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INTERACTION OF ^{28}Mg WITH Ca AND K IN THE SMOOTH MUSCLE OF GUINEA-PIG TAENIA COLI

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SUMMARY

1. The uptake of ^{28}Mg, and the total tissue content of Mg, Ca, Na and K have been determined in the smooth muscle of the guinea-pig taenia coli. The Mg content was 6·56 m-mole/kg fresh wt. immediately after dissection, falling slowly to 5·11 after 6 hr immersion in Krebs solution at 37° C.

2. The Mg content rose to 15·4 m-mole/kg fresh wt. during immersion in isotonic sucrose containing only $MgCl_2$. It was independent of the Mg^{2+} concentration in this solution, but was depressed when K^+ or Ca^{2+} ions were added.

3. ^{28}Mg uptake showed three separate phases, extracellular, intermediate and slow. The size of the extracellular phase was proportional to the Mg^{2+} concentration in the solution, but the size of the slow phase was constant. The size of the intermediate phase, exchanging with a half-time of a few minutes, was depressed when K^+ or Ca^{2+} ions were added.

4. The results are compatible with a competition between Mg in the intermediate phase of tracer exchange, and K^+ or Ca^{2+} ions for fixed anionic sites in the tissue.

INTRODUCTION

There is evidence that cations may compete with one another for fixed negative sites in muscle. The contractility of cardiac muscle is dependent on a competition between the external Ca^{2+} and Na^+ concentrations, and it has been proposed that these ions may compete for a superficial negatively charged carrier R^- in the membrane (Wilbrandt & Koller, 1948; Niedergerke & Lüttgau, 1957). It has also been shown that Ca and Mg exert antagonistic actions on the contractile and the electrical properties of

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smooth muscle (Sperelakis, 1962; Marshall, 1964; Edman & Schild, 1962), and an interaction has been observed between Na^+, K^+ and Ca^{2+} which could be due to a competition between these cations for fixed negative sites in the tissue (Goodford, 1965, 1966, 1967).

An attempt has now been made to establish whether Mg also competes with Ca, Na and K in smooth muscle and, furthermore, whether the characteristics of this competition are compatible with the assumption that there is a constant number of similar anionic sites in the tissue.

A preliminary communication has been made to the Physiological Society (Sparrow, 1969).

METHODS

Solutions

Modified Krebs solutions A, F and G (Table 1) were equilibrated with a CO_2/O_2 mixture to give a pH of $7{\cdot}3 \pm 0{\cdot}1$. In solutions B, C, D and E, a trace of $Mg(OH)_2$ was added initially to adjust the pH to this value, and these solutions were only gassed with O_2. Their pH varied at the most by 0·3 pH units during a 6 hr period, as shown by a continuous pH recording.

Radioactive $^{28}MgCl_2$ in HCl was obtained from Brookhaven National Laboratory, U.S.A. It was checked for purity by radioactive decay measurements of both β and γ activity, and only a single isotopic species (^{28}Mg) could be detected during 5 half-lives after the biological experiments had been completed. No residual β nor γ activity could be detected after 20 half-lives. Efforts were made to detect the trace metals Cu, Ni, Pb and Al in the concentrated isotopic solution (30 μc/ml.) by atomic absorption spectroscopy, and these observations showed that such metal impurities would be present at less than 1 part in 10^8 in the bathing solutions. Furthermore, strips of muscle were observed to contract normally and spontaneously for at least 5 hr when suspended in a normal Krebs solution (A, Table 1) which contained a concentration of ^{28}Mg ten times higher than that normally used.

[^{14}C]sorbitol was obtained from the Radiochemical Centre, Amersham, and was counted by liquid scintillation in order to measure the extracellular space.

Procedure

White guinea-pigs weighing between 300 and 450 g were stunned, bled and eight or nine 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 a thread and attaching a glass bead weighing 1·5 g to each muscle, they were suspended in the appropriate solution at 37° C, and left for several hours 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. Total time of exposure to solutions between dissection and the final weighing was 6–8 hr in most experiments. When measuring the extracellular space during a ^{28}Mg uptake experiment, [^{14}C]sorbitol was present in the solution during the last 15 min before removing the muscle for the final weighing.

Muscles were extracted using a modification of the method of Sparrow & Johnstone (1964). Strips of taenia coli were placed in Jena glass tubes No. 20, and 0·25 ml. of a solution of trichloracetic acid dissolved in glacial acetic acid (1 g/ml.) was added. The tubes were heated in a hot air oven at 160° C for 10 min until the muscle had almost dissolved, and about 2 ml. double-distilled water was added. They were warmed again until nearly boiling, and were then cooled and capped and the γ

TABLE 1. The composition of the solutions (mM) which were oxygenated at 37° C to give a pH of 7·3

Solution	Na	K	Mg	Ca	Cl	HCO_3	Sucrose	Glucose	Sorbitol	Ionic strength	Isotonicity
A	137	5·9	1·25	2·5	144·5	5·9	—	11	1	154	309
B	—	—	1·2	—	2·4	—	292	11	1	3·6	308
C	—	—	2	—	4	—	290	11	1	6	308
D	—	3	1	—	5	—	287	11	1	6	308
E	—	—	1	1	4	—	290	11	1	6	308
F	145	1	1·25	0·1	142·7	6	—	11	1	151	308
G	130	1	1·25	10·0	147·5	6	—	11	1	165	308

radiation from the ^{28}Mg was counted in a NaI crystal well-type scintillation counter. The contents of the tubes were made up to 10 ml., and agitated and centrifuged. 0·5 ml. was withdrawn for ^{14}C determination and counted by liquid scintillation after the ^{28}Mg had decayed. The remainder of the supernatant was used for the determination of the electrolyte content of the tissue, Mg, K, Ca and Na being measured on an atomic absorption spectrophotometer (Techtron, AA4) with adequate controls and precautions against interference. The electrolyte content of tissue was expressed as the average amount of electrolyte per unit weight of freshly dissected tissue, i.e. m-mole/kg fresh wt.

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: $6{\cdot}56 \pm 0{\cdot}06$ (46). Means have been compared on a probability scale so that smaller values of P correspond to increasingly significant differences. Exponentials were fitted by the method of least squares (Goodford, 1966).

RESULTS

Mg content in normal Krebs solution (A)

The average tissue Mg content of strips of freshly dissected taenia coli in preliminary experiments was $6{\cdot}56 \pm 0{\cdot}06$ (46) m-mole/kg fresh wt. Analysis of variance showed a highly significant variation among animals ($P < 0{\cdot}001$) but the Mg content of the smooth muscle decreased significantly ($P < 0{\cdot}001$) to $5{\cdot}11 \pm 0{\cdot}05$ (59) m-mole/kg fresh wt. after equilibration in Krebs solution at 37° C for 6 hr. When matched strips of muscle were used in further experiments the Mg content decreased from $6{\cdot}13 \pm 0{\cdot}08$ (12) m-mole/kg fresh wt. for freshly dissected strips to $5{\cdot}19 \pm 0{\cdot}12$ (12) after 3 hr in Krebs solution A and to $4{\cdot}62 \pm 0{\cdot}11$ (12) m-mole/kg fresh wt. after 12 hr (open circles, Fig. 1). Radioactive tracer observations were usually made after about 6 hr immersion when the total tissue Mg was not changing appreciably, and experiments were designed so that the total immersion period of each muscle strip was the same irrespective of the period of exposure to the radioactive tracer.

^{28}*Mg uptake in normal Krebs solution* (A)

Figure 2 shows the total Mg content and the uptake of ^{28}Mg by strips of taenia coli which were equilibrated in Krebs solution (solution A, Table 1). A single exponential function of the form

$$y = A + B(1 - \exp(-Ct)) \qquad (1)$$

was fitted to the radioactive ^{28}Mg observations, where

y = ^{28}Mg uptake at time t min,
A = ^{28}Mg uptake calculated for zero time,
$A+B$ = ^{28}Mg uptake calculated for infinite time,
C = the exponential rate constant for ^{28}Mg uptake in min^{-1}.

The best-fitting values of the parameters A, B and C were

$$y = 0{\cdot}79 + 4{\cdot}62(1 - \exp(-0{\cdot}0017t)) \qquad (1a)$$

and the calculated half-time of the exchange process was therefore some 400 min.

Equation (1*a*) is compatible with a single exponential process for ^{28}Mg uptake approaching an asymptote of $0{\cdot}79 + 4{\cdot}62 = 5{\cdot}41$ m-mole Mg/kg fresh wt., and the total tissue magnesium was in fact $5{\cdot}29 + 0{\cdot}09$ (30)

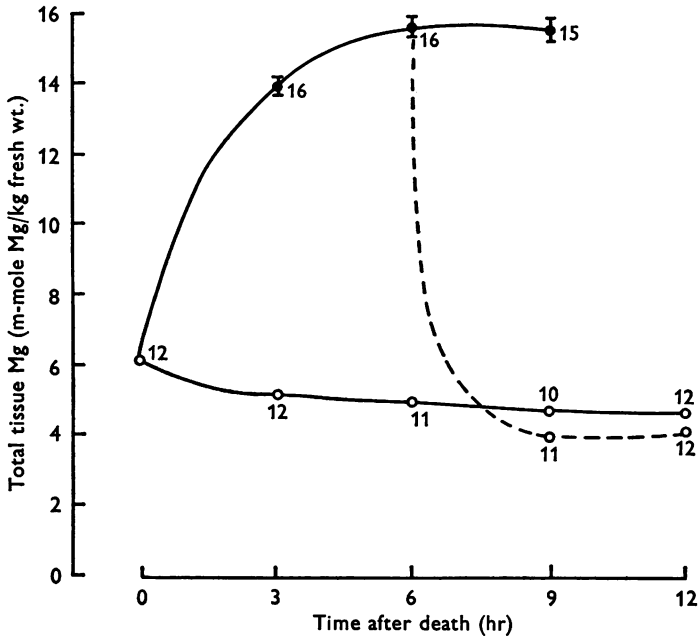

Fig. 1. Observations *in vitro* at 37° C. Abscissa: time in hr after killing a guinea-pig. Ordinate: total tissue Mg in m-mole/kg fresh wt. The number of observations is shown by each point, and the S.E. limits of each mean are marked by horizontal lines if they exceed the size of the printed symbol. This convention is followed in all Figs. When the taeniae were immersed in normal Krebs solution (open circles) there was an initial loss of magnesium after which an almost steady state was established. Immersion in sucrose solution containing 1·2 mM-$MgCl_2$ caused a substantial increase in the total tissue Mg (filled circles) which was reversible in Krebs solution (open circles; interrupted line). Radioactive observations were normally made at 6 hr when the composition of the tissue was not changing appreciably.

m-mole Mg/kg fresh wt. when determined independently in the same tissue samples by atomic absorption photometry. The values of 5·41 and 5·29 did not differ significantly, and so the experiments gave no indication of a still more slowly exchanging fraction of the tissue Mg. One might therefore calculate an average trans-membrane Mg flux of 0·02 p-mole cm^{-2} sec^{-1},

if the slowly exchanging Mg was all exchanging across the cell membrane, and a Mg equilibrium potential of -23 mV if activity coefficients be neglected.

The slow component of ^{28}Mg exchange gave an intercept of 0·79 m-mole/kg fresh wt. at zero time, and the interpretation of this value on the basis of a two compartment system would depend, in general, on whether a series or a parallel arrangement of components be assumed. However, the

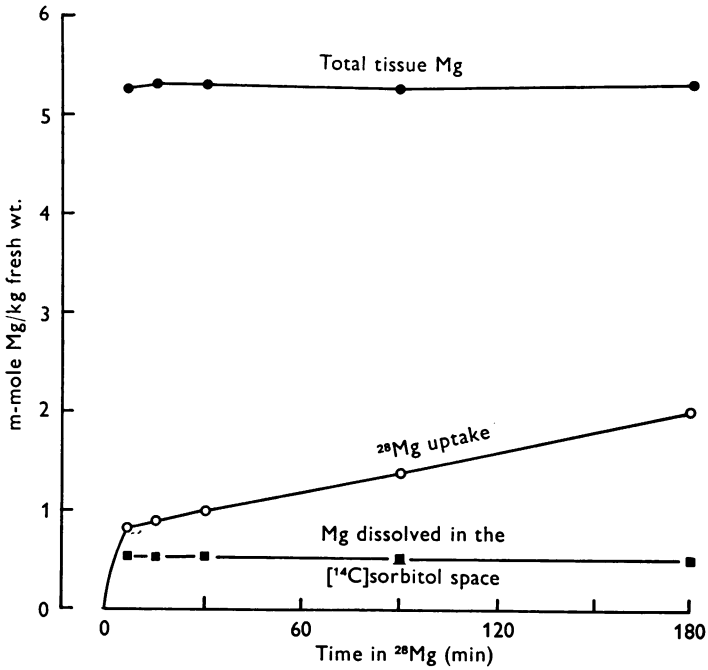

Fig. 2. Observations *in vitro* at 37° C in normal Krebs solution. Abscissa: time in min after immersion in radioactive ^{28}Mg solution. The last 15 min of immersion were always in a solution containing radioactive [^{14}C]sorbitol to measure the extracellular space. Ordinate: m-mole Mg/kg fresh wt. Symbols: ●, total tissue Mg; ○, ^{28}Mg uptake; ■, the amount of Mg freely dissolved in extracellular [^{14}C]sorbitol space. Each point is the mean of six observations, and the S.E. never exceeded the size of the printed symbol. The uptake of ^{28}Mg is slow (see text) and may be described by a single exponential function after the first few minutes.

difference between such interpretations has been neglected in the present case because the amount of Mg in the rapidly exchanging compartment would be exactly 0·79 m-mole/kg fresh wt. for a parallel system, and the correction on the basis of the published formulae for interaction between compartments (Huxley, 1960; Weatherall, 1962; Goodford, 1966) would only increase this value to 0·795 m-mole/kg fresh wt. The amount of rapidly exchanging Mg in the taenia coli in normal Krebs solution was

therefore taken as 0·79 m-mole/kg fresh wt., and an attempt was made to assign a S.E. to this value. The observations were recalculated separately for each guinea-pig and the mean of these individual results was 0·786 ± 0·024 (6), which significantly exceeded ($P < 0·05$) the amount of Mg (0·52 ± 0·02 m-mole/kg fresh wt.) which would be freely dissolved in the extracellular [^{14}C]sorbitol space of 420 ± 19 (30) ml./kg fresh wt. The small variation between 0·795, 0·79 and 0·786 has been neglected, but the difference 0·79 − 0·52 = 0·27 m-mole/kg fresh wt. may be real, and may represent counter cation at superficial anionic sites in the tissue.

Total tissue Mg in solutions of low ionic strength

The amount of Mg at the postulated superficial anionic sites in the taenia coli might have been small in normal Krebs solution because of the preponderance of K^+, Na^+ and Ca^{2+} cations which could also be competing for these sites, and for this reason observations were next made in solutions of reduced ionic strength. The muscle Mg and the exchangeable ^{28}Mg were measured in solution B (Table 1) when all the other cations used to prepare the Krebs solution had been replaced with sucrose. The total tissue Mg rose during this immersion in $MgCl_2$ 1·2 mM, and reached 15·4 ± 0·3 (16) m-mole/kg fresh wt. after 6 hr (filled circles, Fig. 1). No further significant increase occurred after this time, although the Mg uptake was substantially incomplete after only 3 hr. The Na was rapidly lost from the tissue, less than 1 m-mole Na/kg fresh wt. remaining after 3 hr, but the K changes were somewhat slower so that 24·3 ± 0·7 (15) m-mole K/kg fresh wt. remained at the same time. The Na and K contents of frog and toad stomach muscle behaved similarly in sucrose solutions (Bozler & Lavine, 1958; Sparrow, Mayrhofer & Simmonds, 1967).

The striking increase in the Mg content of the smooth muscle in these $MgCl_2$ solutions was found to be rapidly reversible when the muscle was re-equilibrated in Krebs solution, and even after 6 or 9 hr exposure to 1·2 mM-Mg in sucrose the elevated Mg content decreased to a value only slightly lower than that of matched strips of muscle exposed continuously to Krebs solution for the same length of time (Fig. 1). However, the Na and K content of the muscle were not restored so rapidly on equilibrating in Krebs solution, and completely normal values were not re-established even after long periods up to 12 hr.

Mg content at different $[Mg]_o$

Goodford (1966) considered the possibility of a series of ionic interactions at the cell surface of the taenia coli

$$Mg^{2+} + 2NaR = 2Na^+ + MgR_2,$$
$$K^+ + NaR = Na^+ + KR$$

in which R^- represented a single species of fixed anionic charge. In such a system the total amount of counter-cation should always be constant, and if only one cationic species was present in the solution it should be in equilibrium with the same amount of counter-cation, irrespective of its solution concentration. Goodford was therefore concerned to observe that the amount of K counter-cation tended to increase when the K^+ concentration in the solution was raised from 5·96 to 11·92 mM, although he was not prepared to place too much reliance on this particular comparison.

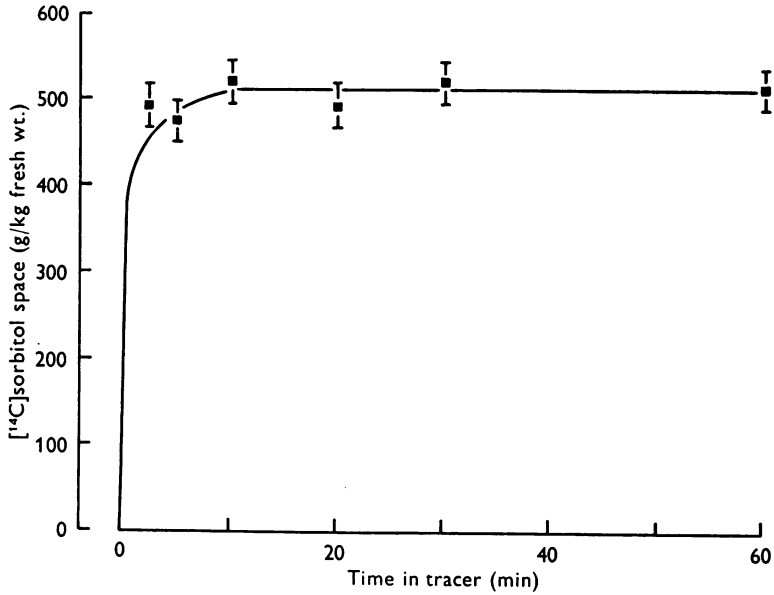

Fig. 3. Observations *in vitro* at 37° C in isotonic sucrose solutions containing 2 mM-$MgCl_2$. Abscissa: time of immersion in radioactive [^{14}C] sorbitol (min). Ordinate: the uptake of [^{14}C] sorbitol by the tissue, measuring the extracellular space in g/kg fresh wt. Each point is the mean of six observations. Note that the first rapid phase of uptake was virtually complete within 10 min. 15 min immersion was therefore taken as standard for the measurement of the extracellular [^{14}C]sorbitol space.

A series of experiments have now been carried out in which taeniae were equilibrated with isotonic sucrose solutions to which $MgCl_2$ was added giving concentrations ranging from 0·5 to 4·7 mM. The amount of extracellular Mg in the tissue was calculated by measuring the [^{14}C]sorbitol uptake after 15 min [^{14}C]sorbitol immersion, since this was long enough for a steady state to be established in these solutions (Fig. 3). The increased Mg content of the [^{14}C]sorbitol space at higher $[Mg]_o$ almost entirely accounted for the corresponding increase in total tissue Mg (Table 2), and the Mg content of the rest of the tissue was therefore virtually constant in

these experiments. For this reason it is tentatively assumed that the anionic sites in the taenia may be associated with a counter cation at all times.

^{28}Mg uptake in 2 mM-$MgCl_2$ sucrose solution

The uptake of ^{28}Mg by strips of taenia coli equilibrated in 2 mM-$MgCl_2$ in sucrose (solution C) is shown in Fig. 4*a*. The total tissue Mg was 16·05 ± 0·13 (61) m-mole/kg fresh wt. as measured by atomic absorption

TABLE 2. Total tissue Mg and the amount of Mg in the [^{14}C]sorbitol extracellular space of the taenia coli, after 8 hr immersion in $MgCl_2$ solutions made isotonic with sucrose. Increasing the Mg concentration in the bathing medium caused the Mg dissolved in the extracellular [^{14}C]sorbitol space to rise, but the Mg in the rest of the tissue was not changed

$[Mg]_o$ (mM)	Total tissue Mg (m-mole/kg fresh wt.)	Extracellular Mg (m-mole/kg fresh wt.)	Remaining Mg (m-mole/kg fresh wt.)
0·5	14·4 ± 0·16 (24)	0·34 ± 0·007	14·1 ± 0·17
1·1	15·4 ± 0·28 (24)	0·62 ± 0·018	14·8 ± 0·31
1·5	15·7 ± 0·18 (23)	0·91 ± 0·014	14·8 ± 0·17
2·0	16·2 ± 0·14 (23)	1·09 ± 0·011	15·1 ± 0·14
4·1	16·7 ± 0·28 (24)	1·80 ± 0·037	14·9 ± 0·26
4·7	17·5 ± 0·32 (22)	2·23 ± 0·045	15·3 ± 0·31

photometry, and the ^{28}Mg observations were subtracted from this value giving differences which were plotted in Fig. 4*b* using the logarithmic ordinate to the right of the Fig. The points still lie on a rising curve as in Fig. 4*a*, and comparison with the left hand ordinate shows that they still measure the uptake of tracer. The distortion from a single exponential function may be very clearly seen with these co-ordinates, and this was confirmed by calculation (Table 3), so that ^{28}Mg observations were reinterpreted using a double exponential function of the form

$$y = A + B(1 - \exp(-Ct)) + D(1 - \exp(-Et)) \qquad (2)$$

in which the best-fitting values for the parameters, determined by the method of least squares, were

$$y = 1{\cdot}89 + 4{\cdot}89(1 - \exp(-0{\cdot}110t)) + 8{\cdot}62(1 - \exp(-0{\cdot}0161t)). \qquad (2a)$$

It would be necessary to consider another interpretation of the observations if the intercept at 1·89 m-mole/kg fresh wt. and the asymptote of 1·89 + 4·89 + 8·62 = 15·40 m-mole/kg fresh wt. were significantly different from the amounts of Mg freely dissolved in [^{14}C]sorbitol extracellular space (1·1 m-mole/kg fresh wt.), and from the total tissue Mg (16·05 m-mole/kg fresh wt.). However, it is an open question whether the differences are

statistically significant; and consideration of the ^{28}Mg results suggests that they are compatible with a double exponential function between the limits 1·1 and 16·05 m-mole/kg fresh wt. These two additional pieces of experimental evidence can be used to constrain the least squares fit, which then becomes

$$y = 1{\cdot}10 + 5{\cdot}95(1 - \exp(-0{\cdot}131t)) + 9{\cdot}00(1 - \exp(-0{\cdot}0131t)). \quad (2b)$$

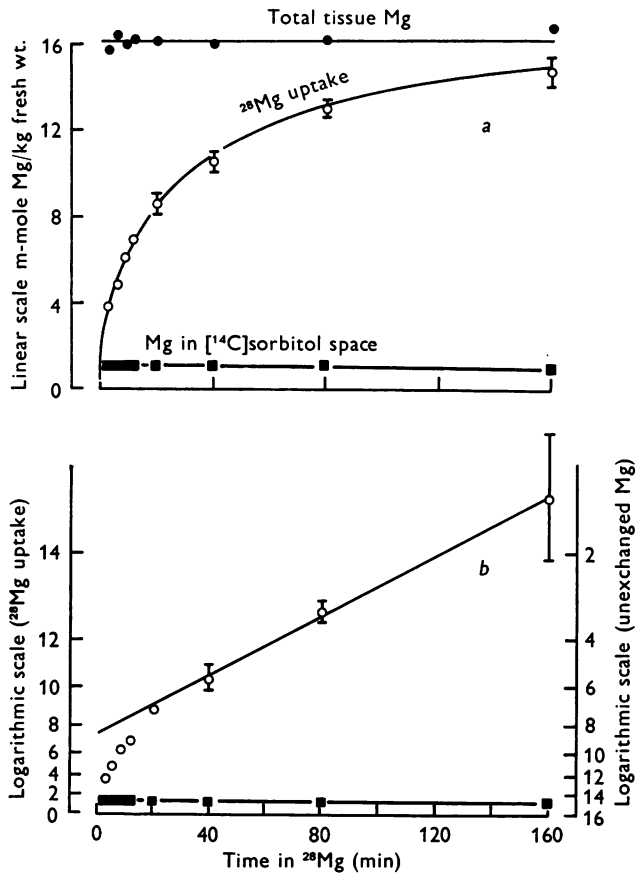

Fig. 4. Observations *in vitro* in isotonic sucrose solution containing 2 mM-$MgCl_2$ at 37° C. Ordinates (linear in Fig. 4*a*; logarithmic in Fig. 4*b*, see text): m-mole Mg/kg fresh wt. Abscissa and symbols as Fig. 2. In Figs. 4 to 7 each point is the mean of at least seven observations. The observations cannot easily be fitted to a single exponential function, but are adequately described by a double exponential curve (see text and Table 3).

Equation (2*b*) can describe one very rapid exchange process followed by two slower phases arranged either in series or in parallel (Weatherall, 1962). In the latter case the amount of Mg in the intermediate phase would

be 5·95 m-mole/kg fresh wt. which substantially exceeds the 0·27 m-mole/kg fresh wt. exchanging similarly in Krebs solution (Table 4). However, appreciable corrections to these values would be needed if the compartments were arranged in series (Huxley, 1960; Weatherall, 1962; Goodford, 1966), and the increase would then be from 0·44 to 7·77 m-mole/kg fresh wt., showing that the change of solution still influenced the size of the intermediate phase of ^{28}Mg exchange.

TABLE 3. Analysis of ^{28}Mg uptake in 2 mM-$MgCl_2$ solution in isotonic sucrose. The observations from 3 to 12 min, and from 20 to 160 min, were fitted separately to the single exponential function $y = A + B(1 - \exp(-Ct))$ assuming that the uptake finally asymptoted to the total tissue Mg. The regressions at early and late times of observation differed significantly ($P < 0·001$) showing that the uptake was *either* not a single exponential *or* that it did not asymptote to the total tissue Mg

Times of observation (min)	Intercept A (m-mole Mg/kg fresh wt.)	Rate constant C (min^{-1})
3–12	2·25 ± 0·19 (8)	0·036 ± 0·0025 (8)
20–160	6·86 ± 0·70 (8)	0·012 ± 0·0016 (8)
Difference	4·61 ± 0·73 (14)	0·024 ± 0·0029 (14)
P	< 0·001	< 0·001

Interaction of Mg with other cations

The previous experiment suggested that the intermediate phase of ^{28}Mg exchange, which had a rate constant of 0·131 min^{-1}, might represent Mg^{2+} ions associated with fixed anionic sites in the tissue. Further experiments were therefore designed in order to measure the size of this component in the presence and absence of other cations which might be competing for the same sites. The slow phase of Mg exchange was measured with ^{28}Mg at times longer than 20 min, and the extracellular Mg was calculated from observations of the [^{14}C]sorbitol space in the same muscle samples, so that the size of the intermediate phase could be calculated by subtracting these quantities from the total tissue Mg.

Effect of $[Mg]_o$ on ^{28}Mg exchange

In the absence of other cations the size of the ^{28}Mg intercept of the slow phase showed a rising trend when increasing concentrations of $MgCl_2$ in sucrose were used (Table 5), in the same way as the total tissue Mg rose. However, there was a corresponding increase in the amount of Mg in the [^{14}C]sorbitol space, and the size of the intermediate phase of Mg exchange was therefore independent of the $MgCl_2$ concentration. This finding was compatible with the view that the anionic sites in the taenia were fully associated with Mg counter-cations at all times in these experiments, since no competing cations had been added to the bathing solutions.

TABLE 4. A comparison of the ^{28}Mg uptake kinetics in normal Krebs solution, and in 2 mM-$MgCl_2$ solution in isotonic sucrose. The main effect of altering the solutions was upon the intermediate phase, which increased from 0·27 to 5·95 m-mole/kg fresh wt., or from 0·44 to 7·77 after Huxley's correction had been applied

	Krebs solution			2 mM-$MgCl_2$ solution		
	Observed rate constant (min^{-1})	Observed size (m-mole/kg fresh wt.)	Corrected size (m-mole/kg fresh wt.)	Observed rate constant (min^{-1})	Observed size (m-mole/kg fresh wt.)	Corrected size (m-mole/kg fresh wt.)
^{28}Mg in						
Slower phase	0·0017	4·50	4·33	0·0131	9·00	7·18
Intermediate phase	0·1	0·27	0·44	0·131	5·95	7·77
[^{14}C]sorbitol space	2·8	0·52	0·52	2·8	1·1	1·1
Total tissue Mg		5·29 ± 0·09 (30)			16·05 ± 0·13 (61)	

Competition between Mg and K

The ionic strength of the low cation solutions was kept constant in the first competition experiments, in order to ensure that the strength of the electrical field in the vicinity of any fixed negative charges would not vary appreciably. Strips of taenia were equilibrated for 8 hr in either solution C or D (Table 1), and the total tissue Mg was reduced in the presence of K (Figs. 5*a* and 5*b*). A small part of this reduction was due to the different Mg contents of the [^{14}C]sorbitol space, and the slowest phase of ^{28}Mg

TABLE 5. ^{28}Mg uptake in different concentrations of $MgCl_2$ in isotonic sucrose. The changes in the intercept of the slow phase are accounted for by the changed Mg content of the [^{14}C]sorbitol space, and the size of the intermediate phase of ^{28}Mg exchange (last column) does not increase appreciably

$[Mg]_o$ (mM)	^{28}Mg intercept	Extracellular Mg (m-mole/kg fresh wt.)	^{28}Mg intercept less extracellular Mg
1	6·91	0·63	6·28
1·5	6·96	0·91	6·05
2·0	7·28	1·14	6·14
4·5	7·97	2·10	5·87
		Average	6·09

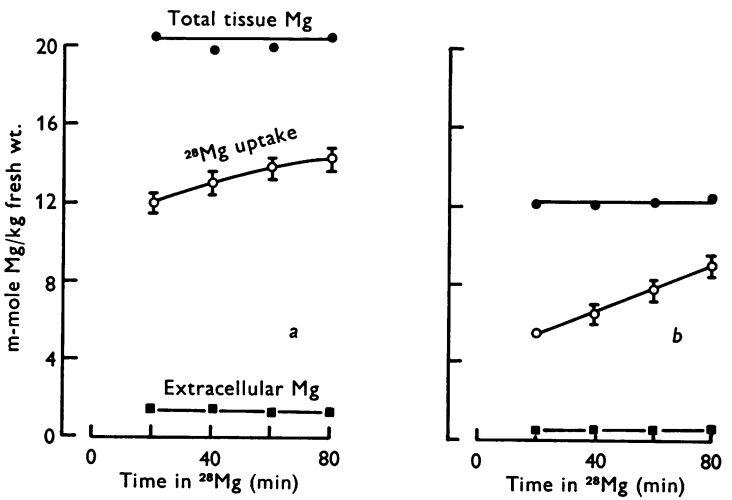

Fig. 5. Observations *in vitro* at 37° C in two different solutions with the same total ionic strength. (*a*) 2 mM-$MgCl_2$ in isotonic sucrose, (*b*) 1 mM-$MgCl_2$ and 3 mM-KCl. Abscissae, linear ordinates, and symbols as in Fig. 2. The uptake of ^{28}Mg was depressed when the K concentration was raised, and this was largely due to a change in the size of the intermediate phase (see text).

exchange was slightly different between the two solutions, but the greatest effect was observed on the size of the intermediate phase which fell from 8·7 to 3·1 m-mole/kg fresh wt., or from 10·0 to 4·1 m-mole/kg fresh wt. after applying Huxley's correction (Table 6).

It would be possible to argue that the changes shown in Fig. 5 and Table 6 were due to changing the Mg concentration from 2 to 1 mM, rather than to the competition of the added K^+ ions, and further experiments were

TABLE 6. Observations in isotonic sucrose solutions C and D in which Mg^{2+} and K^+ were the only cations used, and the ionic strength was maintained constant. The total tissue content of Mg and K did not change when K^+ ions were substituted for Mg^{2+} in the solution, but there was a substantial fall in the Mg content of the intermediate phase of ^{28}Mg exchange

Solution	C	D	Units
$[Mg]_o$	2	1	mM
$[K]_o$	0	3	mM
Ionic strength	6	6	mM
Total tissue Mg	20·1 ± 0·4 (28)	12·3 ± 0·2 (29)	m-mole/kg fresh wt.
Total tissue K	3·7 ± 0·4 (28)	19·6 ± 0·8 (29)	m-mole/kg fresh wt.
Total tissue Mg and K	44·2	44·2	m-equiv/kg fresh wt.
[^{14}C]sorbitol space	637 ± 16	513 ± 13	ml./kg fresh wt.
Corresponding extracellular Mg	1·3	0·5	m-mole/kg fresh wt.
^{28}Mg intercept	10·0	3·6	m-mole/kg fresh wt.
Intermediate phase	8·7	3·1	m-mole/kg fresh wt.
Rate constant of slowest phase	0·00856	0·00938	min^{-1}
Intermediate phase corrected	10·0	4·4	m-mole/kg fresh wt.

therefore made at constant Mg concentrations. When K^+ ions were included in the solution at increasing concentrations the total tissue Mg again declined (Table 7), and this fall was due to a reduction in the size of the intermediate phase of ^{28}Mg exchange (Fig. 6).

Competition between Mg and Ca

Similar observations were made in order to test whether Ca^{2+} ions would also compete with Mg^{2+}. The sum of the total tissue Mg and Ca contents did not change greatly in these experiments (Table 8), but the introduction of Ca^{2+} ions reduced the size of the intermediate phase of ^{28}Mg exchange from 6·46 to 1·37 m-mole/kg fresh wt., or from 8·00 to 2·14 m-mole/kg fresh wt. after applying Huxley's factor.

Competition in normal Krebs type solutions

It was concluded on the basis of the previous experiments that Mg, Ca and K might all be competing for a common site in the tissue, and further-

TABLE 7. Observations in isotonic sucrose solutions containing $MgCl_2$, 1 mM with increasing concentrations of K. The total tissue content of Mg and K rose slightly when K^+ ions were added to the solution, but this was accounted for by the changed content of the extracellular space. There was, however, a systematic fall in the ^{28}Mg content of the intermediate phase

					Units
$[Mg]_o$	1	1	1	1	mM
$[K]_o$	0	1	2	4	mM
Ionic strength	3	4	5	7	mM
Total tissue Mg	18·2 ± 0·4	16·6 ± 0·4	15·1 ± 0·3	13·8 ± 0·3	m-mole/kg fresh wt.
Total tissue K	1·5 ± 0·2	5·9 ± 1·1	12·0 ± 3·6	18·1 ± 1·2	m-mole/kg fresh wt.
Total tissue Mg and K	37·9	39·1	42·2	45·7	m-equiv/kg fresh wt.
[^{14}C]sorbitol space	743 ± 23	732 ± 23	678 ± 19	666 ± 20	ml./kg fresh wt.
Corresponding extracellular Mg	0·74	0·73	0·68	0·67	m-mole/kg fresh wt.
^{28}Mg intercept	7·41	6·43	5·11	4·39	m-mole/kg fresh wt.
Intermediate phase	6·67	5·70	4·43	3·72	m-mole/kg fresh wt.
Rate constant of slowest phase	0·0104	0·0106	0·0100	0·00850	min^{-1}
Intermediate phase (corrected)	8·41	7·20	6·01	4·99	m-mole/kg fresh wt.

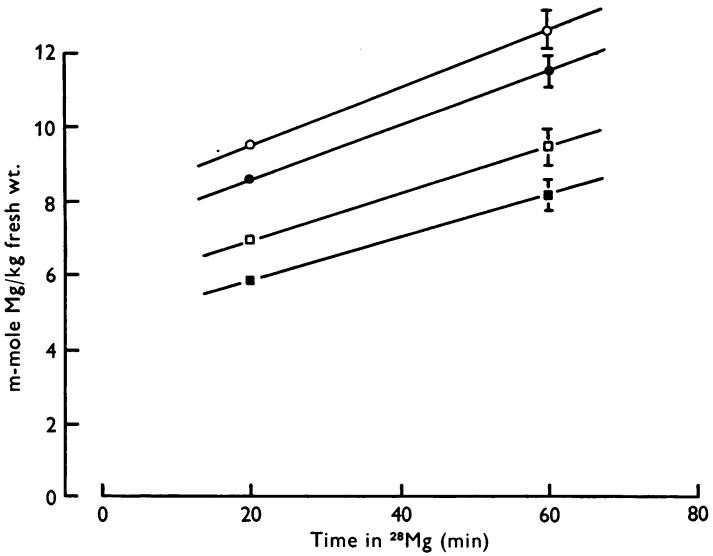

Fig. 6. Observations on the uptake of ^{28}Mg in isotonic sucrose solutions containing 1 mM-$MgCl_2$ with increasing concentrations of K. Abscissa: time in min after immersion in radioactive ^{28}Mg solution. Linear ordinate: the uptake of ^{28}Mg (m-mole/kg fresh wt.) Symbols: ○, no added K; ●, with 1 mM-KCl; □, with 2 mM-KCl; ■, with 4 mM-KCl. Increasing the K concentration depressed the uptake of radioactive Mg and reduced the size of the intermediate phase of Mg exchange (see text).

TABLE 8. Observations in isotonic sucrose solutions C and E in which Mg^{2+} and Ca^{2+} were the only cations used. The total tissue content of Mg and Ca did not change greatly when Ca^{2+} ions were substituted for Mg^{2+} in the solution, but there was a substantial fall in the Mg content of the intermediate phase of ^{28}Mg exchange

Solution	C	E	Units
$[Mg]_o$	2	1	mM
$[Ca]_o$	0	1	mM
Ionic strength	6	6	mM
Total tissue Mg	18·2 ± 0·31	6·7 ± 0·09	m-mole/kg fresh wt.
Total tissue Ca	0·8 ± 0·05	10·1 ± 0·25	m-mole/kg fresh wt.
Total tissue Mg and Ca	38·0	33·6	m-equiv/kg fresh wt.
[^{14}C]sorbitol space	595 ± 14·7	433 ± 16·2	ml./kg fresh wt.
Corresponding extracellular Mg	1·20 ± 0·03	0·43 ± 0·02	m-mole/kg fresh wt.
^{28}Mg intercept	7·66	1·8	m-mole/kg fresh wt.
Intermediate phase	6·46	1·37	m-mole/kg fresh wt.
Rate constant of slowest phase	0·00942	00·00952	min^{-1}
Intermediate phase (corrected)	8·00	2·14	m-mole/kg fresh wt.

more that this competition was primarily a characteristic of the intermediate phase of the ^{28}Mg exchange. Such competition should be most marked in solutions of low ionic strength such as those used up to now, but it was of interest to see if any similar effects could be demonstrated in more normal media. The experiments shown in Fig. 7 were therefore carried out in Krebs solutions which contained 1 mM-K^+, but which were

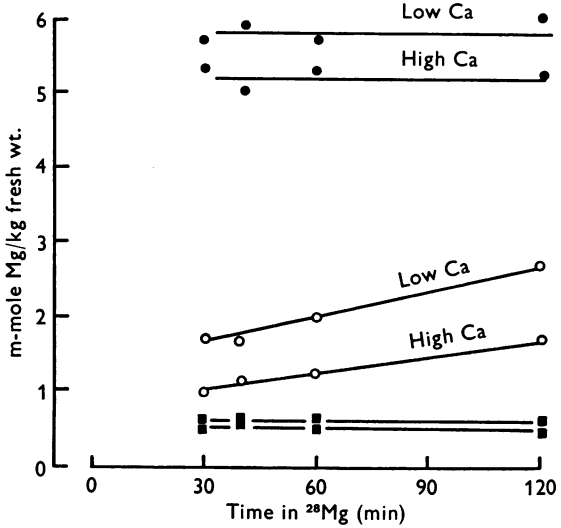

Fig. 7. Observations *in vitro* in Krebs solution at 37° C. Abscissa: time in min after immersion in radioactive ^{28}Mg solution. Ordinate: m-mole/kg fresh wt. Symbols: ●, total tissue Mg; ○, ^{28}Mg uptake; ■, the calculated amount of Mg freely dissolved in the [^{14}C]sorbitol extracellular space. ^{28}Mg uptake and the total tissue Mg were depressed in high Ca solution, although the amount of Mg in the [^{14}C]sorbitol space was not changed. The alteration was largely due to a decrease in the size of the intermediate phase of the ^{28}Mg exchange (see text).

in all other respects normal (solutions F and G, Table 1). The total tissue Mg content increased when the solution Ca concentration was lowered from 10·0 to 0·1 mM, and this change was almost entirely associated with an alteration in the intermediate phase of ^{28}Mg exchange, showing that the effect also occurred under these less extreme physiological conditions.

DISCUSSION

The Mg content of freshly dissected guinea-pig taenia coli in the present experiments (6·56 m-mole/kg fresh wt.) was similar to the content of cat intestinal muscle (6·81 ± 0·07 (36) m-mole/kg fresh wt., Potter & Sparrow, 1968) and toad stomach muscle (6·01 m-mole/kg fresh wt., Sparrow &

Simmonds, 1965), but in all these tissues it fell during immersion in Krebs type solutions in contrast to the Ca content which tended to rise (Bauer, Goodford & Hüter, 1965; Sparrow & Simmonds, 1965). The uptake of ^{28}Mg also contrasted with ^{45}Ca uptake since the former showed three separate components in the present experiments (extracellular, intermediate, and slow) while the uptake of ^{45}Ca only showed two significant phases (Bauer *et al.* 1965). However washout experiments with ^{45}Ca have demonstrated that there is a very small slowly-exchanging component in the taenia coli (Goodford, 1965), and it may be that the two cations Mg^{2+} and Ca^{2+} actually have three phases of exchange each, but phases of different magnitudes. Thus each has an extracellular exchange component; a slowly exchanging component of some 5 m-mole/kg fresh wt. for Mg but only some 0·16 m-mole/kg fresh wt. for Ca (Bauer *et al.* 1965); and an intermediate phase exchanging with a half-life of minutes. Bauer *et al.* (1965) postulated that this intermediate Ca phase might be 'in equilibrium with the ionized extracellular calcium', and it may in fact represent Ca^{2+} ions which are competing with the other cations in the tissue.

The present experiments were designed to investigate the hypothesis that Mg^{2+} ions might be competing for fixed anionic sites in the smooth muscle of the guinea-pig taenia coli. Such a competition may be described by a series of chemical equilibria of the type

$$MgR_2 + Ca^{2+} = Mg^{2+} + CaR_2, \quad (3)$$

$$MgR_2 + 2K^+ = Mg^{2+} + 2KR \quad (4)$$

in which R^- represents a single type of fixed negative charges. If the amount of R^- in the tissue was constant, and if Mg^{2+} was the only cation available, each site would have the option either of being vacant or of being associated with Mg. The results in Tables 2 and 5 show that the amount of associated Mg was essentially constant when the concentration $[Mg]_o$ in the bathing solution was varied tenfold, and the simplest interpretation of this finding would be that the available sites were all occupied by Mg in the absence of other cations. In this case the amount of fixed anion available for cation competition should be equal to the amount of associated Mg, which is the amount of ^{28}Mg in the intermediate phase of exchange in the absence of other cations; i.e. some 6·09 m-mole/kg fresh wt. or 12·2 m-equiv/kg fresh wt.

Previous estimates of the amount of fixed anion in the smooth muscle of the guinea-pig taenia coli have been of the order of 5 m-equiv/kg fresh wt. when radioactive ^{42}K rather than ^{28}Mg was used (Goodford, 1966, 1967). This is substantially lower than the present results and might suggest that the fixed anionic groups R^- in the tissue are not all identical. It is possible that some may have a more specific affinity for Mg, so that

the Mg^{2+} ions were not displaced by K^+ in Goodford's experiments. However, two other factors which could account in part for the discrepancy should be considered. Goodford's observations were made in solutions buffered with the CO_2/HCO_3^- system so that bicarbonate was the predominant anion rather than chloride and, furthermore, Goodford was not able to measure the tissue Mg which might still have been present in his experiments in a sufficient extent to occupy a proportion of the groups R^- without his being aware of its presence.

The amount of ^{28}Mg in the intermediate phase was reduced from 6·46 m-mole Mg/kg fresh wt. in the absence of Ca, to 1·37 m-mole Mg/kg fresh wt. when the concentration of Ca^{2+} and Mg^{2+} ions in the bathing solution were equal (Table 8). In this special case the selectivity coefficient for eqn. (3) may be written

$$\frac{ca}{mg} = \frac{[Mg^{2+}][CaR_2]}{[Ca^{2+}][MgR_2]} = \frac{[CaR_2]}{[MgR_2]} = \frac{6{\cdot}46 - 1{\cdot}37}{1{\cdot}37} = 3{\cdot}7$$

which shows that the anionic sites have on average four times higher affinity for Ca than for Mg. Similarly the results in Table 6 may be used in order to calculate the selectivity coefficient for eqn. (4) as

$$\frac{k^2}{mg} = \frac{[Mg^{2+}][KR]^2}{[K^+]^2[MgR_2]} = \frac{1[17{\cdot}4 - 6{\cdot}2]^2}{3 \times 3[3{\cdot}1]} = 4{\cdot}5 \text{ l./kg fresh wt.}$$

It has been concluded on the basis of these findings that Mg^{2+} ions in the intermediate phase of exchange of the smooth muscle of the guinea-pig taenia coli are competing with Ca^{2+} and K^+. This competition can be described by the mass-action laws applicable to ion exchange phenomena, but further experiments are needed to establish whether there are more than one type of binding site in the tissue, and where the sites are located.

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