taenia coli only one tenth as much calcium would be needed for contraction. This would amount to 0·01–0·025 m-mole Ca/kg fresh wt., taking the cell volume to be 500 ml./kg fresh wt., and on the basis of the previous calculation this calcium could be accommodated as a counter-action on less than 1 % of the cell surface sites. It is therefore possible that the rhythm of contraction and relaxation in smooth muscle may be associated with a tidal movement of calcium from sites at the cell membrane to other sites inside the cells, and such a flow of calcium ions could be associated with a flow of electric charge.

The $3{\cdot}3 \times 10^6$ cm^2/kg fresh wt. of cell membrane in the taenia coli have a capacity of 3 μF/cm^2 (Tomita, 1966) charged to a potential of 50 mV in the resting state (Holman, 1958), and some 0·7 C of electricity/kg tissue would be needed to reverse the potential by 20 mV at the height of an action potential. This electric charge could be carried by $0{\cdot}7\ (2 \times 96{,}500)^{-1}$ mole of divalent calcium ions, where 96,500 C/mole is Faraday's constant, so that a flow of 0·004 m-mole Ca/kg fresh wt. passing across the cell membrane would account for the rising phase of the action potential, in the absence of other current flow. This calcium could partly activate the contractile mechanism, and five or ten such events would produce full activation according to the present calculations.

It is suggested as a working hypothesis that a primitive mechanism of excitation–contraction coupling is quantitatively feasible in the taenia coli, and that each action potential may be associated with an inward movement of calcium ions from a small proportion of the superficial anionic sites. This calcium flow may both initiate the contractile response and contribute significantly to the depolarizing current across the cell membrane, the movement of other ions possibly being responsible for the characteristic shape of the action potential itself.

I am indebted to the U.S. Public Health Service for grant GM 10404 which supported part of this work, and to Dr E. Bülbring and Dr T. Tomita for many valuable discussions.

REFERENCES

ASHLEY, C. C., CALDWELL, P. C., LOWE, A. G., RICHARDS, C. D. & SCHIRMER, H. (1965). The amount of injected EGTA needed to suppress the contractile responses of single *Maia* muscle fibres and its relation to the amount of calcium released during contraction. *J. Physiol.* **179**, 32–33*P*.

BAUER, H., GOODFORD, P. J. & HÜTER, J. (1965). The calcium content and ^{45}calcium uptake of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **176**, 163–179.

BOZLER, E. (1962). Osmotic phenomena in smooth muscle. *Am. J. Physiol.* **203**, 201–205.

CHUJYO, N. & HOLLAND, W. C. (1963). Potassium induced contracture and calcium exchange in the guinea-pig's taenia coli. *Am. J. Physiol.* **205**, 94–100.

FREEMAN-NARROD, M. & GOODFORD, P. J. (1962). Sodium and potassium content of the smooth muscle of the guinea-pig taenia coli at different temperatures and tensions. *J. Physiol.* **163**, 399–410.

GOODFORD, P. J. (1965*a*). An interaction between potassium and calcium in smooth muscle. *J. Physiol.* **180**, 19–20*P*.

GOODFORD, P. J. (1965*b*). The distribution of calcium in intestinal smooth muscle. In *Muscle*, ed. PAUL, W. M., DANIEL, E. E., KAY, C. M. and MONCKTON, C. London: Pergamon.

GOODFORD, P. J. (1966). An interaction between potassium and sodium in the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **186**, 11–26.

GOODFORD, P. J. & HERMANSEN, K. (1961). Sodium and potassium movements in the unstriated muscle of the guinea-pig taenia coli. *J. Physiol.* **158**, 426–448.

GOODFORD, P. J. & LEACH, E. H. (1966). The extracellular space of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **186**, 1–10.

GORTER, E. & GRENDEL, F. (1925). On bimolecular layers of lipoids on the chromocytes of the blood. *J. exp. Med.* **41**, 439–443.

HARRIS, E. J. & BURN, G. P. (1949). The transfer of sodium and potassium ions between muscle and the surrounding medium. *Trans. Faraday Soc.* **45**, 508–528.

HOLMAN, M. E. (1958). Membrane potentials recorded with high resistance micro-electrodes and the effects of changes in the ionic environment on the electrical and mechanical activities of the smooth muscle of the taenia coli of the guinea-pig. *J. Physiol.* **141**, 464–488.

HUXLEY, A. F. (1960). Compartmental methods of kinetic analysis. Appendix 2. *Mineral Metabolism*, vol. 1, Part A, ed. COMAR, C. L. & BRONNER, F., pp. 163–166. New York: Academic Press.

KREBS, H. A. (1950). Body size and tissue respiration. *Biochim. biophys. Acta* **4**, 249–269.

MANERY, J. F. (1954). Water and electrolyte metabolism. *Physiol. Rev.* **34**, 334–417.

NEEDHAM, D. M. & WILLIAMS, J. M. (1963). The proteins of the dilution precipitate obtained from salt extracts of pregnant and non-pregnant uterus. *Biochem. J.* **89**, 534–545.

NEWMAN, W. F. & NEWMAN, M. W. (1958). *The Chemical Dynamics of Bone Mineral*, 1st edn., pp. 1–38. University of Chicago Press.

NIEDERGERKE, R. (1963). Movements of Ca in frog heart ventricles at rest and during contracture. *J. Physiol.* **167**, 515–550.

NIEDERGERKE, R. & LÜTTGAU, H. C. (1957). Antagonism between calcium and sodium ions. *Nature, Lond.* **179**, 1066–1067.

SCHAECHTELIN, G. (1961). Der Einfluss von Calcium und Natrium auf die Kontraktur des M. rectus abdominis. *Pflügers Arch. ges. Physiol.* **273**, 164–181.

SCHATZMANN, H. J. (1961). Calciumaufnahme und Abgabe am Darmmuskel des Meerschweinchens. *Pflügers Arch. ges. Physiol.* **274**, 295–310.

TOMITA, T. (1966). Membrane capacity and resistance of mammalian smooth muscle. *J. theor. Biol.* **12**, 216–227.

WEATHERALL, M. (1962). Quantitative analysis of movements of potassium in rabbit auricles. *Proc. R. Soc.* B **156**, 57–82.

WEBER, A. & HERTZ, R. (1963). The binding of calcium to actomyosin systems in relation to their biological activity. *J. biol. Chem.* **238**, 599–605.

WILBRANDT, W. & KOLLER, H. (1948). Die Calcium Wirkung am Froschherzen als Funktion des Ionengleichgewichts zwischen Zellmembran und Umgebung. *Helv. physiol. pharmac. Acta* **6**, 208–221.