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Kinetic Studies of Calcium Movements in Intestinal Cells: Effects of Vitamin D Deficiency and Treatment*

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Summary. The calcium distribution among three cellular calcium pools was studied by kinetic analyses in intestinal cells isolated from normal, vitamin D-deficient and vitamin D-repleted chicks. Vitamin D deficiency significantly reduces the cellular ^{45}Ca uptake by reducing the intracellular exchangeable calcium pool. Calcium efflux from the cells varies depending on the onset of the vitamin D deficiency: at four weeks calcium efflux is greater than control but after the fifth week it decreases and remains significantly lower than control. The cytoplasmic calcium pool follows the same biphasic pattern: it is higher at 4 weeks and lower after 5 weeks of D-deficiency. The mitochondrial calcium pool and calcium efflux from this compartment do not show a biphasic pattern. They are markedly depressed from the 4th week of D-deficiency. Eighteen hours after vitamin D administration the cytoplasmic and mitochondrial calcium pools return toward normal; after 42 hours the cytoplasmic pool and calcium efflux from the cell are normal. These data suggest that in D-deficiency, mitochondrial calcium uptake is depressed producing first a rise and then a drop in cytoplasmic calcium. The cytoplasmic calcium is responsible for the changes in cellular calcium efflux. The decreased calcium uptake may be partially due to a decreased cellular exchangeable calcium pool.

The mechanism of action of vitamin D on calcium transport in intestinal cells is still controversial. An increased mucosal uptake (Harrison & Harrison, 1965; Martin & DeLuca, 1969; Melancon & DeLuca, 1970) or an increased serosal transport (Schachter, Kowarski, Finkelstein & Ma, 1966; Wasserman & Taylor, 1969) are usually implicated. There is also some evidence that mitochondria, which may be involved in calcium transport (Borle, 1971*a*, *b*, 1972, 1973), may play a role in vitamin D action (Engstrom & DeLuca, 1962, 1964; Kimberg & Goldstein, 1967; Kimmich & Rasmussen, 1969; Hamilton & Holdsworth, 1970; Sampson, Matthews, Martin & Kunin, 1970).

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By kinetic analyses of ^{45}Ca movements in and out of isolated cells, it is possible to study the distribution and the fluxes of calcium among several internal and external compartments of the intact cell: mitochondria, cytoplasm and extracellular binding (Borle, 1970, 1972). This study performed with isolated chick intestinal cells, is an attempt to determine the distribution of calcium and its fluxes between various cellular compartments in normal, vitamin D-deficient and D-repleted animals. It was found that in D-deficiency (1) calcium uptake by the cells is decreased, (2) the mitochondrial and the cytoplasmic calcium pools are severly depleted, and (3) calcium efflux from the cell is depressed. After vitamin D administration, the cytoplasmic pool and calcium efflux from the cell reach control values in less than 42 hr. The largest effects of vitamin D depletion and of vitamin D treatment are observed in the mitochondrial compartment.

Materials and Methods

Animals

Male leghorn chicks were obtained at 1 day of age. For the first week they were kept in a 37 °C incubator room equipped with tungsten illumination. They were divided into four groups: Group I was the control and was fed a commercial purified chick feed. Group II was the vitamin D-deficient group and was fed from day one, a commercial rachitogenic diet containing 1% calcium and 1% phosphorus (Nutritional Biochemical Corp.). Group III was a vitamin D_3-treated group. It was fed the same rachitogenic diet and received one dose of 500 international units of vitamin D_3 (Hydrosol, Wander A.G., Bern, Switzerland) administered with olive oil by stomach tube 18 hr before sacrifice. Group IV was another vitamin-D treated group. It was fed the rachitogenic diet and received 500 IU vitamin D_3 42 hr before sacrifice. All chicken were kept in a windowless animal room. The aim was to produce a moderate vitamin D deficiency and not to produce severe rickets since this would have interfered with the normal growth and the general health of the animal and with the absortive function of its intestinal tract. After 3 to 4 weeks, the experimental animals showed definite signs of vitamin D deficiency: their knee joints were swollen, and when standing the chicken were obviously "knock-kneed." Their feathers were ruffled and sparse. Despite these typical signs of vitamin D deficiency, their duodenum was of normal size and showed no macroscopic sign of atrophy.

Cell Preparation

Two chicken were used for each experiment. They were killed by decapitation. The proximal end of the duodenum, measuring 6 to 8 cm in length, was removed, slit longitudinally and washed in an ice-cold Tris-buffered Krebs-Ringer's solution containing 1 mM calcium and 1% bovine serum albumin. Isolated epithelial cells were prepared by the method employed by Kimmich (1970) and by Shain (1972). The duodenal segment was incubated at 37 °C for 20 min in the same Tris-buffered Krebs-Ringer's with the addition of 1 mg/ml of hyaluronidase. After the incubation the segment was removed, gently washed in ice-cold buffer without hyaluronidase to rinse the enzyme from the

tissue. The tissue was then vigorously shaken in fresh, ice-cold medium. The cell suspension obtained was filtered through a nylon mesh, centrifuged at $150 \times g$ for 2 min and washed. Two wash cycles followed by centrifugation were enough to produce a clean homogenous cell suspension. After isolation 85 to 95% of the cells excluded the dye erythrosin B which differentiates between viable and nonviable cells. The calcium uptake curves obtained with such cells demonstrate that they are viable for 4 to 6 hr without significant decay. Indeed the first sign of cell damage and of cell death is an immediate and exponential rise in calcium uptake. This was not observed in isolated intestinal cells even after 6 hr. The cell protein concentration of the suspension was not different between groups and did not significantly decrease during the experiments which is another evidence of cell viability. Furthermore, Shain (1972) has tested the metabolic integrity of chicken intestinal cells isolated by this procedure. He measured their ability to accumulate sugars and to oxidize glucose to CO_2 up to 6 hr and concluded that cells isolated by this method are capable of performing complex cellular functions for a prolonged interval during incubation at 37 °C.

Uptake Experiments

The method for the determination of calcium influx has already been reported in detail (Borle, 1969, 1970). The cells were suspended in a Krebs-Ringer's Tris-HCl buffer containing 1 mM calcium, 1 mM phosphate, 1 mM magnesium, 5 mM potassium, 1 mM sulfate and 145 mM sodium. The gas phase consisted of 95% O_2 and 5% CO_2. A 50-ml cell suspension containing approximately 1 mg of cell protein/ml of medium was stirred at 37 °C with a magnetic Teflon bar driven by an immersible magnetic stirrer. After a preincubation period of 30 min, 50 μC of ^{45}Ca were added to the cell suspension. One-ml aliquots of the suspension were taken from 1 to 180 min, added to centrifuge tubes containing 40 ml of ice-cold medium and immediately centrifuged at $1{,}000 \times g$ for 45 sec. The supernatant was decanted and the cell pellet homogenized in 3 ml of deionized water with an ultrasonic probe. The cell protein content, total calcium and radioactivity were measured in duplicate aliquots of the cell sonicate. The cell protein was determined by the method of Oyama and Eagle (1956), the cell calcium by the method of Borle and Briggs (1968) and the radioactivity by scintillation spectrophotometry on an L-100 Beckman instrument using Aquasol® as scintillation medium. The kinetic analyses of calcium uptake curves were performed by computer as reported previously (Borle, 1970).

Calcium Efflux

The method for the determination of mitochondrial and cytoplasmic pools by desaturation experiments has also been published (Borle, 1972). The cells were suspended in the same buffer as described above and labeled with ^{45}Ca for exactly 60 min. After the labeling period, the cells were separated from the radioactive medium by centrifugation at $600 \times g$ for 45 sec, washed and then desaturated for 3 to 4 hr by repeated washout. Pool sizes and fluxes were calculated by computer from each isotopic desaturation curve as previously published (Borle, 1972).

Results

Comparison Between Intestinal and Kidney Cells

Table 1 compares the calcium fluxes, the exchangeable calcium pools of freshly isolated intestinal cells and of kidney cells grown in culture (LLC-

Table 1. Kinetic parameters of calcium fluxes of freshly isolated intestinal cells and of kidney cells grown in culture (LLC-MK_2)

A. Determined by Ca influx	Intestine (7)	Kidney (5)
Compartment 1		
Influx (pmoles/mg protein × min)	653 ±111	1125±112
Pool size (nmoles/mg protein)	1.94±0.44	2.10 ±0.19
Compartment 2		
Influx (pmoles/mg protein × min)	144 ±14	96 ±6
Pool size (nmoles/mg protein)	7.40±0.91	3.54 ±0.14
B. Determined by Ca efflux	**Intestine (14)**	**Kidney (9)**
Compartment 2		
Efflux (pmoles/mg protein × min)	177 ±12	83.4 ±8
Pool size (nmoles/mg protein)	6.47±0.6	1.84±0.22
Compartment 3		
Efflux (pmoles/mg protein × min)	58.4±6	11.1 ±1
Pool size (nmoles/mg protein)	8.0±0.7	2.54±0.43

Values are mean ± SE. The number of determinations are shown in parentheses. The first phase of calcium efflux is too fast to be described here and has been omitted.

MK_2). The first compartment of calcium influx represents extracellular calcium binding to the cellular glycocalyx. There is little difference between intestine and kidney cells considering the fact that the intestinal cells were exposed to hyaluronidase for 20 min. The second compartment of calcium influx represents calcium transport into the cell. Influx in intestinal cells is 50% greater than in kidney and the intracellular pool is twice as large. This may not necessarily represent a characteristic of intestinal cells. The possibility that hyaluronidase-treated intestinal cells are more "leaky" should be recognized. In intestinal cells the fluxes and the pool size of this second phase are in very good agreement when determined either by influx or by efflux techniques. The fluxes are, respectively, 144 and 177 picomoles mg $protein^{-1}$ min^{-1} and the pool size 7.40 and 6.47 nanomoles/mg protein. The third calcium compartment which can only be detected by efflux measurements, is significantly greater in intestinal cells than in kidney cells, 8.0 compared to 2.54 nanomoles/mg protein. It represents a subcellular pool that we have previously identified as a mitochondrial exchangeable calcium pool (Borle, 1972). This larger compartment may possibly reflect the significant sequestration of calcium in the mitochondria of intestinal

cells observed by electron-micrography by Sampson *et al.* (1970), or it may represent an increased calcium transport by intestinal cells and its buffering by mitochondria.

Effect of Vitamin D Deficiency on Calcium Uptake

Calcium uptake was measured in chicken placed on the rachitogenic diet from 3 to 7 weeks. There was no difference between the results obtained after 3 weeks of D-deficiency and those observed at 7 weeks. Therefore, all the results obtained between 3 and 7 weeks were pooled. Fig. 1 shows that ^{45}Ca uptake by freshly isolated intestinal cells is significantly reduced in D-deficiency. After 40 min of uptake each point of the experimental curve is significantly different from its control ($p < 0.01$). The mean and standard error of the calcium fluxes and the pool sizes derived from each individual

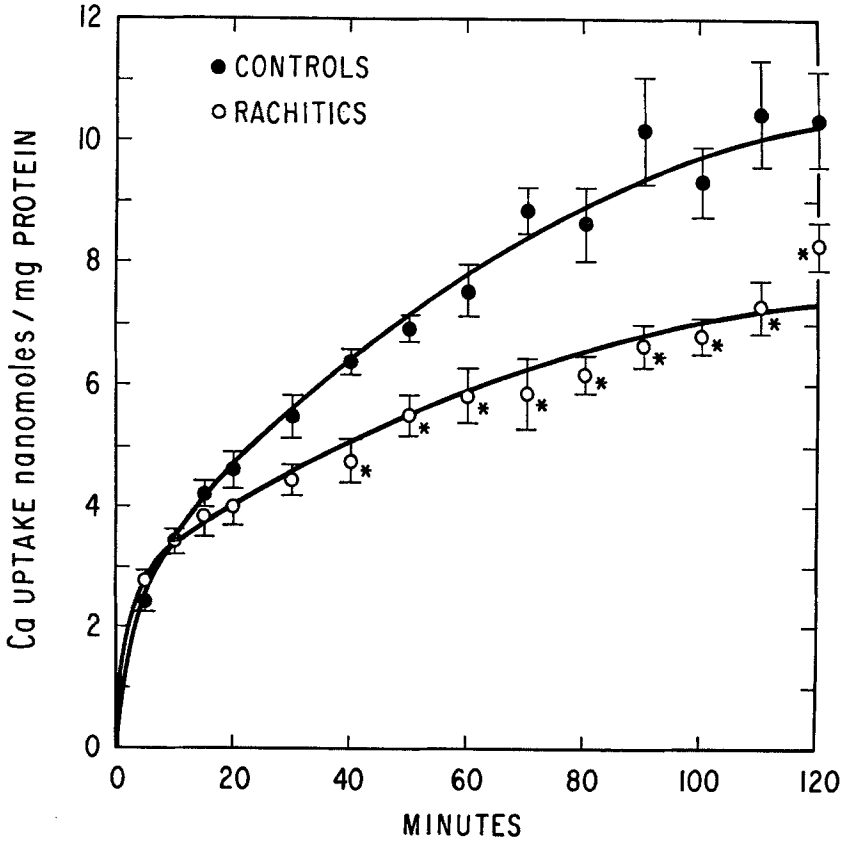

Fig. 1. Calcium uptake in intestinal cells isolated from normal and vitamin D-deficient chicks. From 40 to 120 min the values of the rachitic group are statistically different ($^*p < 0.01$) from control

Table 2. Ca influx in intestinal cells derived from control and vitamin D-deficient chicks

	Control (7)	Vitamin D-deficient (6)
Compartment 1		
Influx (pmoles/mg prot^{-1} min^{-1})	653 ±111	1733 ±280[a]
Pool (nmoles/mg prot)	1.94 ±0.43	2.64 ±0.25
Compartment 2		
Influx (pmoles/mg prot^{-1} min^{-1})	144 ±14	144 ±26
Pool (nmoles/mg prot)	7.40 ±0.91	5.57 ±0.46[b]

Values are mean ± SE. The number of determinations is in parentheses.
[a] $p < 0.01$.
[b] $p < 0.05$.

curve are shown in Table 2. In vitamin D deficiency, the fast phase, representing extracellular binding is significantly increased. This may reflect alterations in the properties of the plasma membrane. Indeed, Sampson *et al.* (1970) published electron-micrographs showing that duodenal microvilli from untreated rachitic rats showed an abundance of calcium granules attached to the inner portion of the limiting membrane in contrast to the controls treated with vitamin D. On the other hand, calcium influx into the second compartment is not affected but the intracellular exchangeable calcium pool is significantly decreased. Consequently, the depressed ^{45}Ca uptake obtained in vitamin D deficiency appears to be due to a decreased intracellular calcium pool and not to an impaired calcium influx into the cell.

Effect of Vitamin D Deficiency on Calcium Efflux

Unlike calcium uptake, calcium efflux from vitamin D-deficient intestinal cells varies between 3 and 7 weeks. For instance, Fig. 2 shows that calcium efflux from the second compartment depends on the degree of vitamin D deficiency or at least on the length of time of exposure to a vitamin D-deficient diet. In control intestinal cells, calcium efflux from the second compartment is constant throughout the same period. At 3 weeks there is no difference between control and experimental animals. During the fourth week of D-deficiency calcium efflux increases before falling sharply after the fifth week. It appears that during the development of vitamin D deficiency there may be a stage during which calcium transport out of the cell may transiently increase and therefore exceed calcium influx. In this case,

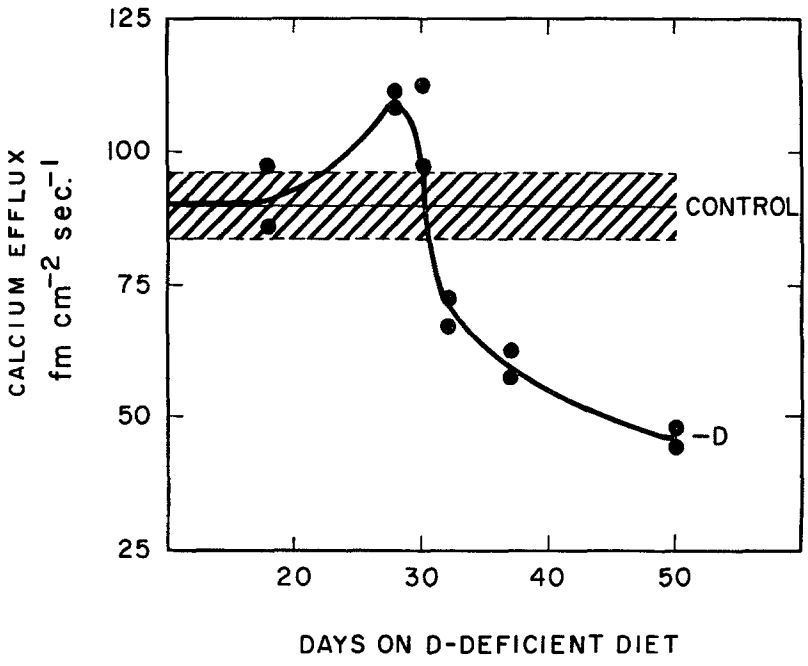

Fig. 2. Influence of the duration of vitamin D deficiency on calcium efflux from isolated intestinal cells. Phase 2 efflux represents calcium transport from the cytoplasm to the medium. The mean and SE of the controls are indicated in the dotted line and shaded area. The days indicate both the chicken age and the length of the vitamin D deficiency

one may expect calcium depletion to occur in some intracellular compartment. Table 3 shows that the third calcium compartment is indeed significantly decreased during the fourth week. This compartment reflects the mitochondrial exchangeable calcium pool (Borle, 1972). When vitamin D deficiency is well established between 5 and 7 weeks, the second phase of calcium efflux, which represents the exchange of calcium between the cytoplasm and the extracellular medium across the plasm membrane (Borle, 1972), is depressed 33% and the cytoplasmic pool is reduced 32%. Both results are statistically significant. The decreased efflux can be totally accounted for and is probably due to the low cytoplasmic calcium. At the same time, the third phase of calcium efflux is dramatically and significantly decreased. Calcium efflux drops 74% while the pool size falls 63%. The depression of calcium efflux from this mitochondrial pool could also be due to the fall in mitochondrial calcium. The depressive effects of vitamin D deficiency on the mitochondrial phase precedes and is twice as severe as its effects on the cytoplasmic phase. This suggests that the primary defect of

Table 3. Effect of vitamin D deficiency and vitamin D treatment on intracellular calcium distribution and on calcium efflux

Group	Compartment 2		Compartment 3	
	Efflux [c]	Pool size [d]	Efflux [c]	Pool size [d]
Controls (14)	177±12	6.47±0.6	58.4±6.0	8.0 +0.7
D-depleted				
4th week (4)	217±7 [a]	7.28±0.2	12.3±1.4	3.89±0.8 [a]
5th to 7th week (7)	119±8 [a]	4.44±0.5 [a]	15.5±1.2 [a]	2.95±0.4 [a]
D-treated				
18 hours (10)	142±24	5.04±07	22.6±3.0	4.1 ±0.6
42 hours (8)	194±19.4 [b]	7.04±09 [b]	24.2±3.8 [b]	3.7 ±0.9

Values are mean±SE. The number of determinations are indicated in parentheses.

[a] $p<0.01$ when compared to controls.

[b] $p<0.01$ when compared to 5th to 7th week D-depleted.

[c] pmoles/mg protein × min.

[d] nmoles/mg protein.

D-deficiency may reside in the mitochondria and that the low calcium of the cytoplasm and the decreased efflux from the cell may be secondary to a defect in mitochondrial calcium transport.

Effect of Vitamin D Treatment

Eighteen hours after the *in vivo* administration of 500 IU of vitamin D, important changes are already taking place in all intracellular compartments. Table 3 shows that the cytoplasmic pool and calcium efflux are increased 14 and 18%, respectively. However, the effects of vitamin D on the mitochondrial pool are much more pronounced, both flux and pool are increased 46%. Although these results fail to reach statistical significance, they indicate a trend which will be confirmed 42 hr after vitamin D administration. Fourty-two hours after one single dose of 500 IU, the cytoplasmic pool and calcium efflux from the cell have increased 59 and 63%, respectively, when compared to the D-deficient group (Table 3). They reach and even slightly exceed the control levels. Calcium efflux from mitochondria and the mitochondrial calcium pool are also greater than in the D-deficient group but this compartment is still depleted when compared with the control group. Since vitamin D deficiency may be a progressive and slow process, as shown earlier, the replenishment of calcium stores in the mitochondria may also be slow and progressive and may require more than 42 hr.

Discussion

The results of these kinetic analyses of ^{45}Ca fluxes in freshly isolated intestinal cells are similar to those obtained in established cultured cell lines. The calcium pools and fluxes are about the same as those measured in monkey kidney cells, dog kidney cells and human HeLa cells (Borle, 1969, 1971*a*, 1972). The effects of vitamin D deficiency and of D-repletion obtained in isolated intestinal cells do not differ from most of the results observed in other species with other techniques.

It is well known that vitamin D deficiency decreases the uptake of ^{45}Ca by the intestinal mucosa and that vitamin D administration increases it. Harrison and Harrison (1960, 1965) proposed that vitamin D increases the permeability of the mucosal membrane for calcium. DeLuca and his collaborators believe that calcium uptake by the intestine is a metabolically dependent process and that vitamin D stimulates this active transport (Martin & DeLuca, 1969; Melancon & DeLuca, 1970). However, the active or passive nature of the entry step of calcium into the mucosal cell and the effects of metabolic inhibitors on the action of vitamin D on calcium uptake are still controversial (Borle, 1974). The results presented here also demonstrate that calcium uptake by isolated intestinal cells is depressed in vitamin D deficiency (Fig. 1). A decreased uptake of ^{45}Ca can be obtained in two situations: when calcium influx into the cell is depressed or when the intracellular calcium pool with which ^{45}Ca exchanges is reduced. Table 2 shows that the decreased ^{45}Ca uptake observed in D-deficient intestinal cells may not be due to a decreased calcium influx into the cell but to a reduced intracellular calcium pool. This fall in intracellular calcium is confirmed by efflux experiments (Table 3).

Calcium efflux from intestinal cells is presumably an active transport process; it is depressed in vitamin D deficiency and stimulated by vitamin D (Wasserman, 1963, 1968; Holdsworth, 1965; Schachter *et al.*, 1966; Wasserman & Taylor, 1969). The present data not only confirm these findings but they further show that the cytoplasmic calcium pool is equally affected: it is depressed in vitamin D deficiency and replenished after vitamin D administration. The question arises whether vitamin D influences calcium efflux directly as it is usually assumed or whether the effect of vitamin D on calcium efflux is a consequence of changes in cytoplasmic calcium. Regardless of the active or passive nature of calcium efflux out of the cell, it will be dependent upon the cytoplasmic calcium activity. It is thus possible that the decreased calcium uptake and the decreased calcium efflux observed in vitamin D deficiency may be due at least in part to a fall in intracellular calcium.

Some effects of vitamin D on mitochondria have been observed for more than a decade. Engstrom and DeLuca (1962, 1964) first reported that vitamin D stimulates the release of calcium accumulated in mitochondria isolated from liver, kidney and intestine. On the other hand, Kimberg and Goldstein (1967) reported that vitamin D enhances the rate of calcium uptake by isolated liver mitochondria and facilitates the subsequent release of calcium when ATP is depleted. They suggest that the failure of other investigators to observe an effect of vitamin D on calcium uptake by mitochondria may be due to an excess of mitochondria relative to the calcium available in the medium, masking the rate-limiting effect of D-depletion on the uptake process. Kimmich and Rasmussen (1969) also observed that the calcium content of freshly isolated mitochondria from liver and kidney is dramatically decreased in vitamin D deficiency. Sampson *et al.* (1970) demonstrated by electron-microscopy that the mitochondria of intestinal mucosa cells contain granules of calcium phosphate. In D-deficiency, there is a complete disappearance of the granules. After vitamin D administration, the mitochondria are loaded with these electron-dense calcium granules. Hamilton and Holdsworth (1970) showed that calcium binding protein (CaBP) stimulates the loss of ^{45}Ca from isolated mitochondria. They suggest that vitamin D could increase the calcium turnover in mitochondria. Our results are in agreement with these findings. The exchangeable calcium pool of mitochondria loses 2/3 of its original exchangeable calcium in vitamin D deficiency. After vitamin D administration, the fastest change again occurs in the mitochondria pool. This exchangeable calcium pool is not identical to the calcium mass present in mitochondria or to its calcium activity, and the changes represent trends rather than quantitative measurements of mass or activity. This is evident in Table 3 showing a cytoplasmic exchangeable calcium pool at control levels while the mitochondrial pool is still depressed 42 hr after vitamin D administration. The discrepancy does not exclude a complete restoration of mitochondrial calcium activity.

If cellular calcium transport is controlled by the calcium activity of the cytoplasm, one should consider the possibility that cytoplasmic calcium is regulated by calcium exchange between mitochondria and cytoplasm (Borle, 1973). The effects of vitamin D on calcium uptake, on calcium efflux and on the mitochondria could be somehow related. It is of course possible that the effects observed in mitochondria are only a reflection of changes in calcium transport. Alternatively, the changes in calcium transport may be the consequence of alterations in mitochondrial function. It has already been proposed that mitochondria may play the role of an intracellular buffer for calcium ion and may control and regulate the cytoplasmic calcium

activity (Lehninger, 1964; Rasmussen, 1966; Borle, 1971*a*, *b*, 1972, 1973). Such a model for intracellular calcium homeostasis could readily account for the experimental results presented here. It proposes that the calcium activity of the cytoplasm is mainly controlled by the exchange of calcium between mitochondria and cytoplasm and minimally by the calcium fluxes in and out of the cell. In turn, calcium efflux and calcium transport is regulated by the cytoplasmic calcium activity. According to this model (Borle, 1973) the calcium activity of the cytoplasm (Ca_c) is a direct function of the mitochondrial calcium activity (Ca_m) and of the rate constant of efflux (k_{mc}) and it is inversely proportional to the rate constant of calcium uptake (k_{cm}):

$$Ca_c = \frac{Ca_m \cdot k_{mc}}{k_{cm}}. \tag{1}$$

In normal intestinal cells, the mitochondrial calcium activity (Ca_m) will remain constant since it will be fixed by the solubility product of the mineral phase of calcium phosphate, as long as mitochondrial calcium granules are present. If one assumes that vitamin D is necessary for the uptake of calcium in mitochondria, as shown by Kimberg and Goldstein (1967), vitamin D deficiency would reduce the rate constant of calcium uptake (k_{cm}). According to Eq. (1), the first result would be an increase in cytoplasmic calcium activity and a temporary increase in calcium efflux from the cell. This could explain the transient increase in calcium efflux observed during the fourth week of vitamin D deficiency (Fig. 2 and Table 3). The net result would be a slow depletion of intracellular stores of calcium, primarily of mitochondria. This is also supported by the results of Kimmich and Rasmussen (1969) and by Sampson *et al.* (1970). After the disappearance of the mineral phase of calcium phosphate in mitochondria, their calcium activity (Ca_m) can no longer be maintained at a fixed level and it will start to decrease. According to Eq. (1), as soon as the progessive fall in mitochondrial calcium activity (Ca_m) exceeds the depression in the rate of calcium uptake (k_{cm}), the cytoplasmic calcium activity as well as calcium efflux from the cell will decrease. This could explain the difference between the results obtained at 4 weeks and after 5 weeks of vitamin D deficiency. At the later stage calcium efflux and calcium transport will be reduced, because of the low cytoplasmic calcium activity. In addition, ^{45}Ca uptake will be reduced because of the significant drop in intracellular exchangeable calcium. Thus, in vitamin D deficiency, the mitochondrial buffer system may be failing, and recent results from Wong, Adams, Roberts and Norman (1970) seem to support this interpretation. They showed that filipin, a polyene antibiotic which

increases the permeability of membrane to calcium, stimulates intestinal calcium transport in vitamin D-deficient chicken but has no effect on D-treated animals. It is possible that in D-deficiency, an increased calcium influx cannot be buffered by the deficient mitochondrial system; this would increase the cytoplasmic calcium and calcium transport. In D-treated animals, the increased calcium influx would be buffered by the mitochondria which would maintain a normal cytoplasmic calcium level and a normal calcium transport. Vitamin D administration would of course restore the mitochondrial ability to take up calcium (Kimberg & Goldstein, 1967; Sampson *et al.*, 1970), and raise the mitochondrial calcium activity back to normal. Vitamin D may not replenish immediately the stores of calcium phosphate usually trapped in mitochondria. This could account for the lag observed 42 hr after D administration between the cytoplasmic and the mitochondrial calcium pools.

This interpretation does not exclude other effects of vitamin D on other functions of the cell metabolism. It could reconcile several conflicting ideas regarding the effect of vitamin D on intestinal calcium transport, and on the active or passive nature of calcium uptake. We do not know, however, if the calcium binding protein discovered by Wasserman could be implicated in the transport of calcium into the mitochondria of intestinal cells (Wasserman & Taylor, 1969); we do not know whether the increased ATPase activity reported by Melancon and DeLuca (1970) could be linked to an increased cytoplasmic calcium activity or whether the alteration of microvillar membrane lipid composition shown by Goodman, Haussler and Rasmussen (1972) could be in any way connected with a primary disturbance in cellular calcium homeostasis. The present hypothesis is only an alternative to the many proposals published in the literature.

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