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Calcium Absorption in the Rat in Relation to Excessive Vitamin D and Cortisone. (27682)

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Our initial studies(1), measuring active transport of calcium across the intestinal wall of the rat by an *in vitro* intestinal sac technic, indicated that excessive Vit. D increased active transport in all segments studied, but cortisone decreased this transport in the duodenal segment only. This technic would be most helpful in investigation of factors affecting calcium absorption if the results obtained by this *in vitro* technic correlated with those obtained by studying calcium absorption in the intact animal. It was considered important therefore to extend our initial *in vitro* experiments, to study *in vivo* calcium absorption under the same experimental conditions, and to compare the results of the two procedures, in an attempt to determine: 1) whether excessive amounts of Vit. D will increase intestinal calcium absorption *in vivo* above that induced by physiologic amounts, 2) whether cortisone will inhibit calcium absorption *in vivo* in presence of either physiologic or excessive amounts of Vit. D, 3) whether changes in intestinal active transport of calcium determined *in vitro* can be used as an accurate index of calcium absorption *in vivo*.

Materials and methods. Male Sprague-Dawley rats weighing 100-130 g were treated with daily doses of 20,000 units of Vit. D* by stomach tube, or 2.5 mg of cortisone acetate† subcutaneously, or a combination of both drugs. A control group received cottonseed oil and saline, which were the vehicles for the Vit. D and cortisone respectively. During treatment, the rats were maintained on distilled water and a nutritionally complete diet containing a physiologic amount (148 USP units/100 g diet) of Vit. D.

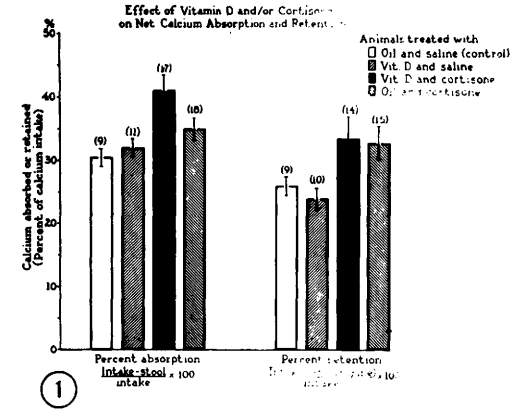
Calcium metabolic balance studies were carried out from the 2nd through the 5th day of the treatment period. On the 6th day blood was obtained by orbital sinus puncture, the rats were killed by decapitation and a 3-cm segment of duodenum, jejunum, and ileum was quickly removed and prepared for *in vitro* active transport studies as previously described(1). In some of the animals, serial segments of small intestine (beginning at the pylorus) were studied for active transport. Calcium was extracted from the feces with trichloroacetic acid as described by Harrison and Harrison(2). The calcium content of serum, stool extract, and urine was determined by a modification of the method of Kingsley and Schaffert(3).

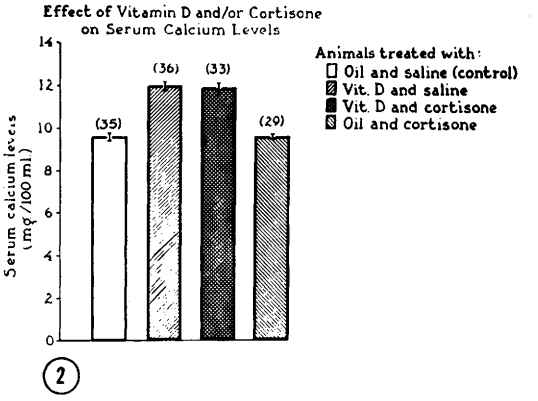
Using the above methods, a comparison of the effects of equal doses of cortisone and corticosterone on net calcium absorption, serum calcium level and duodenal active calcium transport was carried out, each treatment group consisting of 9 animals.

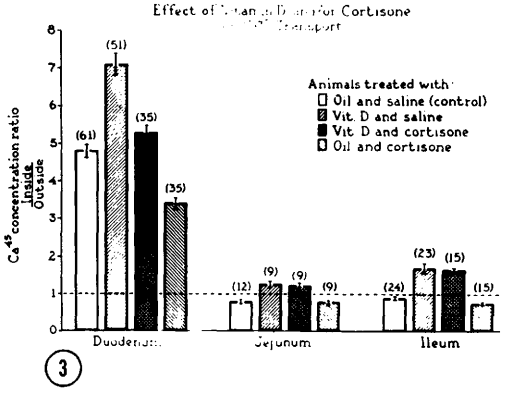
Results. In vivo net calcium absorption

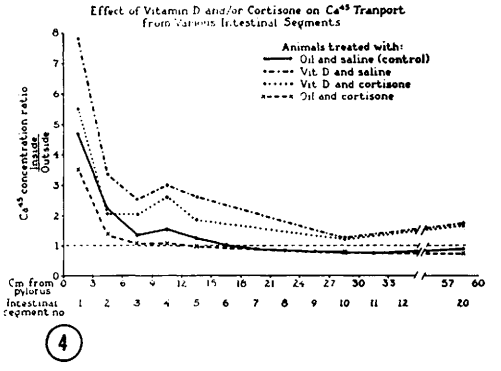
* Kindly furnished by Brewer and Co., Inc., Worcester, Mass.

† Kindly furnished by Sharp and Dohme, Division of Merck and Co., Inc., Philadelphia, Pa.









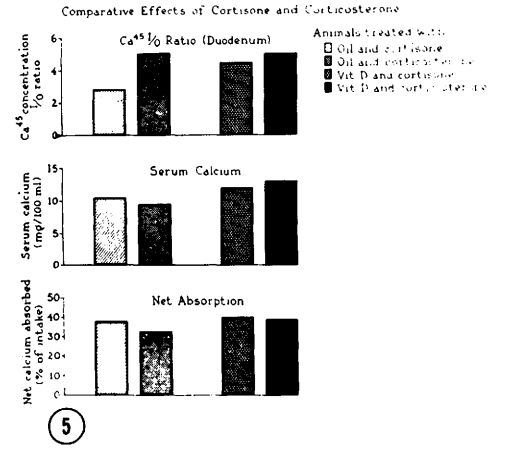


FIG. 1. Effect of Vit. D and/or cortisone on net calcium absorption and retention. Each bar represents mean value, with stand. error, for number of animals studied (in parentheses).

FIG. 2. Effect of Vit. D and/or cortisone on serum calcium levels. Each bar represents mean value, with stand. error for number of animals studied (in parentheses).

FIG. 3. Effect of Vit. D and/or cortisone on active transport of calcium. Dotted line indicates I/O ratio existing in all sacs before incubation. Each bar represents mean post-incubation value, with stand. error, for number of animals studied (in parentheses).

FIG. 4. Active transport of calcium from serial intestinal segments.

FIG. 5. Comparison of effects of cortisone and corticosterone on intestinal active transport, serum calcium level, and net calcium absorption.

was 30.4% and calcium retention was 26.0% of calcium intake for the control rats (Fig. 1). Absorption and retention were 32.1% and 24.2% respectively for the Vit. D-treated rats, neither being significantly different from those of the control rats. Absorption and retention were 42.1% and 33.7% respectively for the rats receiving Vit. D plus cortisone. Therefore, these rats absorbed significantly more calcium ($P<0.01$) than the control rats and those receiving Vit. D alone, and also retained more ($P<0.05$) than the latter group. Absorption and retention of the rats receiving cortisone alone were 35.1% and 32.8% re-

spectively, indicating greater retention ($P < 0.05$), but not greater absorption, than the control rats.

The mean serum calcium level (Fig. 2) of the control rats was 9.6 mg/100 ml and of the Vit. D-treated group was 12.0 mg/100 ml, indicating a statistically significant increase ($P < 0.001$). Concomitant administration of cortisone did not alter this hypercalcemia, nor did cortisone alone alter the serum calcium level from that of the control level.

As shown in Fig. 3 (which includes the initially reported studies(1) plus the current studies) active calcium transport determined *in vitro* was much greater in the duodenal segment than in the other 2 intestinal segments under all conditions studied. In the duodenal segment, the mean I/O ratio was 4.8 for the control rats and 7.1 for the excessive Vit. D-treated rats, indicating a statistically significant increase ($P < 0.001$) in calcium transport. Cortisone significantly decreased calcium transport of Vit. D-treated rats to 5.3 and of non-vitamin D-treated rats to 3.4 ($P < 0.001$ in both instances). Active transport in the distal control segments was much less, even in the adjacent segment, than in the first (duodenal) segment, and was insignificant in the 3rd and succeeding segments (Fig. 4). Active transport in the other treatment groups followed the same pattern of rapid decrease distal to the first intestinal segment. In the jejunal and ileal segments (Fig. 3), there was no calcium transport in the control rats, but significant transport ($P < 0.001$) in the Vit. D-treated rats. Cortisone did not depress the Vit. D-induced transport of these two segments.

Duodenal active transport of calcium was greater in the rats receiving corticosterone alone than in those receiving cortisone alone ($P < 0.01$); but there was no difference in active transport between the rats receiving Vit. D plus corticosterone and those receiving Vit. D plus cortisone. There was also no difference between corticosterone and cortisone in their effect on serum calcium levels and net calcium absorption (Fig. 5).

Discussion. The *in vivo* stable calcium absorption observations indicate that neither net absorption nor retention of calcium was significantly increased by excessive Vit. D, in spite of the development of hypercalcemia and increased urinary calcium. Therefore the source of the added serum calcium must have been extra-intestinal, presumably bone. On the other hand, cortisone plus Vit. D treatment resulted in an increase in calcium absorption.

There are apparent discrepancies between the *in vivo* absorption observations in the rat reported here and those in man reported by others(4,5,6), suggesting that species differences in calcium absorption may exist. However, many of the more recent studies of Vit. D and cortisone effects on calcium absorption in man have involved patients with hypercalcemia due to sarcoidosis. Though there is evidence that some of these patients have increased intestinal absorption of calcium and have apparent increased sensitivity to Vit. D (5,6), it has not been conclusively shown that these manifestations are the same as those in a person who has received excessive amounts of Vit. D. Also Jackson and Dancaster(7) have recently reported that a) large doses of Vit. D do not increase calcium absorption (in spite of increased serum and urinary calcium) in man, except those who are rachitic, and b) cortisone does not prevent or reverse the effects of excessive Vit. D while the latter is still being administered. Thomas and Morgan(8) have also reported the ineffectiveness of cortisone to modify Vit. D-induced hypercalcemia in the rat and in 1 out of 2 patients studied during the continued administration of Vit. D. By comparison with these reports (7,8) the net calcium absorption and serum calcium observations in the rat reported here are not discrepant with those in man.

As was initially reported(1) and confirmed in this study, in the presence of physiologic amounts of Vit. D, active calcium transport was demonstrable only in the proximal intestine, with marked reduction immediately distal to the duodenum. Excessive Vit. D increased active transport of calcium in the proximal segments, and initiated active transport in the more distal segments. Cortisone reduced calcium transport in the proximal segments, in the presence of either physiologic or excessive Vit. D. However, cortisone did

not decrease the Vit. D-induced calcium transport in the more distal segments.

Because corticosterone, rather than cortisone, is the physiologic adrenocortical hormone in the rat, the possibility of differences in response to these hormones was considered, and evaluated. However, corticosterone gave the same responses as those observed with cortisone in all aspects of this study in the presence of excessive Vit. D, and in all aspects except duodenal active transport, in the presence of physiologic amounts of Vit. D.

The present study therefore reveals apparent disparities between the *in vivo* net calcium absorption data and the *in vitro* active calcium transport data in the rat. This *in vitro* system has the advantages of eliminating bone and kidney effects on calcium metabolism and of allowing precise adjustment of the experimental conditions, and has been helpful in studying physiologic aspects of calcium movement across intestinal membranes (9-12). However, this intestinal sac technic has the disadvantage of being unphysiological, because a) the contribution of endogenous calcium to the intestinal contents *via* digestive juices is absent, b) removal of absorbed calcium from the intestinal wall *via* vascular channels does not exist, and c) calcium transfer through the entire thickness of the intestinal wall, rather than through the mucosa only, is measured. The *in vitro* method as used here, measures only active transport, rather than all processes which may be involved in calcium absorption. This active transport is limited to a small area of proximal intestine, where transit of intestinal contents is rapid and time available for absorption is short in the intact animal. Some of these factors are undoubtedly involved in the discrepancies between our *in vivo* and *in vitro* observations. Specific reasons for these discrepancies are not clear. No explanation is apparent for the increased calcium absorption and retention observed in animals under Vit. D plus cortisone treatment.

Summary. Calcium absorption studied *in vivo* was not increased by excessive Vit. D, nor decreased (actually increased) by concomitant cortisone or corticosterone administration. Active calcium transport studied *in vitro* was confined to the proximal small intestine, which was increased by excessive Vit. D and decreased by concomitant cortisone or corticosterone. Therefore, with the drugs, dosages, and time relationships used in this study, the *in vitro* intestinal sac technic is not considered a valid index of net calcium absorption in the intact animal.

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