Influence of Sodium and Parathyroid Hormone on Calcium Release from Intestinal Mucosal Cells

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ABSTRACT The uptake and release of ^{45}Ca from the intestinal mucosal epithelium were investigated under a variety of conditions. The initial rate of uptake characterized a calcium pool with a half-time of saturation of less than 2 min. The entry of ^{45}Ca into this pool was inhibited by NaCN and ethacrynic acid and was stimulated by the removal of Cl^- from the incubation. The initial rate of ^{45}Ca release was also inhibited by NaCN and removal of Na^+ from the incubation. Parathyroid hormone administration enhanced the release of ^{45}Ca from cells prepared from parathyroidectomized animals. These observations suggest that calcium transport across the brush border and basal-lateral membranes are identifiable components of the kinetics of ^{45}Ca uptake and release and that parathyroid hormone stimulates a sodium-dependent mechanism of calcium transport across the basal-lateral membranes.

INTRODUCTION

The intestinal transport of calcium has been the subject of extensive investigation over the past decade. Despite this effort, the mechanism of this important transport process and specifically the roles of sodium and parathyroid hormone in intestinal calcium transport remain largely obscure. It is generally accepted that the intestine is capable of moving calcium from lumen to serosal surface against a concentration and electrical potential gradient. This process is saturable and energy dependent (1–3). Recent observations have demonstrated that sodium is required for this transport process (4, 5). The regulation of intestinal calcium absorption and the adaptation to dietary calcium deprivation has been postulated to be mediated in part by parathyroid hormone (6–10), although there has been some difficulty in demonstrating this phenomenon in experimental models (11, 12). Consequently the site of action of the hormone on the transport process is unknown. Furthermore, existing methods for the study of the calcium transport process have not permitted the adequate description of events occurring at the base or serosal surface of the intestinal mucosal cell where the rate-limiting step in calcium transport is postulated to exist (5, 13). In order to further elucidate the role of sodium and parathyroid hormone in the calcium transport process a method was developed which permitted the direct measurement of both the rate of uptake and release of ^{45}Ca by mucosal cells. These studies suggest that parathyroid hormone stimulates the energy-dependent translocation of calcium across the base of the mucosal cell by a sodium-dependent process.

METHODS

Mucosal tissue was prepared from the duodenum of 3½-wk-old male Wistar rats (National Animal Laboratories, St. Louis, Mo.). A 4-cm segment of the everted duodenum was partially distended with 0.3 ml of the incubation medium and tied at each end. The incubation medium contained NaCl, 135 mM; calcium, 1.4 mM; phosphate, 0.2 mM; glucose, 2 mM in 10 mM Tris-HCl buffer at pH 7.3. The duodenal segment was then placed in a 25-ml Erlenmeyer flask containing 5 ml of the incubation medium and incubated at 37°C in an atmosphere of 100% O_2 with gentle shaking for 4–10 min. After the appropriate period of preincubation, the everted intestinal segment is removed, blotted dry, washed by repeated immersion in the buffer, and transferred to a second flask containing buffer and 1.0–2.0 μCi/ml ^{45}Ca. After the appropriate time interval, the intestinal segment is removed, blotted, and washed as before. The mucosa is then scraped from the underlying muscularis mucosae and crypts with a spatula and rapidly suspended in 10 ml of buffer at 0°C by applying to a Vortex mixer (Scientific Industries, Inc., Queens Village, N. Y.) for 4 s. The mucosal cell suspension is then poured onto a Whatman no. 541 low-ash filter paper under suction. The retained cells are washed immediately with an additional 10 cm^3 of 5 mM $CaCl_2$ in saline at 0°C. The time required to complete the preparation of cells after removal of the tissue from the incubation is approximately 35–40 s. The cells are dissolved from the filter paper with

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1 N KOH. One aliquot is retained for protein determination (14). After acidification with HCl, a second aliquot is added to Bray's solution (15) for determination of radioactivity using a liquid scintillation spectrometer. The rate of ^{45}Ca uptake by the mucosal cells is expressed as the counts per minute per milligram protein accumulated by the cells during 2 min of incubation in the labeled buffer at 37°C.

To study the rate of calcium release from the mucosal cell, the intestinal sac was incubated for 6 or 12 min in the buffer labeled with ^{45}Ca with a calcium concentration of 1.4 mM, then after thorough rinsing of the tissue as described above, the tissue is transferred to isotope-free medium for an additional period of incubation. The cells are dispersed, collected, and washed by filtration as previously described. The amount of radioactivity released from the cells during a given time interval is equivalent to the radioactivity per milligram protein present in the cells at the beginning of the incubation in the isotope-free buffer (R_i) minus the radioactivity per milligram protein from the cells during incubation in the isotope-free buffer (R_i-R_f) and is expressed as a fraction of the initial radioactivity (R_i-R_f/R_i) to give a fractional rate of release. Routinely, eight animals were used to define a rate of uptake and, therefore, 16 animals were used to determine the fractional rate of release with an average SE of ±3%. In some studies metabolic inhibitors, NaCN (0.1 mM) or ethacrynic acid (1.0 mM) (Upjohn Co., Kalamazoo, Mich.), were added to the preincubation medium and subsequent incubations.

Preparation of animals. The rats were maintained for 3 days before use on a low calcium diet (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) unless stated otherwise. The vitamin D studies were performed with 6-wk-old animals maintained for the last 3 wk on vitamin D-deficient rat chow (Nutritional Biochemical Corp., Cleveland, Ohio). The rats were given orally either 200 U of vitamin D_3 (Phillips-Duphar, Amsterdam, Holland) in cotton seed oil or cotton seed oil alone. Parathyroidectomy was performed by hot wire cautery 24 h before sacrifice. 4 h before sacrifice the parathyroidectomized animals were given subcutaneously 30 U of parathyroid extract (Eli Lilly and Co., Indianapolis, Ind.) or 0.3 ml of 0.2% phenol in saline and 1.6% glycerine. After parathyroid surgery, the animals were maintained on the calcium-deficient diet to which 3% $CaCO_3$ had been added. In order to simulate the rise in serum calcium resulting from the hormone injection, the control animals were given intraperitoneally 17 μmol and 35 μmol calcium gluconate at 3.5 and 1.0 h before sacrifice. The animals receiving parathyroid hormone were given the equivalent dose of sodium gluconate at the same time intervals. Serum for calcium determinations (16) was obtained at the time of sacrifice. Animals with serum calcium values that were > 1 SD from the mean of the experimental group were excluded from the calcium transport data.

RESULTS

To characterize the movement of calcium across the brush border as well as the basal-lateral membrane, the rate of uptake and release of ^{45}Ca by duodenal epithelial cells was measured. The incubations were carried out with intact intestinal segments, thus, perserving the normal structural morphology and cell-to-cell interactions. In terminating the incubation for either uptake or release studies, the mucosal epithelial tissue was removed to the level of crypt-villus junction and then was rapidly dispersed. The resulting suspension consists of single as well as clumps of columnar epithelial cells. The subsequent collection of these epithelial cells on filter paper permitted an effective and rapid removal of the tissue extracellular fluid and contaminating radioactivity (Table I). Examination of the filtrate, which contained 3–4% of the protein filtered, revealed pre-

TABLE I
Characteristics of the Filtration Step

Tissue preparation	Incubation time +^{45}Ca	Incubation time −^{45}Ca	Total cpm added to filter	Total cpm retained	% of total retained
	min				
A. Cell suspension	0	0	50,480	960	1.6
B. Intact strip	2	0	45,650	34,466	75.5
C. Intact strip	2	6	38,240	13,135	34.3
D. No tissue	—	—	50,480	21	0.04

The efficiency of the filtration step in the removal of extracellular ^{45}Ca was evaluated under the following experimental conditions. (A) A known quantity of radioactivity was added to a suspension of mucosal cells at 0°C and applied immediately to the filter. (B) The intact intestinal strip was incubated for 2 min at 37°C with ^{45}Ca, the mucosal cells then removed, suspended, and added to the filter. (C) The intact intestinal strip was incubated at 37°C with ^{45}Ca, then transferred to buffer without ^{45}Ca for an additional 6 min before removal and suspension of the mucosal cells and addition to the filter. (D) The radioactivity in A was added to the filter in the absence of cells. Total radioactivity added in B and C was determined from the sum of that filtered and that retained on the filter. Each value represents the mean of four experiments.

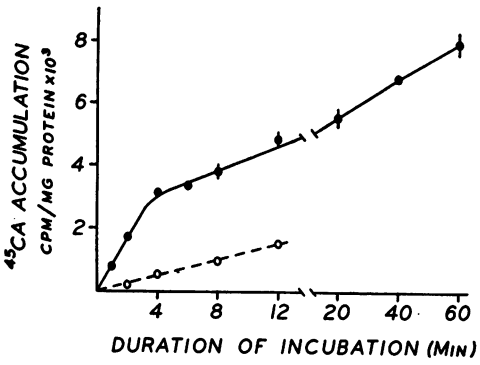

FIGURE 1 The uptake of ^{45}Ca per milligram protein by the mucosal cells was studied at 37°C (●——●) and at 0°C (○- - -○) as a function of time. Each point is the mean of five determinations, the SEM being indicated by the vertical bars.

dominately intact round cells (lymphocytes) and occasional erythrocytes. In addition, 30% of the cells were mucosal epithelial cells identified by the presence of the microvillus tuft. These findings suggest that the filter permits preferentially the passage of the smaller erythrocyte and lymphocyte and retards the passage of the larger columnar epithelial cell.

The efficiency of the dispersal and filtration procedure in removal of extracellular radioactivity was evaluated in a series of experiments outlined in Table I. In order to estimate what fraction of radioactivity retained on the filter could be attributed to extracellular radioactivity trapped by the tissue, epithelium scraped from unlabeled intestine was added to buffer containing ^{45}Ca at 0°C and then dispersed and suspended in the buffer with the Vortex mixer as usual. The cells were filtered immediately and the radioactivity per milligram protein retained was determined. Only 1.6% of the total radioactivity filtered was retained. This compares to a retention by cells prelabeled with the isotope for 2 min of 75.5 and 34.3% of the total radioactivity applied to the filter. The latter value was derived from that tissue incubated for an additional 6 min in the absence of isotope. Since the extracellular radioactivity is small relative to the apparent intracellular radioactivity retained, no correction for extracellular binding or contamination with ^{45}Ca have been made in calculations of uptake and release rates.

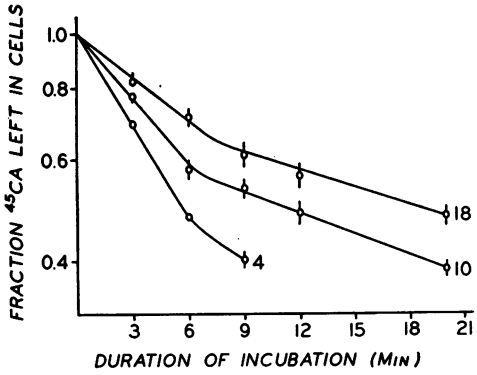

FIGURE 2 The fraction of ^{45}Ca released by the mucosal cells per milligram protein, as a function of time, was studied after incubating the intestinal segments in ^{45}Ca labeled buffer for 4 min, 10 min, and 18 min. Each point is the mean of three determinations, the SEM being indicated by the vertical bars.

The kinetics of the ^{45}Ca uptake by the mucosal epithelial cells are characterized by an initial rapid and linear phase of isotopic accumulation completed within 4 min (Fig. 1). This initial rate of uptake is markedly inhibited at 0°C. The release of ^{45}Ca from the mucosal epithelial cells is also characterized by an initial rapid phase followed by a much slower rate of release. When the fraction released is plotted semilogarithmically, it appears to conform to a single exponential for the first 6 min (Fig. 2). The absolute magnitude of this initial

TABLE II

Estimation of Mucosal Cell-to-Lumen and Mucosal Cell-to-Serosal Surface Calcium Flux

Incubation	Time	Tissue ^{47}Ca	Medium ^{47}Ca	Weight of segment
	min	*cpm*	*cpm*	*g*
A.				
37°C	0	11,989	0	0.7359
37°C	6	10,283	4,090	0.7272
0°C	6	3,410	2,418	0.7395
Total ^{47}Ca $F_{M\to L}$		1,706	1,672	
Fraction of total released to medium = 0.14				
B.				
Fraction of total (^{45}Ca) released from cell = 0.58				
Therefore of the total ^{45}Ca released from cell in 6 min:				
24% = $F_{M\to L}$				
76% = $F_{M\to S}$				

The relative contribution of the mucosal cell to lumen calcium flux ($F_{M\to L}$) and the mucosal cell to serosal surface calcium flux ($F_{M\to S}$) to the total calcium released from the cell was estimated. Everted intestinal segments of uniform length were incubated in buffer containing ^{47}Ca for 2 min at either 37°C or 0°C. The intestinal segment was then transferred to isotope-free buffer at either 37 or 0°C. The 0 time intestinal segments were processed immediately, the remainder after 6 min of incubation. Total ^{47}Ca $F_{M\to L}$ was calculated from either the differences in tissue content of ^{47}Ca or medium content of ^{47}Ca release from extracellular sites. In study B animals from the same group were used to determine the fraction of ^{45}Ca released from the mucosal cell in 6 min after preloading the mucosa for 2 min with ^{45}Ca by using the method described in the text. Each number is the mean of four animals.

TABLE III

Effect of Metabolic Inhibitors on the Rate of Uptake and Release

Incubation	Uptake		Release		Fr. rate
	cpm/mg protein/2 min	*% Control*	*cpm/mg protein/6 min*	*% Control*	
Control buffer	1,979±104	100	2,293±112	100	0.58±0.02
−Na, +mannitol	3,028±168*	153	1,633±94*	71	0.27±0.04*
−Na, +choline	1,980±108	100	1,067±126*	46	0.27±0.05*
+Ouabain (1 mM)	1,859±101	94	2,301±145	100	0.57±0.02
Control buffer	2,223±108	100	1,012±32	100	0.45±0.02
$-Cl^- + HCO_3^-$	2,725±181‡	123	1,089±90	108	0.40±0.04
Control buffer	1,843±72	100	2,208±152	100	0.49±0.01
NaCN (0.1 mM)	1,161±96*	63	1,146±130*	52	0.32±0.02*
Ethacrynic acid (1 mM)	1,235±140*	67	1,958±84‡	89	0.51±0.03
Control buffer	2,156±160	100			
N_2	1,401±136*	65			

The rate of uptake and release of ^{45}Ca was examined under a variety of experimental conditions as indicated. The intestinal strips were preincubated in the appropriate buffer without isotope for 6 min before transfer to the labeled buffer. In studying the influence of anaerobiosis, the isotope was added to the sealed flasks equilibrated with either O_2 or N_2. In the experiments in which Cl^- was replaced with HCO_3^- the flasks were gased with 95% O_2–5% CO_2. In determining the rate of release the cells were incubated for 6 min in the presence of the isotope except in the case of NaCN and ethacrynic acid studies which were incubated for 12 min in the presence of the isotope. The rate of release is expressed as both the total radioactivity released in 6 min and this value as a per cent of control and finally as a fraction of the total radioactivity available for release at time 0 (Fr. rate). Each value represents the mean±SEM of a least 12 determinations.

* Indicates a $P < 0.01$.

‡ $P < 0.05$.

phase of ^{45}Ca release is not altered by increasing the duration of preincubation in the presence of the isotope from 4 to 18 min. This suggests that the ^{45}Ca efflux during the first phase of release is derived from a pool which is uniformly labeled within 4 min. In an effort to estimate what proportion of the ^{45}Ca released represented efflux across the brush border, everted duodenal segments were incubated in the presence of ^{47}Ca in order to determine total tissue uptake and that fraction of the total tissue ^{47}Ca which was released into the medium under the conditions employed in these studies. From the calculations given in Table II, it is estimated that 75% of the measured release of calcium represents efflux across the basal-lateral membranes.

To identify the physiologic relevance of the initial phase of ^{45}Ca uptake and release, the response to several metabolic perturbations were examined (Table III). In the study of the influence of various metabolic inhibitors on ^{45}Ca release, the tissue was labeled by incubating in the presence of the isotope for a period of 6 or 12 min before transfer to the isotope-free calcium-free medium. By omitting calcium from the release incubation medium, the influence of altered rates of calcium uptake on the intracellular labeled calcium pool was minimized during the 6 min of release. The 4-min preincubation used in uptake studies was omitted in release experiments. In the presence of sodium cyanide both the rate of uptake and release were inhibited. The rapid phase of uptake was also inhibited by ethacrynic acid but not ouabain. These inhibitors of sodium transport had no effect on the initial phase of calcium release. To insure access of the ouabain to the mucosal epithelium, the intestinal segments were preincubated for as long as 45 min in 1 mM ouabain inside and outside the sac with similar results. The removal of sodium and its replacement by isotonic mannitol resulted in a marked acceleration of the measured rate of ^{45}Ca uptake which was associated with an equally significant inhibition of the initial phase of ^{45}Ca release. The substitution of choline chloride for sodium in the incubation medium also inhibited the release of ^{45}Ca from the mucosal cells. However, ^{45}Ca uptake was not increased by the replacement of sodium with choline. Substitution of the anion, chloride, of the sodium chloride did result in an increased uptake of the isotope.

The response of the ^{45}Ca uptake and release rates to vitamin D and parathyroid hormone, two physiologic factors which influence intestinal calcium absorption,

TABLE IV
Influence of Vitamin D on ^{45}Ca Uptake

	Time after treatment		
	4 h	8 h	8 h
	cpm/mg protein/2 min		*Serum Ca (mg/100 ml)*
−Vitamin D	1,579±191	1,485±80	11.4±0.2
+Vitamin D	1,554±90	1,751±84*	11.5±0.2
% Change	0	18	0

Vitamin D-deficient animals were given 200 U of vitamin D_3 in cotton seed oil orally at 4 and 8 h before sacrifice. The control, D-deficient animals were given cotton seed oil at 4 and 8 h before sacrifice. The intestinal sacs were preincubated for 4 min before transfer to the labeled buffer for 2 min. Each value represents the mean±SEM of eight determinations.
* $P < 0.05$.

were studied. The influence of vitamin D_3 on epithelial cell uptake of calcium was examined in 6-wk-old rats maintained for 3 wk on a vitamin D-deficient diet (Table IV). ^{45}Ca uptake was enhanced in those cells obtained 8 h after vitamin D_3 administration but not in those cells obtained 4 h after vitamin D_3 treatment. The influence of parathyroid hormone on the uptake and release process was examined in rats parathyroidectomized 24 h before sacrifice (Table V). In determining both the rate of uptake and release, the intestinal segments were preincubated for 10 min in the appropriate buffer. Removal of sodium from the incubation markedly enhanced the rate of ^{45}Ca uptake. This effect was independent of whether the animal had received parathyroid hormone before sacrifice. In contrast, the fractional rate of ^{45}Ca release by cells from parathyroidectomized animals was not significantly altered by the restoration of sodium to the incubation buffers. However, the fractional rate of ^{45}Ca release by cells from animals given parathyroid hormone was markedly stimulated by the replacement of mannitol with sodium. Thus in the absence either of parathyroid hormone treatment or of sodium in the incubation buffers, ^{45}Ca release is significantly impaired. Maximal rate of ^{45}Ca efflux requires both the hormone and sodium.

TABLE V
Influence of Parathyroid Hormone (PTH) and Sodium on Release of ^{45}Ca

	Experimental conditions			
PTH given	yes	yes	no	no
Sodium in buffer	yes	no	yes	no
Serum Ca, *mg/100 ml*	10.1±0.5		8.9±0.3	
Uptake, *cpm/mg protein/6 min*	6,302 ±380	7,429 ±434	5,798 ±452	7,732 ±699
Release, *cpm/mg protein/6 min*	3,277 ±163	1,756 ±285	1,777 ±183	1,870 ±325
Fractional rate of release	0.48 ±0.02	0.24 ±0.03	0.31 ±0.02	0.24 ±0.03
P value		<0.05		NS

All animals were parathyroidectomized 24 h before study. Parathyroid hormone was given to one-half the animals 4 h before sacrifice. Sodium in the incubation buffers was replaced in two groups by the substitution of isotonic mannitol. Uptake is the radioactivity of ^{45}Ca per milligram of mucosal cell protein accumulated in 6 min of incubation. Release is expressed both as total radioactivity of ^{45}Ca per milligram mucosal protein released in 6 min and as a fraction of the total ^{45}Ca accumulated to give a fractional rate of release. Each value is the mean±SEM of 16 determinations from four different sets of animals. Student's *t* test was applied to the fractional rate of release derived from the means of the four experimental sets.

DISCUSSION

In studying calcium translocation across the intestinal mucosa cell a variety of in vitro techniques have been utilized. These techniques may be characterized in general as either utilizing intact intestinal segments or isolated cells. However with both approaches there exist significant limitations which have been reviewed by Bray and Clark (17). With intact intestinal tissue, mucosal-serosal flux is influenced by diffusion barriers, the muscularis and serosa, which are nonexistent in vivo because of the cation removal by the submucosal capillary circulation. Determination of isotopic accumulation by mucosal scrapings reflects not only the rate of calcium transport across the brush border but also the rate of calcium efflux and the subsequent accumulation of isotope in the extracellular fluid. Finally, intact tissue preparations do not permit the independent and direct assessment of calcium translocation across the basal-lateral membrane and brush border. For this purpose it was considered necessary to develop an isolated cell preparation. However, isolated cell preparations described previously by Hashim and Clark (18), Kimmich (19), and Rasmussen, Waldorf, Dziewiatkowski, and DeLuca (20) do not permit the analysis of calcium uptake across the brush border specifically. Secondly they are generally inadequate for the description of the kinetics of the initial rapid phase of calcium uptake and release. In addition, the time required to prepare the cells, with and without enzymatic treatment, leads to morphologic changes and eventual disruption of the epithelial cell (17).

The method described in this report was devised to circumvent the major difficulties encountered in the currently available techniques. The transport studies are carried out with intact tissue, thus, minimizing disruption or alteration of the membrane and cell physiology. In terminating the incubation by rapid dispersal and

subsequent collection by filtration of the mucosal epithelial cell suspension more effective removal of extracellular calcium is achieved. In addition lymphocytes, erythrocytes, and cell fragments which are poorly retained by the filter are removed from the final tissue preparation, thus, enhancing the homogeneity of the intact columnar epithelial cells. Finally, by limiting the total incubation time to 18 min or less, the integrity and viability of the cell is more completely preserved.

Two characteristics of the kinetics of the calcium fluxes observed in these studies deserve comment. The first is the rapid initial phase of ^{45}Ca uptake which appears to achieve equilibrium within 4 min with a calcium pool associated with the cell. The kinetics of this phase of uptake and the size of the rapidly equilibrating calcium pool are similar to the initial phase of uptake described by Borle with a half-time of 2 min and a pool size of 1.82 nmol/mg protein (*21*). Our observations indicate that this initial phase of ^{45}Ca uptake is (*a*) inhibited by ethacrynic acid, anaerobic conditions, sodium cyanide, and lowering of the incubation temperature, (*b*) accelerated by reduction of extracellular chloride concentration, and (*c*) stimulated by vitamin D treatment of the vitamin D-deficient animal. Consequently, these data suggest that the initial phase reflects, at least in part, the rate of accumulation of intracellular, and presumably cytoplasmic, isotope. Since complete inhibition of the initial phase of uptake was not observed, a part of the isotope accumulation may also reflect the nonspecific absorption of calcium to the glycocalyx on the luminal surface of the mucosal epithelial cell (*22*).

The kinetics of the rate of isotopic release from the cells are also consistent with the interpretation that this rapidly saturable calcium pool represents, at least in part, cytoplasmic calcium. Release of ^{45}Ca is characterized by an initial rapid rate with a half-time of desaturation of 3 min. This initial phase of release is also inhibited by sodium cyanide, is inhibited by the removal of sodium from the extracellular fluid, and is stimulated by parathyroid hormone. This rapidly exchangeable calcium pool defined by the initial rates of ^{45}Ca uptake and release must therefore be an intracellular calcium pool associated with the intestinal mucosal cell since the exchange of calcium between this pool and the extracellular fluid is dependent on temperature, oxidative phosphorylation, and sodium and may be altered by ethacrynic acid, vitamin D, and parathyroid hormone. On this basis, the assumption is then made that the initial rate of ^{45}Ca uptake by the cell preparation is a function of the movement of calcium across the brush border and that the initial rate of ^{45}Ca release from the cell is a function of the movement of calcium across the basal-lateral membrane. Admittedly the methodology employed does not permit the functional delineation of the release process to the basal-lateral membrane exclusively, although the majority of ^{45}Ca is clearly released across the basal-lateral membrane as shown in Table II.

Release of ^{45}Ca from the cell during the first 6 min conforms to a single exponential decay which would be predicted if one assumes that the calcium is derived from a single intracellular compartment. Thus the contribution of the much larger and presumably mitochondrial calcium pool to the rapidly exchangeable pool is not significant during the first 6 min of the initial phase of release. This assumption is supported by the observation that increasing the duration of incubation with isotope does not increase the total ^{45}Ca released in the first 6 min. On this basis the fractional rate of release for the first 6 min has been used in these studies as a measure of the plasma membrane transport of calcium from the cell.

Having identified those components of the kinetics of ^{45}Ca uptake and release which best describe the movement of calcium across brush border and basal-lateral membranes, we can further characterize the transport processes occurring at the two membrane sites. The mechanism of calcium transport at either site has not been established. A calcium-activated vitamin D-dependent ATPase has been described in preparations of brush border fragments (*23*). The role, if any, in calcium transport of this phosphatase, which becomes activated at calcium concentrations 1,000 times that of the intracellular calcium concentration, has not been established. Its participation in the energy-dependent influx of calcium would be redundant since the movement of calcium from the intestinal lumen across the brush border would be facilitated by an electrical potential gradient across the cell membrane which is generated by an energy-dependent sodium efflux. Inhibition of the sodium pump would be expected to inhibit calcium uptake as was observed in the presence of ethacrynic acid and sodium cyanide. Removal of sodium from the extracellular environment would be expected to enhance sodium efflux and, therefore, enhance the electrical potential gradient across the brush border. Thus the accelerated uptake of calcium in the absence of sodium observed in these studies is also consistent with this model.

The failure to accelerate calcium uptake when sodium chloride was replaced with choline chloride is of some interest. One explanation for this observation is that an exchange of ^{45}Ca at anionic binding sites of the membrane is facilitated in the absence of the cations Na^+ and choline, resulting in an apparent increase in ^{45}Ca uptake by the mucosal cells. Alternatively the chloride anion (Cl^-) may be an essential determinant of the role of a ouabain-insensitive, ethacrynic acid-sensitive so-

dium gradient across the intestinal epithelial cell membrane. It is of interest that Proverbio, Robinson, and Whittembury (24) have demonstrated in the kidney that ethacrynic acid has essentially no effect on Na^+, K^+ exchange but completely inhibits the extrusion of Na^+ accompanied by Cl^-. Thus one can postulate that it is this Na^+ pump, dependent on Cl^- efflux, which primarily conditions the entry of calcium into the mucosal cell. Accordingly the substitution of extracellular Cl^- with HCO_3^-, in contrast to the substitution of Na^+ with choline, facilitated the extrusion of intracellular Na^+ with the subsequent increase in the electrical potential gradient across the cell membrane. The failure to inhibit mucosal calcium uptake by ouabain, which inhibits intestinal as well as renal (Na, K) ATPase and, therefore, Na^+, K^+ exchange, is also consistent with this model of Na^+ efflux.

Efflux of calcium across the basal-lateral membrane is also an energy-dependent process and inhibited by sodium cyanide. This step in mucosal calcium transport was found to be dependent on the presence of sodium in the extracellular environment. The failure of ethacrynic acid and ouabain to significantly alter calcium release suggests that the classic sodium transport mechanisms are not involved in the calcium efflux process. In the absence of a demonstrable effect of ouabain on ^{45}Ca uptake or release, the question arises as to the availability of ouabain to the basal-lateral membrane and the (Na, K) Mg ATPase. Under identical conditions of incubation used in these studies, net water flux was inhibited by ouabain at a time when there was no demonstrable effect of the inhibitor on net calcium flux (25). In the present studies, prolonged preincubation of the everted intestine, exposed to ouabain on both the serosal and mucosal surfaces for 45 min, failed to influence ^{45}Ca release. These data suggest that sodium may participate in the energy-dependent efflux of calcium across the basal-lateral membrane through the activation of an enzyme complex mediating the translocation of calcium across the cell membrane, analogous to the (Na, K) Mg ATPase enzyme complex (26). This mechanism differs from the sodium-dependent efflux of calcium from nerve and muscle in which the driving force of the calcium pump is generated by the sodium gradient (27). Thus the intestinal calcium pump would appear to be more similar to the red cell calcium pump which is mediated by a magnesium-dependent calcium-activated ATPase and is independent of the sodium gradient (28).

Since calcium efflux across the basal-lateral membrane of the mucosal cell is probably the rate-limiting step in intestinal calcium transport process under ordinary conditions, physiologic control would be expected to be mediated at this step. The observations presented in this report suggest that parathyroid hormone may act at this step in the transport process. In the absence of either in vivo parathyroid hormone or the addition of sodium to the incubation medium, ^{45}Ca release from the mucosal epithelial cell preparation was markedly inhibited. These data are consistent with the interpretation that the stimulation of ^{45}Ca release from the mucosal epithelial cell by parathyroid hormone is mediated through a sodium-dependent active transport of calcium. In light of the recent observations of Tanaka and DeLuca and co-workers (29) consideration of an indirect action of parathyroid hormone on the intestine must be entertained. It is, however, unlikely that the parathyroid hormone administered could enhance renal 1,25-dihydroxycholecalciferol production rapidly enough to influence intestinal calcium transport within 4 h. No effect on mucosal calcium absorption has been observed until 6 h after i.v. administration of 1,25-dihydroxycholecalciferol (30).

In summary, a method has been developed which permits the measurements of calcium uptake and release by intestinal mucosal epithelial cells. The kinetics of these two transport processes have been delineated so that it is possible to determine the rate of calcium movement in either direction across the cell membrane as a specific function of the membrane-associated transport process. These studies have demonstrated that the entry of calcium into the cell, presumably across the brush border, is dependent on active chloride-dependent sodium transport. Transport of calcium from the cell is also an energy-dependent process which requires sodium but which is insensitive to inhibitors of sodium transport. This sodium-dependent calcium efflux is also dependent on parathyroid hormone and may, therefore, mediate the rate-limiting hormone-dependent step in the intestinal transport of calcium.

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