VITAMIN D STIMULATED, CALCIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE FROM BRUSH BORDERS OF RAT SMALL INTESTINE[1]

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Summary: A calcium dependent-adenosine triphosphatase in the brush borders of rat small intestine is markedly increased after the administration of vitamin D to vitamin D deficient rats. This system may be responsible for the well-known vitamin D directed absorption of calcium in the intestine.

Convincing evidence has now accumulated that calcium is transported across intestinal mucosa by a cation-oriented active transport mechanism (1, 2, 3). Clearly vitamin D or its active form in some way induces the formation of a protein component of this system by a mechanism which involves "unmasking" of DNA (4), synthesis of nuclear RNA (5), and protein synthesis (6, 7, 8). The nature of the calcium transport system is poorly understood and especially that portion which is induced by vitamin D. An apparent breakthrough was registered when Wasserman and co-workers (9, 10, 11, 12) discovered a calcium binding protein in chick mucosa after vitamin D administration. They have studied this protein extensively and indeed it appears to be formed in response to vitamin D. In spite of all our

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efforts it has not been possible to show that the appearance of the binding protein correlates with the onset of calcium absorption in response to vitamin D (13). Instead, it seems likely that some other factor must be formed in response to vitamin D. Martin and DeLuca (3) demonstrated that initial calcium uptake across the brush border surface of intestine is an oxygen dependent, vitamin D stimulated process which is carrier mediated. At least one function of vitamin D must therefore be at the brush border surface. Direct evidence has now been obtained by the demonstration of a calcium dependent ATPase of intestinal brush border which is induced by vitamin D.

Male weanling rats were obtained from the Holtzman Company of Madison, Wisconsin. They were housed in hanging wire cages and given a purified vitamin D-deficient diet 11 (14) for 4-5 weeks. At this time the rats were severely deficient as revealed by low serum Ca^{++} and retarded growth. One-half the rats were given 500 i.u. of vitamin D_3 in 0.25 ml of cottonseed oil. After 40 hours they were killed by decapitation, the first 8 cm of the small intestine removed, slit open and rinsed in ice cold 5 mM EDTA, pH 7.4. The mucosal tissue was blotted dry with absorbant tissue paper and the mucosa scraped from the muscle coat with a glass slide. Brush borders were prepared essentially as described by Forstner, et al. (15) and used in the experiments. Ten micrograms of brush border protein was incubated in 1 ml of medium that contained 40 mM Tris pH 7.4, 5 mM ATP (made to pH 7.4 with NaOH), 5 mM $MgCl_2$, and 0 or 40 mM $CaCl_2$. The incubation was stopped by the addition of 1 ml of ice cold 5 N N_2SO_4 and PO_4 was determined by the method of Gomori (16). Brush border protein was determined by the method of Lowry, et al. (17).

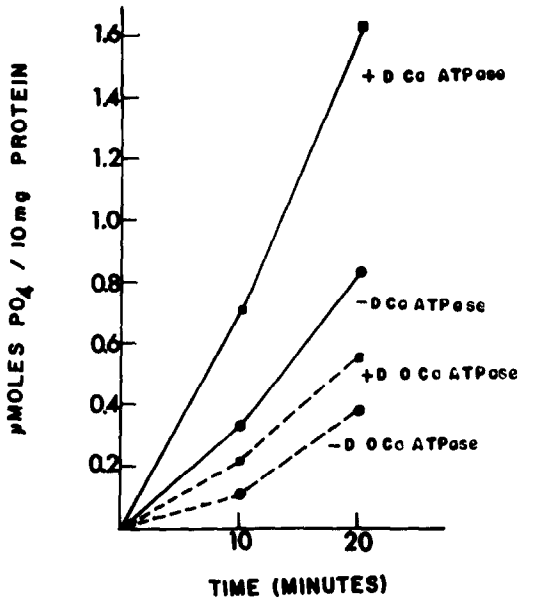

Fig. 1. Rate of ATP hydrolysis in the presence and absence of calcium by brush borders of duodenum from either vitamin D deficient rats or those given 500 i.u. vitamin D_3 orally 40 hours before. Each point represents triplicate determinations and the rate curves have been reproduced in 3 separate experiments.

The time course of ATP hydrolysis is demonstrated in Figure 1. Clearly ATP hydrolysis is markedly stimulated by the addition of calcium. This calcium dependent ATPase requires the presence of magnesium but not of sodium. Of greatest importance is that the calcium dependent ATPase is either low or absent from the brush border preparations from vitamin D deficient rats.

The results in Table 1 demonstrate that this effect of vitamin D is highly significant ($p < 0.001$). A similar, but more dramatic effect of vitamin D on calcium dependent ATPase has also been found in the brush borders of chick intestinal mucosa. Furthermore the presence of this calcium dependent ATPase exactly correlates with the appearance of vitamin D induced calcium transport in chick intestine. These results verify the conclusion

Table 1

Calcium dependent ATPase in brush borders of mucosa from duodenum of either vitamin D deficient rats or those given vitamin D

µmoles	PO_4 Released/10 µg Protein/10 Min.	
	-D	+D
0 Ca	0.11 ± 0.002	0.16 ± 0.029*
40 mM Ca.	0.29 ± 0.013	0.66 ± 0.029*
Ca-ATPase	0.19	0.50

Each value is the average of 2 experiments in which triplicate incubations were performed. Many other experiments at differing calcium concentration or incubation times etc. have revealed the vitamin D stimulated ATPase.

The values as given ± standard error of the mean.

*At 0 calcium, the difference due to vitamin D has a significance = $0.2 > P > 0.1$ while at 40 mM calcium, the significance = $P < 0.001$. Evaluation was carried out according to the Students "T" test.

of Martin and DeLuca (3) reached earlier that one if not the major site of vitamin D action on intestinal calcium transport is the transfer of calcium across the brush border surface of the columnar epithelial cell.

Experiments are now in progress to delineate the time course of appearance of the calcium dependent ATPase in response to vitamin D and 25-hydroxy vitamin D. It is hoped that this finding will plot a new and major course in the study of intestinal calcium transport and especially the action of vitamin D.

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