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CALCIUM EXCHANGE IN ISOLATED INTESTINAL VILLI

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SUMMARY

1. A study of Ca exchange in isolated intestinal villi was carried out in an effort to further define certain parameters of Ca transport in the intestine.

2. The exchange of Ca between isolated villi and the medium is controlled by at least three processes: (a) an active uptake of Ca which is dependent upon oxidative metabolism; (b) a passive uptake which is a function of the external Ca^{2+} concentration; and (c) a temperature-dependent release.

3. By appropriate control of the temperature it is possible to demonstrate an effect of parathyroidectomy upon the release process. Villi from parathyroidectomized animals release their Ca more slowly than villi from normal animals.

4. The relationship between Ca exchange in villi and Ca exchange in isolated mitochondria is discussed.

INTRODUCTION

There is increasing evidence that Ca is transported across the intestinal wall by an active process[1,2] which is stimulated by vitamin D (refs. 1, 3) and parathyroid hormone[3–5]. Either isolated loops *in situ*[6] or isolated everted sacs of intestine *in vitro*[7,8] have been employed to study this process. A great disadvantage of the latter technique is that the submucosal, muscular, and serosal layers of this tissue are significant diffusion barriers between the probable site of active transport, the mucosal cells, and the serosal fluid. In an effort to circumvent some of these difficulties, SCHACTER, DOWDLE AND SCHENKER[9] used slices of intestine to study various parameters of this process. Unfortunately the results of studies with slices are also difficult to interpret because of the presence of several nontransporting but actively metabolizing tissue layers. Recently, CRANE AND MANDELSTAM[10] have employed isolated intestinal villi to study sugar transport. This type of preparation seemed to offer possible advantages for the study of Ca transport. Therefore, a study of Ca exchange in isolated intestinal villi was undertaken in an effort to better define certain parameters of intestinal Ca transport.

MATERIALS AND METHODS

Male Wistar or Holtzman rats weighing between 100–130 g were fed a low-Ca diet for 4–6 days. When indicated, rats were parathyroidectomized under anesthesia 4–5 h before an experiment. Sham-operated controls were also employed in several experiments. The animals were killed by decapitation; the duodenum and upper 3–4 cm of the jejunum were excised immediately, slit open, and washed in cold saline (140 mM NaCl, 4 mM KCl). The intestinal segment was then laid on a glass slide, mucosal side up, and the mucosa separated from the remainder of the wall by scraping with a glass slide. The villi from groups of 10 intact or parathyroidectomized rats were pooled in cold saline, and dispersed by taking them up into and expelling them from a 5-ml serological pipette. The preparations were centrifuged at 100 × *g* at 0° in a refrigerated PR-2 International centrifuge for 5 min, washed twice with cold saline and then once with the cold buffer in which they were to be incubated. They were made up as 10 % (v/v) suspensions in buffer, placed in tall beakers, which in their turn were placed in a thermostatically controlled bath. The suspension was stirred throughout the course of the experiment with a magnetic stirrer. When the desired temperature was attained, a measured amount of ^{45}Ca was added to each suspension, and to an equal volume of buffer, as a standard. O_2 was the gas phase when phosphate buffers were used, and a constant stream of 5% CO_2–95% O_2 was added to the glass beaker with a polyethylene tube when bicarbonate buffers were employed. Standard Krebs–Ringer phosphate and Krebs–Ringer bicarbonate buffers were used except that the Ca^{2+} concentration was 0.1 mM, and Mg^{2+} was omitted except as described in the experimental protocols.

Samples were taken after intervals of time from 1 to 90 min by the use of a 5-ml Luer–Lok syringe, a three-way stopcock, and a modified Sweeney Millipore syringe adapter, one side of which had been cut away. One arm of the three-way stopcock was connected to the syringe, and the second to the Millipore adapter, and the third to a length of polyethylene tubing (PE-320). The tubing was inserted into the suspension, 1–2 ml were withdrawn into the syringe, the stopcock was turned, and the mixture forced into the Millipore adapter. A No. SMWPO2500 Millipore filter in the adapter retained the cells and allowed sufficient supernatant solution to pass in 10–30 sec so that a 50–100-μl sample could be taken for analysis. These were plated, dried and counted with a Nuclear-Chicago D-47 gas-flow Geiger–Müller counter equipped with a micromil window.

To test that the disappearance of radioactivity from the medium represented an uptake of isotope by the villi rather than exchange, stable calcium (^{40}Ca) was determined simultaneously with the ^{45}Ca in several experiments. The $^{40}Ca^{2+}$ concentrations were measured by a spectrophotometric titration technique, using EDTA as titrant and murexide as the indicator[11].

RESULTS

When villi were incubated at 33° in Krebs–Ringer phosphate containing 0.1 mM Ca^{2+} and 0.1 μC ^{45}Ca there was a rapid uptake of approx. 25 % of the ^{45}Ca (Fig. 1) and then a gradual return of a small amount of isotope until a nearly constant distribution of Ca was achieved between cells and supernatant with approx. 20 % of the ^{45}Ca in the cells. However, when the incubation was carried out at 4° nearly 90 % of the ^{45}Ca

disappeared from the medium (Fig. 1). This ^{45}Ca was retained by the cells for at least 60 min if the temperature was maintained at 4°. However, if the suspension was warmed to 33° then the ^{45}Ca reappeared in the supernatant (Fig. 1) and soon reached a constant value similar to that obtained when the incubation temperature was 33° throughout the course of the incubation.

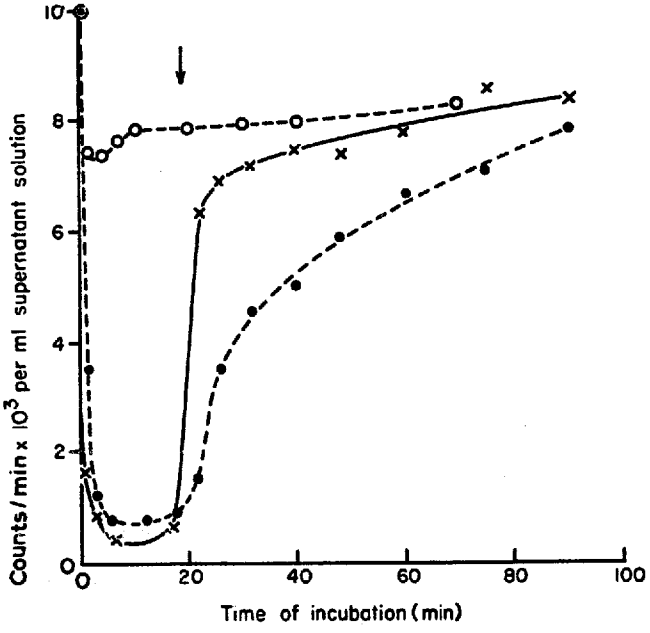

Fig. 1. Uptake and release of ^{45}Ca by isolated intestinal villi from intact and parathyroidectomized rats. Villi from intact rats (×—×) and parathyroidectomized rats (●– – –●) were first incubated at 4° for 18 min, then warmed to 33° (↓) and kept at this temperature until the experiment was terminated. The time required to warm the suspensions to 33° was 60–75 sec. In a parallel experiment, villi from intact rats (O– – –O) were incubated only at 33°. A 10% suspension of villi was incubated in Krebs–Ringer phosphate containing 0.1 mM Ca^{2+} and 0.1 μC ^{45}Ca.

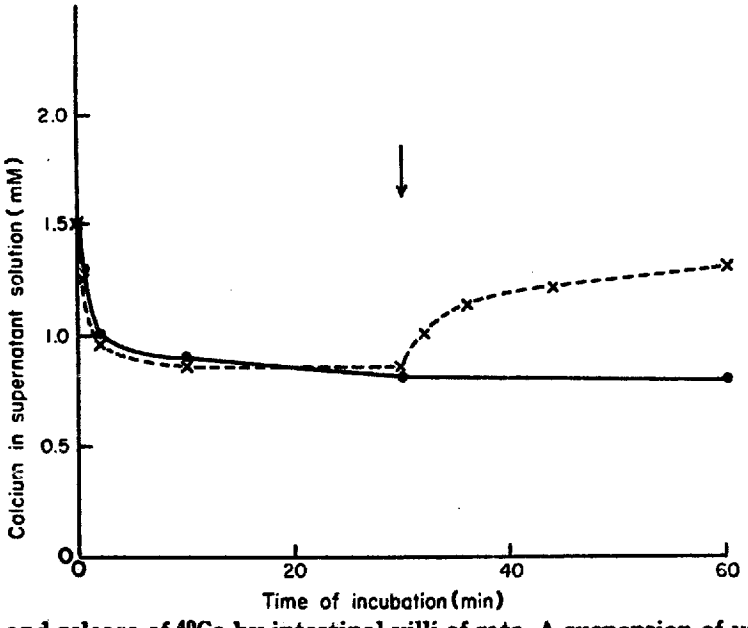

Fig. 2. Uptake and release of ^{40}Ca by intestinal villi of rats. A suspension of villi was divided into two equal portions. Both were incubated at 4° for the first 30 min; then one (●—●) was continued at this temperature while the other (×– – –×) was warmed to 33° and maintained at the elevated temperature. The concentration of Ca^{2+} in the cell-free supernatant is plotted against time of incubation.

The uptake of ^{45}Ca represented a net uptake of Ca and not merely an isotropic exchange as shown in Fig. 2. Incubation of villi at 4° in Krebs–Ringer phosphate containing 1.5 mM Ca^{2+} caused a fall to 0.85 mM Ca^{2+} in the supernatant and this concentration was maintained for 60 min if the villi were kept at 4°. However, if the villi were warmed to 33° the stable Ca returned to the medium until a concentration of 1.35 mM was reached.

As shown in Fig. 1, villi prepared from parathyroidectomized rats took up nearly the same percentage of ^{45}Ca as did villi from normal controls when both were incubated at 4°. The rate of release of the accumulated Ca was slower from the villi of parathyroidectomized animals as compared to controls when the villi were warmed to 33°. It has not been possible to increase the rate of release by the addition *in vitro* of parathyroid hormone to villi from normal or parathyroidectomized animals.

To further define the nature of the process of Ca uptake at 4°, the effect of a variety of metabolic inhibitors upon the process was examined. The results, shown in Table I, indicate that most uncouplers of oxidative phosphorylation, inhibitors of electron transport, and inhibitors of substrate oxidation prevented the uptake of the bulk of the ^{45}Ca. It is noteworthy that ouabain, an inhibitor of Na transport, and oligomycin, an inhibitor of oxidative phosphorylation, were without effect.

Sr competed with Ca in the uptake process, but Ca uptake was favored by a factor of nearly 3:1. K^+ concentrations as high as $4 \cdot 10^{-2}$ M had little effect upon Ca uptake, nor did variations in Na^+ concentration. The uptake was depressed 28–37% by $1 \cdot 10^{-2}$ M Mg^{2+}. This concentration of Mg^{2+} had no effect upon the subsequent release of Ca when the villi were warmed to 33°.

The uptake of ^{45}Ca as a function of stable Ca^{2+} concentration was determined in

TABLE I

THE EFFECT OF METABOLIC INHIBITORS UPON THE UPTAKE OF ^{45}Ca BY ISOLATED INTESTINAL VILLI

Incubation time 12 min at 4° after addition of ^{45}Ca. The inhibitor was added 15 min before ^{45}Ca. A 10% suspension of villi was incubated in Krebs–Ringer phosphate containing 0.5 mM Ca^{2+} and 2.0 μC ^{45}Ca/l.

Inhibitor	*Concentration (M)*	*Counts/min/100 µl supernatant*	*Inhibition (%)*
Control without cells	—	73 800	—
—	—	31 400	0
Ouabain	$1 \cdot 10^{-5}$	30 250	0
PCMB	$1 \cdot 10^{-4}$	30 300	0
NaF	$1 \cdot 10^{-2}$	29 700	0
N-Ethylmaleimide	$1 \cdot 10^{-4}$	34 200	7
Caffeine	$1 \cdot 10^{-2}$	35 700	10
Warfarin	$1 \cdot 10^{-4}$	37 500	14
Oligomycin	$1 \cdot 10^{-5}$	40 000	20
2,3-Dimercaptopropanol	$1 \cdot 10^{-3}$	40 000	20
NaCN	$1 \cdot 10^{-3}$	61 600	71
Malonate	$1 \cdot 10^{-2}$	64 500	78
Antimycin A	$1 \cdot 10^{-6}$	65 200	79
Dicumarol®	$1 \cdot 10^{-5}$	66 300	83
Dinitrophenol	$2 \cdot 10^{-5}$	67 600	85
Sodium azide	$1 \cdot 10^{-3}$	67 300	84
$SrCl_2$	$1 \cdot 10^{-4}$	33 800	4
	$2 \cdot 10^{-3}$	73 200	98

the presence and absence of Dicumarol® (Fig. 3). From these results the original and final concentrations of Ca^{2+} in the medium, and the concentrations of cellular Ca^{2+} were estimated (Table II). The total uptake of Ca in μmoles/g dry weight are plotted in Fig. 4. These results indicate that in the presence of Dicumarol® the intracellular Ca^{2+} concentration is nearly equal to the extracellular concentration at all concentrations of extracellular Ca. In the absence of Dicumarol® the intracellular Ca was

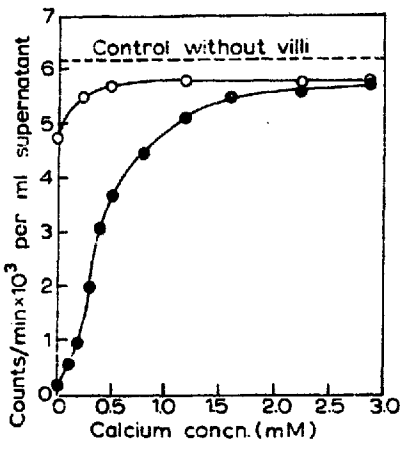

Fig. 3. The uptake of ^{45}Ca by intestinal villi as a function of external Ca^{2+} concentration in the presence (O—O) and absence (●—●) of $1 \cdot 10^{-5}$ M Dicumarol®. A 10% suspension of villi was incubated for 15 min at 4° in Krebs–Ringer phosphate containing 0.1 μC ^{45}Ca and varying amount of ^{40}Ca.

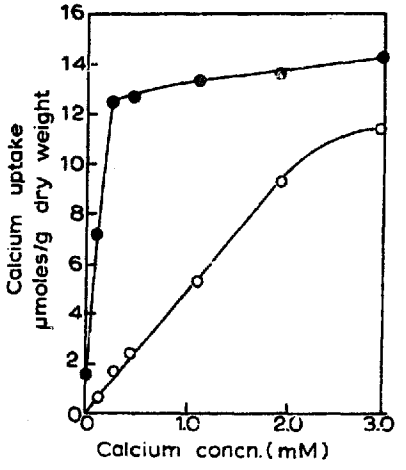

Fig. 4. The uptake of stable Ca by isolated intestinal villi as a function of external Ca^{2+} concentration in the presence (O—O) and absence (●—●) of Dicumarol® ($1 \cdot 10^{-5}$ M).

always greater than the extracellular concentration. The maximum active uptake was approx. 14.0 μmoles/g dry weight of tissue. These estimations have neglected the original Ca content of the cells, but there is apparently a rapid equilibration of ^{45}Ca with this pool because the specific activity of Ca in the villi at the end of an experiment was the same as that in the medium.

TABLE II

DISTRIBUTION OF Ca BETWEEN ISOLATED INTESTINAL VILLI AND THE EXTERNAL MEDIUM IN THE PRESENCE AND ABSENCE OF DICUMAROL®

A 10% suspension of villi was incubated at 4° for 15 min in Krebs–Ringer phosphate containing 0.1 μC ^{45}Ca. Concentration of Dicumarol®, $1 \cdot 10^{-5}$ M.

Medium Ca^{2+} concentration (μmoles/ml)			*Cells Ca^{2+} concentration (μmoles/ml)*		*Ratio cellular Ca to Ca in medium*	
Original	*Final no Dicumarol®*	*Dicumarol®*	*no Dicumarol®*	*Dicumarol®*	*no Dicumarol®*	*Dicumarol®*
0.12	0.012	0.11	1.8	0.18	150	1.6
0.28	0.09	0.25	3.1	0.46	35	1.8
0.47	0.28	0.43	3.2	0.60	11.3	1.4
1.12	0.92	1.04	3.3	1.3	3.6	1.3
1.96	1.77	1.82	3.4	2.3	1.8	1.2
2.98	2.76	2.86	3.5	2.8	1.3	0.9

Villi from all portions of the small intestine displayed a qualitatively similar uptake and release of Ca, although villi obtained from the duodenum and proximal jejunum took up a higher percentage of Ca at all Ca^{2+} concentrations than did villi from other portions of the small bowel.

DISCUSSION

The data recorded in this paper indicate that the distribution of Ca between isolated intestinal villi and the surrounding medium is the result of at least three separate processes: (1) a Dicumarol®-sensitive uptake of Ca which is relatively temperature independent; (2) a Dicumarol®-insensitive uptake which is proportional to the external Ca^{2+} concentration; and (3) the release of Ca by a highly temperature-dependent process.

The data recorded in Table I, Table II, and Fig. 3 indicate that at low external Ca^{2+} concentrations (0.5 mM or less) the cellular uptake of Ca is predominantly active in nature even at 4°. The pattern of inhibition suggests that oxidative energy and/or coupling to oxidative phosphorylation are required for the uptake of Ca by isolated villi. However, it is difficult to explain the lack of inhibition by oligomycin and Warfarin, both inhibitors of oxidative phosphorylation. It is noteworthy that neither of these agents inhibit the uptake of Ca in isolated mitochondria[12]. More recent unpublished studies from this laboratory have shown that the accumulation of Ca by isolated mitochondria is brought about by at least two different mechanisms, one depending upon ATP and the other upon electron transport and oxidative phosphorylation. A similar complexity is to be expected in the case of villi and probably accounts for the complex inhibition pattern observed in the present study.

It is of interest that ouabain, an effective inhibitor of Na transport in a variety of systems, is without effect upon Ca uptake in either villi or mitochondria.

The striking similarity between the behavior of the isolated villi and isolated mitochondria is further confirmed by the effects of temperature and of parathyroidectomy upon the two processes. In both systems uptake occurs at low temperature and subsequent release is brought about by an elevation in temperature. Villi or kidney mitochondria from parathyroidectomized animals release their accumulated Ca more slowly than do their appropriate controls. Also parathyroid hormone, added *in vitro*, stimulates Ca release from the mitochondria. It has not yet been possible to demonstrate a similar effect of added hormone on the isolated cells.

The one major unaccounted for difference between the two systems is the fact that although it has been possible to demonstrate an effect of vitamin D deficiency upon Ca release from mitochondria[13], it has not been possible to demonstrate a similar phenomenon with isolated villi. This is particularly surprising in view of the fact that it can be demonstrated that mitochondria prepared from isolated villi respond to vitamin D in a manner similar to kidney mitochondria (WALDORF AND DELUCA, unpublished). This particular problem requires further investigation.

Although the Ca in the cells has been considered to be in solution and uniformly distributed throughout the cell water for the calculations reported in Table II, this undoubtedly is not the actual state of affairs. As shown by the work of THIERS AND VALLEE[14], a large percentage of the intracellular Ca exists in the mitochondrial fraction. This observation as well as the similarity between cellular and mitochondrial

uptake of Ca suggest that most of the actively accumulated Ca is probably within the mitochondria. It seems likely that a part at least of this Ca does not exist in free form[15].

The relationship of the exchange of Ca in isolated villi and isolated mitochondria to that of the net transport of Ca across the intestinal wall remains to be established. All are inhibited when oxidative metabolism is suppressed; Sr competes with Ca in all systems; and in general the behavior of the systems is similar. However, there are unexplained differences, the resolution of which await further experimentation.

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