J. Physiol. (1973), **229**, *pp.* 733–749
With 5 text-figures
Printed in Great Britain

UPTAKE OF CALCIUM AND MAGNESIUM BY RAT DUODENAL MUCOSA ANALYSED BY MEANS OF COMPETING METALS

BY J. M. O'DONNELL AND M. W. SMITH
From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

(*Received 13 September 1972*)

SUMMARY

1. The short-term uptake of Ca and Mg by rat duodenal mucosa consisted of two parts, one apparently complete within 1 min (uptake I) and a second which increased linearly with time from 1 to 12 min (uptake II).

2. Uptake II for both Ca and Mg showed a curvilinear dependence on concentration. Uptake I was related linearly to concentration up to the highest concentration tested.

3. Mg caused a significant inhibition of Ca uptake II. Ca was without effect on Mg uptake.

4. Mn reduced significantly both uptakes I and II for Ca; Ba and Sr were without effect on Ca uptake.

5. Two lanthanides, La and Pr, both inhibited uptakes I and II for Ca. Inhibition by Pr could be reversed by increasing the concentration of Ca. Some uptake II for Ca still persisted when uptake I was completely abolished by 3 mM-La.

6. It is suggested that a small part of Ca uptake I may be associated with the subsequent mediated entry into the mucosa, since inhibition of Ca uptake consistently involves both forms of uptake. However, since some Ca still enters the mucosa when uptake I is completely inhibited, it appears that some entry of Ca can occur by diffusion.

INTRODUCTION

The phenomenon of net absorption of divalent metals by the intestine is both complex and variable. In the most extensively studied instance, that of uptake of Ca in rat duodenum, net absorption appears to occur principally by an active transfer mechanism in certain circumstances, i.e. in young, growing animals, and in adults on diets deficient in Ca or treated

with vitamin D following depletion of that vitamin (Schacter, Dowdle & Schenker, 1960). In normal adult animals on adequate diet, and in general when a high luminal concentration of available Ca obtains, net absorption may be accounted for by diffusion. Active transfer appears to be carrier-mediated, and the carrier is thought to be a specific Ca-binding protein, induced in demonstrable quantity in the intestinal mucosa by appropriate manipulations of the vitamin D status of the animal (Wasserman, Corradino & Taylor, 1969).

The kinetics of Ca transport across the duodenum have already been investigated in some detail and unequivocal evidence of active transfer obtained using the everted-sac technique. It is difficult, however, to obtain early measurements of transport using everted sacs and so conclusions regarding the initial stages of transport have had to remain speculative until recently. Martin & DeLuca (1969) recognized this problem and devised a method of measurement of short-term influx of Ca in mucosal tissue, using a technique in which only the mucosal surface of excised rat duodenum was exposed to solutions containing tracer ^{45}Ca. Papworth & Patrick (1970) used rings of tissue for the same purpose, but access of radioactive medium was not in their case restricted to the mucosal surface. The former investigators concluded that a one-component system, showing simple Michaelis–Menten kinetics, was adequate to account for the observed uptake of Ca. They did not attempt to estimate the contribution of extracellular isotope to the tissue radioactivity. Papworth & Patrick (1970), using a wide range of Ca concentration, derived an expression of Ca uptake in two components. One of these terms showed Michaelis–Menten kinetics and was saturable, while the second phase of entry was shown not to saturate but to increase linearly up to the highest concentration of Ca employed, 90 mM. Although these workers determined the extent of the contribution of extracellular Ca to the total measured tissue uptake, they did not apply this correction in their analysis of data. There is then still some confusion concerning the interpretation of these supposedly more definitive types of measurement.

The apparatus devised by Schultz, Curran, Chez & Fuisz (1967) may readily be adapted to the study of the short-term kinetics of uptake processes in duodenum. Excised tissue is rapidly mounted for the experiment, the mucosal surface only is exposed to perfusion solutions, changes of perfusing media and washes can be rapidly made, and multiple incubations may be obtained with a segment of intestine taken from a single animal. We have studied the uptake of Ca by tracer technique, using a modification of their apparatus and correcting every individual tissue radioactivity for any extracellular component, in an attempt to clarify the initial events taking place during the absorption of Ca.

Although there is indirect evidence of interaction between the absorption of Mg and that of Ca (Hendrix, Alcock & Archibald, 1963) much less is known of Mg absorption in detail, in part because of the restricted availability of the radioactive tracer ^{28}Mg. Aldor & Moore (1970) have recently concluded, from everted-sac studies of transfer of ^{28}Mg in rat colon and intestine, that Mg transfer is, like that of Ca, probably effected by simple diffusion at high luminal concentrations of Mg, and by active transfer when available Mg is in low concentration. This conclusion, and the finding that glucose but not Ca had an inhibitory effect on Mg transfer at all sites in the intestine where such transfer took place, is in conflict with earlier reports (Ross, 1962; Hendrix *et al.* 1963). Aldor & Moore (1970) have pointed out that leakage and/or exchange of tissue magnesium can distort the findings in studies of uptake and transfer of Mg by gut sac preparations. We have therefore determined the kinetics of uptake of Mg by rat duodenal mucosa by an approach identical to that described for Ca above, using tracer ^{28}Mg. These experiments allowed a comprehensive analysis of the reciprocal relations in the uptake of the two metals; in addition, the selectivity of divalent metal absorption was investigated using Sr, Ba and Mn as competitors. The effects of the metals lanthanum and praseodymium were also investigated; and because these are electron-dense metals, tissues so treated were also examined in the electron microscope. A preliminary account of part of this work has already been published (O'Donnell & Smith, 1972).

METHODS

Animals and apparatus

All experiments were performed on male rats of hooded Lister strain, weighing about 200 g. Animals (9–12 weeks old) were obtained from Animal Supplies (London) Ltd, Welwyn, Herts., several days before use, and fed a normal laboratory diet (Oxoid 41 B) and tap water until the day prior to experiment. Food but not water was withheld from the previous afternoon, and the animals killed by decapitation on the following morning. That segment of intestine extending to 10 cm distal to the pyloric sphincter was rapidly excised and rinsed through with phosphate-free balanced salt solution (see below). The intestine was then cut open and mounted serosal face downwards on a sheet of 'Benchkote' paper (W. & R. Balston Ltd, Maidstone) which was itself cut to fit over the base-plate of an apparatus designed similar to that described by Schultz *et al.* (1967). The tissue was kept at its equilibrium stretch by being impaled on pinpoints protruding upwards from the base-plate. Intestinal segments from two animals were mounted at a time, each yielding six incubations. Two expected sources of variance in duodenal metal uptake, that between individual rats and that due to longitudinal variation in location on the intestine, were accommodated by distributing replicate determinations (at least six) at random for any given set of conditions.

When mounted, the tissues were immediately superfused with appropriate perfusion medium at 37° C, for 5 min of preincubation. They were then rapidly

drained and rinsed with isotope-free incubation medium (where this differed from the perfusion solution) and the radioactive medium applied. A volume of 0·2 ml. was in contact with a mucosal surface of area 0·28 cm^2. Where different loci on the same segment were exposed to radioactive medium for different periods of time the incubations were arranged so as to end together, so that the tissues were all perfused or incubated for the same total length of time. At the conclusion of incubation the tissue was drained of radioactive solution by suction, washed with 2 ml. ice-cold isotonic mannitol solution, stamped out with a cylindrical cutter (bevelled to avoid taking crushed edges of tissue) and transferred to scintillation counting vials containing 0·3 ml. 0·1 N-HNO_3.

Incubation media

Straight uptake. For these experiments on rates of uptake of both Ca and Mg a phosphate-free balanced salt solution of the following composition was used (all concentrations mM): NaCl 117·5, KCl 5·7, $MgCl_2$ 1·2, $CaCl_2$ 1·0, glucose 15, $NaHCO_3$ 25. Ca or Mg were varied between 0·03 or 0·01 respectively and 12 mM in determining the concentration dependence of uptake with all other conditions constant. This medium ('A') was gassed with 95 % O_2: 5 % CO_2 and equilibrated at pH 7·4.

Inhibition/competition studies. For experiments on competition between different divalent metals it was necessary to have a medium free both of phosphate and bicarbonate in order to ensure that metals did not precipitate, therefore a medium ('B') was employed which was the same as 'A' except that 25 mM-Tris-HCl was substituted for $NaHCO_3$ as buffer, and the solution was gassed with pure O_2. In these experiments the superfusion medium always contained Ca 1·0 and Mg 1·2 mM, but the 'cold' wash and radioactive incubation media were modifications of this Tris-buffered medium as required, and in some instances the experimental design required that these be free from Ca or Mg. In all competition experiments the chlorides of Ca, Mg, Sr, Ba or Mn were employed. Where available Analar grade reagents were used.

Radioisotopic methods

^{3}H-labelled inulin (Radiochemical Centre, Amersham) was incorporated in trace concentration in 'hot' solutions in order to yield an estimate of mucosal 'extracellular' space. Inulin space was invariably less than ^{45}Ca or ^{28}Mg spaces but it did show a progressive rise with time. In a comparison of inulin with mannitol as indicators of extracellular space, mannitol gave a small, consistent and significantly greater value. ^{45}Ca was obtained from the Radiochemical Centre, Amersham and ^{28}Mg came from the Brookhaven National Laboratory, Upton, L.I., New York, U.S.A. Isotopes were freed from tissue samples by standing the samples in dilute nitric acid for 3–4 hr at ambient temperature. ^{45}Ca and ^{3}H were counted simultaneously by liquid scintillation in a Packard Tricarb spectrometer. Isotope discrimination and quench correction were performed on raw count data using a programme kindly prepared by Mr M. J. Moore for an Olivetti P 101 calculator. For counting of ^{25}Mg, tissues were first suspended in 7 ml. dioxan and counted by Cerenkov radiation. After ^{28}Mg had decayed concentrated scintillator was added to the vials which were then counted for ^{3}H in the normal way.

Computation of results

Each individual sample count was corrected for ^{45}Ca or ^{28}Mg corresponding to its inulin space, using the counts ratio of isotopes in a standard sample of the incubation medium. Tissue metal isotope counts were converted to quantity of Ca or Mg and influx calculated and expressed per unit surface area of mucosal tissue. Uptake curves are plots of this quantity against time. In plotting the rates of uptake against

time at different concentrations, five time intervals between 1 and 12 min were used. At least six replicates were taken for each time.

For competition and inhibition experiments, uptake measurements were reduced to three time intervals, 1, 6 and 12 min, randomly distributed, in the presence and absence of a second metal in each animal. Thus each animal could serve as its own control. All data from these experiments were first analysed for variance (through the GENSTAT programme, courtesy of Mr D. E. Walters, School of Applied Biology, Cambridge). The results were assessed for the effects of individual animals, time, change of medium from 'A' to 'B', and presence of a second metal, on the uptake of Ca or Mg. This preliminary analysis indicated that variation between animals, and alteration of medium, were not significant and that variation of uptake with time was significant and overwhelmingly linear. Where second metals inhibited uptake over-all, there was also a significant interaction between inhibitor and time. Straight uptake (i.e. no second metal present) and its concentration dependence, was therefore treated as linear with time and analysed using pooled replicates for linear regression. This resulted in a significant zero-time intercept for all experiments. The effect of second metals on this intercept was assessed from these regression analyses and their effect on time-dependent uptake analysed separately using paired-sample data from individual animals.

RESULTS

Rates of uptake of Ca and Mg. All experiments under this heading were performed using a bicarbonate buffered phosphate-free solution (medium 'A') in which either Ca or Mg concentration was varied while Mg or Ca was held constant at, respectively, 1·2 or 1·0 mM, and all other conditions constant. Fig. 1*A* shows the uptake of Ca at concentration 1·0 mM over an incubation period of 12 min. From 1 to 12 min the data could be fitted to a straight line which did not pass through the origin. For the purpose of analysis of data, the initial, non-linear portion of uptake was treated as time-independent and its magnitude and statistical significance calculated as an intercept given by the regression equation of the linear portion. From the pooled data (Fig. 1*A*) the intercept had a value $4{\cdot}31 \pm 0{\cdot}76$ n-mole cm^{-2}, which was significantly different from zero ($P < 0{\cdot}01$). A significant intercept was also found at all other concentrations of Ca used, and it was therefore decided to treat this component of Ca uptake, without implication as to possible mechanism, as an entity distinct from the subsequent, time-dependent component. For convenience it is referred to below as uptake I, and the linear uptake is called uptake II. For the pooled data from experiments at Ca 1·0 mM, uptake II had a value $0{\cdot}85 \pm 0{\cdot}10$ n-mole cm^{-2} min^{-1} (Fig. 1*A*).

When Ca concentration was varied over the range 0·03–12 mM and the uptake measured it showed the same pattern as that found at Ca 1·0 mM, that is a rapid (uptake I) and a slower, time-dependent portion (uptake II). The magnitude of uptake I increased with concentration and was not saturated at the upper limit of this range of concentrations (Table 1). The

linear uptake (II) behaved less simply, and the variation of rate with Ca concentration is shown in Fig. 2*A*. The plot of the relationship is curvilinear, so that while the increase in rate tended to fall off at higher Ca concentrations it did not attain a plateau within this range and, perhaps significantly, the variance in the data was greater at 12 mM than at lower concentrations.

The time dependence of Mg uptake, at a concentration of 1·2 mM, and with all other conditions identical to those used to determine Ca uptake,

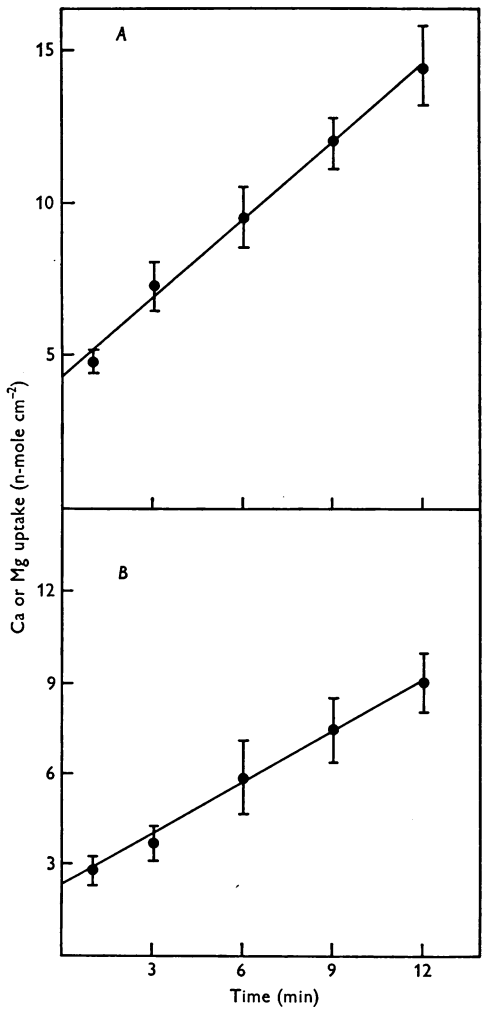

Fig. 1. *A*. Time course of uptake of Ca from medium 'A' at 37° C. Ca concentration 1·0 mM. Each point is the mean of fourteen observations, vertical bars give S.E. of mean. *B*. Corresponding data for uptake of Mg, at concentration 1·2 mM. Means of six observations.

is shown in Fig. 1*B*. As with the uptake of Ca, the influx of Mg was divisible into two distinct components. These will also be referred to as uptakes I and II. The statistical treatment of the data, by which the mean numerical values of these were obtained, was identical to that used for Ca. For the data of Fig. 1*B* uptake I was $2{\cdot}19 \pm 0{\cdot}70$ n-mole cm^{-2}, and uptake II was $0{\cdot}58 \pm 0{\cdot}10$ n-mole cm^{-2} min^{-1}; thus, although Mg uptake was of the same general form as that of Ca, the absolute values, for a greater carrier concentration, were lower for both fractions of uptake than were those for Ca.

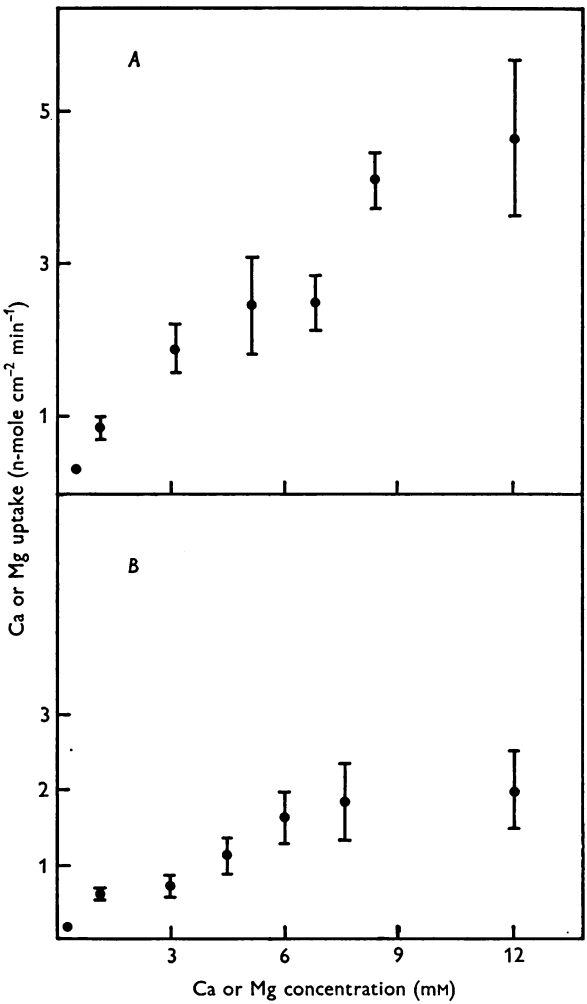

Fig. 2. *A*. Variation of rate of Ca uptake (uptake II) with Ca concentration in medium 'A'. *B*. Corresponding data for uptake of Mg.

The uptake of Mg was determined over a range of concentrations from 0·01 to 12 mM; as with Ca, the intercept at time zero, defining the magnitude of uptake I, was significant at all concentrations of Mg in the range (Table 1). This uptake increased with concentration. Uptake II also increased with concentration, in the manner illustrated in Fig. 2*B*. The increases in both forms of uptake of Mg, like the absolute values, were smaller than those for Ca. The relationship of uptake II to concentration, while curvilinear like that of Ca, was much less steep, particularly at higher concentration.

TABLE 1. Uptake I of Ca and Mg, at various concentrations in incubation media

Concentration in medium (mM)	Ca	Mg
	(n-mole cm^{-2})	
0·1	0·72 ± 0·13	0·32 ± 0·05
0·3	2·42 ± 0·66	1·06 ± 0·26
1·0	4·31 ± 0·76	—
1·2	—	2·19 ± 0·70
3·0	6·90 ± 2·44	4·62 ± 1·15
6·0	—	11·61 ± 2·61
6·7	20·0 ± 5·1	—
12	81·1 ± 16·7	15·4 ± 3·4

Calculated mean values (± S.E.) obtained from regression analysis described in text. Medium 'A'.

Competition and inhibition

Ca and Mg. Uptake of Ca was measured in the presence and absence of 10 mM-Mg, over a concentration range of Ca from 0·03 to 12 mM. Uptake I, in the absence of Mg, increased with increasing Ca concentration in a similar manner to that seen in the first series of experiments (Table 1). The presence of Mg gave lower mean values for this form of uptake at all Ca concentrations employed (Table 2), although these reductions were not statistically significant.

The presence of Mg did, however, inhibit the time-dependent uptake of Ca at concentrations of Ca from 0·03 up to and including 0·3 mM, but not at 1·0 mM. The reduction in the rate of uptake was highly significant at all but the upper limit of the range, but the degree of inhibition was not otherwise correlated with the ratio of Ca to Mg (Table 3).

In the corresponding experiments with Mg uptake, Mg was used in a range from 0·01 to 1·0 mM, and uptake determined in the presence and absence of 10 mM-Ca. Again the variance in data for Mg uptake was relatively larger, and the absolute values lower, than had been found for the uptake of Ca. Uptake I was not influenced by the presence of Ca. Uptake II

was consistently reduced in the presence of Ca, and the reduction persisted up to Mg concentration 1·0 mM, but these effects did not attain significance due to the magnitude of the variance.

From these results it was concluded that the uptake of calcium was susceptible to interference by Mg, but the reciprocal interaction could not be reliably demonstrated. Either the duodenal uptake of Mg is by a mechanism completely independent of that for Ca, or that fraction associated

TABLE 2. Influence of the presence of Mg (10 mM) on the rapid uptake (I) of Ca over a range of Ca concentrations

Calcium (mM)	Calcium uptake (n-mole cm^{-2})	
	−	+
0·03	0·227 ± 0·063	0·141 ± 0·039
0·04	0·206 ± 0·066	0·110 ± 0·045
0·055	0·276 ± 0·099	0·211 ± 0·069
0·10	0·536 ± 0·096	0·390 ± 0·077
0·30	0·989 ± 0·668	0·728 ± 0·301
1·00	3·802 ± 1·510	2·525 ± 1·488

Uptake of Ca was measured in medium 'B' at time intervals 1, 6 and 12 min; −, + indicates absence and presence of Mg. Intercepts calculated as described in text. Means of at least six experiments, ± S.E.

TABLE 3. Influence of presence of Mg on the time-dependent uptake (II) of Ca over a range of Ca concentrations

Calcium (mM)	(*n*)	Calcium uptake (n-mole cm^{-2} min^{-1})		*P*
		−	+	
0·03	(12)	0·087 ± 0·008	0·048 ± 0·007	< 0·001
0·04	(12)	0·093 ± 0·008	0·048 ± 0·005	< 0·001
0·055	(10)	0·136 ± 0·011	0·097 ± 0·015	< 0·05
0·10	(20)	0·153 ± 0·018	0·099 ± 0·013	< 0·05
0·30	(11)	0·637 ± 0·090	0·326 ± 0·038	< 0·02
1·00	(8)	0·816 ± 0·147	0·665 ± 0·100	n.s.

Means ± S.E. of mean. −, + indicates absence and presence of Mg. Uptake rates were calculated for the period of incubation 1–6 min. Comparison of control (−) and test (+) was by paired sample *t* test. Medium 'B'.

with Ca uptake is too small to detect a partial inhibition. Subsequent work using other metal inhibitors was therefore confined to a study of Ca uptake processes.

Sr, Ba and Mn. Strontium is thought to be absorbed by the gut in a similar manner to Ca, and Mn can substitute for Mg in a number of biological processes including enzyme activation (Boyer, Lardy & Myrbäck, 1959). Mn can also parallel some of the physiological actions of Mg, in-

cluding inhibition of neuromuscular transmission (Meiri & Rahamimoff, 1972). The influence of these metals on Ca uptake was therefore assessed as for the effects of Mg on Ca, at Ca concentration 0·1 mM, in the presence and absence of the second metal at 10 mM. Table 4 gives a summary of the results of these experiments.

TABLE 4. Influence of Sr, Ba and Mn (all at 10 mM) on both forms (I and II) of Ca uptake

Competing metal	(*n*)	Uptake I (n-mole cm^{-2}) −	Uptake I (n-mole cm^{-2}) +	*P*	Uptake II (n-mole cm^{-2} min^{-1}) −	Uptake II (n-mole cm^{-2} min^{-1}) +	*P*
Sr	(10)	0·476 ± 0·080	0·328 ± 0·060	n.s.	0·128 ± 0·029	0·102 ± 0·009	n.s.
Ba	(10)	0·501 ± 0·126	0·317 ± 0·067	n.s.	0·153 ± 0·036	0·113 ± 0·018	n.s.
Mn	(10)	0·690 ± 0·128	0·134 ± 0·058	< 0·01	0·231 ± 0·042	0·105 ± 0·017	< 0·01

Mean values, ± S.E., for (*n*) experiments.
Uptakes I and II calculated as described above; −, + indicates absence or presence of competing metals. Medium 'B'.

Sr and Ba reduced the magnitude of both uptake I and uptake II, but the reductions were not statistically significant. Mn gave a mean reduction of 80 % in uptake I, and over 50 % inhibition of uptake II, both effects being highly significant.

La and Pr. These two rare earths have been reported to inhibit the uptake of Ca into mitochondria (Mela, 1969). Pr was found to be relatively more potent than La, while both were considered to act specifically on an active transport process for Ca. Their effects on Ca uptake by the duodenum were measured over a range of concentrations of each lanthanide, at a constant Ca level of 0·1 mM.

Uptake I was not inhibited to a significant extent by La until the La concentration had been increased to 1·0 mM. Further increasing La to 3·0 mM led to a complete abolition of this form of Ca uptake (Fig. 3). Praseodymium inhibition of uptake I was more continuously progressive with Pr concentration, inhibition being significant at concentrations of 0·25, and 0·5 mM as well as at 1·0 mM. At this last concentration, however, the relative effect was less than that of La, and it was not increased by further raising the concentration of inhibitor (Fig. 3).

Uptake II was also inhibited by both lanthanides over the same range of concentrations. The three lower La concentrations gave small and statistically insignificant reductions in rate, but at 1·0 mM and above more than 50 % of uptake II was inhibited. Unlike uptake I, however, uptake II was not fully inhibited even at a La concentration of 3·0 mM (Fig. 4). As in the case of uptake I, effects with Pr on the second fraction of uptake were

significant at all concentrations over the range tested, and these were more closely related to concentration than was the inhibition by La (Fig. 4).

These potent inhibitions, at concentrations much lower than those of the divalent metals which had given inhibition of Ca uptake, were not attributable to alterations in tissue surface topography, since the spaces available to inulin measured after a 60 sec incubation did not differ in the presence of even the highest levels of La or Pr, from those found for control

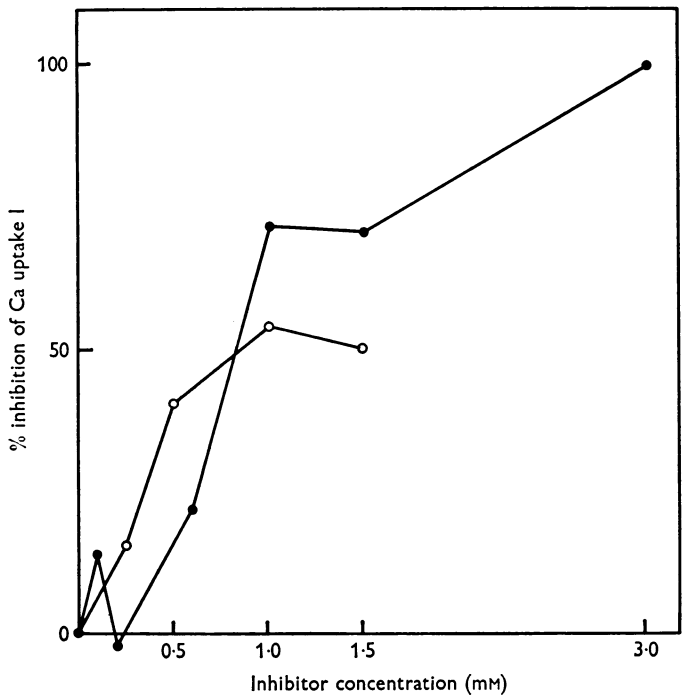

Fig. 3. Effects of various concentrations of $LaCl_3$ and $PrCl_3$, incorporated into medium 'B', on the rapid uptake (I) of Ca at Ca concentration 0·1 mM. ● – La, ○ – Pr.

preparations ($4{\cdot}96 \pm 0{\cdot}46$ and $4{\cdot}96 \pm 0{\cdot}41$ μl. cm^{-2}, respectively). When tissues exposed to La or Pr, in procedures identical to those for measurement of Ca uptake, were examined in the electron microscope it was found that neither metal appeared to have penetrated cells or tight junctions.

Further indication that the effects of lanthanides were specific was obtained when uptake of Ca at a concentration of 12 mM was measured in the presence and absence of 1·5 mM-Pr; inhibition by Pr was overcome by this high concentration of Ca, no difference being found between total control and experimental uptakes measured after 12 min incubation ($84{\cdot}9 \pm 9{\cdot}6$ and $89{\cdot}2 \pm 23{\cdot}8$ n-mole cm^{-2}, respectively).

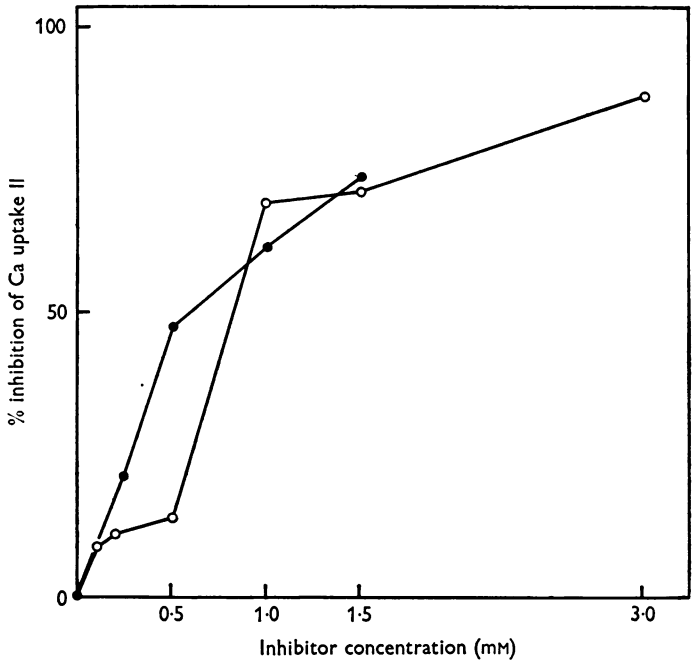

Fig. 4. Effects of $LaCl_3$ and $PrCl_3$ (ranges as in Fig. 3) on uptake II of Ca from medium 'B'. Ca concentration 0·1 mM. ○ – La, ● – Pr.

DISCUSSION

These experiments, designed to elucidate the kinetics of uptake of Ca and Mg by mucosal cells, were carried out by a method which required the assumption that unidirectional flux could be measured by counting the isotope remaining in the tissue following washout of incubation medium and correction for extracellular isotope. The backflux of isotope from tissue to medium was thought to be not appreciable over the time intervals involved in measuring influx. Rapid washing with ice-cold, isotonic mannitol was considered a valid means of removing excess medium and preventing further transfer. The extracellular space correction by inulin, however, might not be exact. Mannitol appeared to penetrate a significantly greater space than did inulin. Inulin has, however, been found adequate in marking the mucosal extracellular compartment in rat when compared to other molecules of medium molecular weight (Sallee, Wilson & Dietschy, 1972) and it has also been used extensively for this purpose in rabbit ileum (see Schultz & Curran, 1970). The difference we found between mannitol and inulin was small and not sufficient to significantly alter the results obtained.

In view of this we felt justified in continuing to use inulin-corrected measurements for calculating calcium uptake.

The tissue influx after these corrections is the sum of processes leading to association of divalent metal with the tissue. Uptake I was rapid and unsaturable over the concentration range used, and therefore distinct operationally from uptake II; primarily, recognition of I as an entity served to yield a clearer measurement of uptake II, and of the effects of concentration and of competition from other species on the progressive, time-dependent uptake of divalent metals. Whatever its functional significance, uptake by the rapid process was not a surface binding wholly specific to Ca, since Mg exhibited similar behaviour, albeit to a lesser extent. Uptake II was linear with time at all concentrations of both metals over the interval one to twelve minutes. In the case of Ca, uptake II was almost certainly itself the sum of two components, since it showed a curvilinear variation with increasing Ca concentration. The two likely components are mediated transfer and diffusion, both of which have been demonstrated in everted-sac preparations from animals of comparable nutritional status (Schacter *et al.* 1960; Helbock, Forte & Saltman, 1966). Although uptake II is a measure of initial influx only and not tissue transfer, it is interesting to compare our results with the rates of unidirectional flux measured across segments of rat duodenum mounted *in vitro* (Walling & Rothman, 1969); to this end we compared by our method mucosal and serosal influx at a Ca concentration of 1 mM. Mucosal influx, calculated for the interval 1–6 min, was greater than its serosal counterpart ($0{\cdot}90 \pm 0{\cdot}12$ and $0{\cdot}58 \pm 0{\cdot}08$ n-mole cm^{-2} min^{-1} respectively; means $\pm$ S.E., six observations). Walling & Rothman (1969) reported values of 1·71 and 0·79 for the corresponding unidirectional fluxes. These comparisons indicate that the net movement of Ca across the duodenum is in the direction required for net absorption to take place though they cannot be extrapolated numerically to describe similar processes operating under the conditions of the physiological situation.

Both forms of uptake, I and II, were smaller for Mg than for Ca. There is evidence that the duodenum is a relatively less important site of net Mg absorption than the more distal segments of the intestine (Hendrix *et al.* 1963; Aldor & Moore, 1970). Although Mg could apparently interact with the mediated uptake of Ca, its own uptake, which remained unaffected by Ca, must be predominantly by a mechanism not shared with Ca. Mg probably enters the duodenum by diffusion, though the tendency of uptake II to saturate means that a separate, mediated entry cannot be completely excluded. An active uptake of Mg has been proposed by some investigators (Ross, 1962; Alcock & McIntyre, 1962).

The over-all picture of Ca uptake, and its interaction with other metals,

suggests that uptake II is partially dependent on uptake I. These interactions could be as represented in Fig. 5. Mn, La and Pr all significantly reduced uptake I at concentrations effective in reducing uptake II. Although the significant inhibition of uptake II by Mg was not accompanied by a corresponding significance in effect on uptake I, mean values

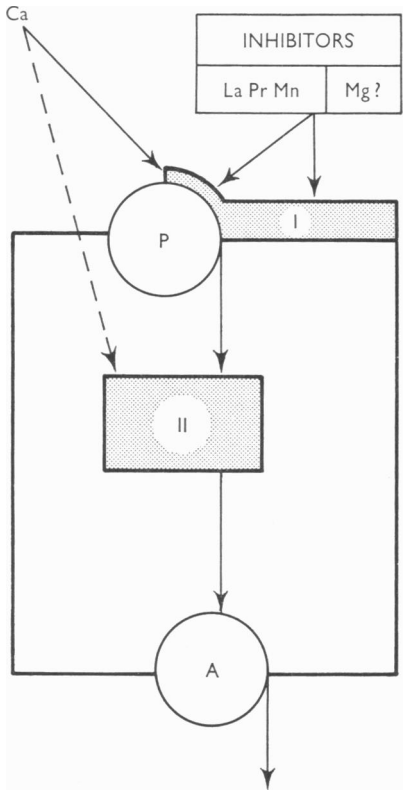

Fig. 5. Schematic representation of Ca uptake by rat duodenum, showing suggested sites of interaction of second metals with the adsorption and entry mechanisms. Mediated entry (P) and active transport (A) at basal-lateral membranes are as outlined by Schacter *et al.* (1970). I and II refer to uptakes of Ca as defined in the text.

of I were, nevertheless, consistently reduced. A small part of uptake I is shown to be directly associated with Ca entry into the mucosa. When uptake I was fully abolished by La, however, a measurable residue of uptake II persisted. This residual uptake seems likely to be by diffusion. It cannot be assumed that the whole of uptake I is directly concerned in the mediated transfer of Ca. Apart from the large amount of Ca taken up by this process, there was no evidence that this uptake could be saturated, as would be expected if all of it were linked to a carrier mechanism. It

could be argued that the part of uptake I which is inhibited along with uptake II represents Ca which has already entered the cells, since Ca transport *across* the mucosal cell is said to be passive at the brush-border membrane and energy-dependent at the basal-lateral membranes (Schacter, Kowarski & Reid, 1970). If this were so, however, one would need to postulate that not only was this entry extremely rapid, but also that it ceased to contribute to overall uptake within one minute of incubation. It is more reasonable to suggest that uptake I is a form of binding to mucosal surfaces, as suggested originally by Schacter, Kowarski, Finkelstein & Ma (1966). An analogy could also be drawn between mucosal and mitochondrial uptake of Ca, where there are both high and low-affinity binding sites on the mitochondrial membranes. Here it is only the much less numerous high-affinity sites which are specifically associated with the mediated entry of Ca (Reynafarje & Lehninger, 1969). Most of uptake I in mucosa probably consists in non-specific binding of Ca to negatively charged membrane proteins and lipids in a way analogous to that seen for the numerous low-affinity sites for Ca binding to mitochondria.

The time-dependent uptake of Ca by rat mucosa bears little resemblance to that in mitochondria. Thus Mg did and Sr did not interfere with mucosal uptake of Ca. Barium was likewise ineffective as a competitor. Everted sacs of duodenum have been shown to discriminate against Ba and Sr as compared to Ca, in establishing concentration gradients (Schacter *et al.* 1960), though Papworth & Patrick (1970) considered that Ca and Sr used the same route of entry into rat duodenum.

The rare earths were much more effective than the divalent metals in reducing both forms of influx of Ca, and these trivalent metals should be considered inhibitors rather than competitors of Ca uptake. The concentration of La which was required to produce substantial inhibition of mucosal Ca influx was of an order greater than that effective for mitochondria (Mela, 1969), but the same as that reported to inhibit tension responses by displacing Ca from surface sites in smooth muscle (Weiss & Goodman, 1969). The greater efficacy of Pr over La at lower concentrations paralleled the situation reported for mitochondria. That the rare earths exert inhibition of Ca uptake by surface displacement was also indicated by the electron micrographic evidence in the present studies. Such studies did not indicate how differences in effectiveness between the two metals at lower concentrations were being brought about. Both rare earths may be considered useful tools for further elucidation of the mechanism of initial uptake of Ca by mucosa.

The present work has shown that uptake of Ca and Mg cannot be adequately described in terms of initial kinetics unless account is taken of the biphasic nature of influx, since single determinations of influx of

isotope at times of incubation between 1 and 12 min will incorporate significant proportions of immediate uptake in addition to time-dependent influx. Two factors in uptake of Ca that were considered relatively unimportant by Papworth & Patrick (1970), namely extracellular fluid isotope and membrane bound Ca, have been shown to be appreciable, and the two forms of uptake described above do not seem to correspond to their two components. The results of the present studies are more similar to those of Martin & DeLuca (1969), in so far as uptake in the first minute is different from subsequent influx. More direct comparisons with the published data of other authors cannot be made here, since the corrections for extracellular space were not allowed for in such studies.

We thank Dr E. A. Munn of this Institute for kindly preparing and interpreting electron micrographs of tissue treated with lanthanum or praseodymium. Miss B. Hick gave excellent technical assistance.

REFERENCES

ALCOCK, N. W. & MCINTYRE, I. (1962). Inter-relation of magnesium and calcium absorption. *Clin. Sci.* **22**, 185–193.

ALDOR, T. A. M. & MOORE, E. W. (1970). Magnesium absorption by everted sacs of rat intestine and colon. *Gastroenterology* **59**, 745–753.

BOYER, P. D., LARDY, H. & MYRBÄCK, K. (1959). *The Enzymes*, 2nd edn. vol. 3, pp. 264–276. New York: Academic Press.

HELBOCK, H. J., FORTE, J. G. & SALTMAN, P. (1966). The mechanism of calcium transport by rat intestine. *Biochim. biophys. Acta* **126**, 81–93.

HENDRIX, J. Z., ALCOCK, N. W. & ARCHIBALD, R. M. (1963). Competition between calcium, strontium, and magnesium for absorption in the isolated rat intestine. *Clin. chem.* **9**, 734–744.

MARTIN, D. L. & DE LUCA, H. F. (1969). Calcium transport and the role of vitamin D. *Archs Biochem. Biophys.* **134**, 139–148.

MEIRI, U. & RAHAMIMOFF, N. (1972). Neuromuscular transmission: inhibition by manganese ions. *Science, N.Y.* **176**, 308–309.

MELA, L. (1969). The reaction of lanthanides with mitochondrial membranes. *Ann. N.Y. Acad. Sci.* **147**, 824–828.

O'DONNELL, J. M. & SMITH, M. W. (1972). Uptake of calcium and magnesium by the duodenum of the rat. *J. Physiol.* **221**, 16–17*P*.

PAPWORTH, D. G. & PATRICK, G. (1970). The kinetics of influx of calcium and strontium into rat intestine *in vitro*. *J. Physiol.* **210**, 999–1020.

REYNAFARJE, B. & LEHNINGER, A. L. (1969). High affinity and low affinity binding of Ca^{++} by rat liver mitochondria. *J. biol. Chem.* **244**, 584–593.

ROSS, D. B. (1962). In vitro studies on the transport of magnesium across the intestinal wall of the rat. *J. Physiol.* **160**, 417–428.

SALLEE, V. L., WILSON, F. A. & DIETSCHY, J. M. (1972). Determination of unidirectional uptake rates for lipids across the intestinal brush border. *J. Lipid Res.* **9**, 184–192.

SCHACTER, D., DOWDLE, E. B. & SCHENKER, H. (1960). Active transport of calcium by the small intestine of the rat. *Am. J. Physiol.* **198**, 263–268.

SCHACTER, D., KOWARSKI, S., FINKELSTEIN, J. D. & MA, R. W. (1966). Tissue concentration differences during active transport of calcium by intestine. *Am. J. Physiol.* **211**, 1131–1136.

SCHACTER, D., KOWARSKI, S. & REID, P. (1970). Active transport of calcium by intestine: studies with a calcium activity electrode. In *Calcium and Cellular Function*, ed. CUTHBERT, A. W., pp. 108–123. London: St Martin's.

SCHULTZ, S. G., CURRAN, P. F., CHEZ, R. A. & FUISZ, R. E. (1967). Alanine and sodium fluxes across mucosal border of rabbit ileum. *J. gen. Physiol.* **50**, 1241.

SCHULTZ, S. G. & CURRAN, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637–718.

WALLING, M. W. & ROTHMAN, S. S. (1969). Phosphate-independent, carrier-mediated active transport of calcium by rat intestine. *Am. J. Physiol.* **217**, 1144–1148.

WASSERMAN, R. H., CORRADINO, R. A. & TAYLOR, A. N. (1969). Binding protein from animals with possible transport function. *J. gen. Physiol.* **54**, 114s–137s.

WEISS, G. B. & GOODMAN, F. R. (1969). Effects of lanthanum on contraction, calcium distribution and ^{45}Ca movements in intestinal smooth muscle. *J. Pharmac. exp. Ther.* **169**, 46–55.