Effects of Vitamin D and Cortisone on Intestinal Absorption of Calcium in the Rat. (26436)

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The importance of Vit. D in promoting intestinal absorption of calcium has long been recognized(1,2), but its mechanism of action remains unknown. The work of several investigators(1-4) strongly suggests that large doses of Vit. D can lead to excessive calcium absorption. However, other studies(1,2,5,6) indicate that calcium mobilization from bone is the predominant cause of the hypercalcemia of Vit. D overdosage. The hypercalcemia can be corrected by cortisone administration(7,8). Though this effect has been attributed(8,9), at least partially, to a decrease in intestinal calcium absorption, the mode of cortisone action in this situation has not been clearly defined.

The present study was designed to determine 1) whether large doses of Vit. D will increase intestinal calcium absorption above that induced by physiologic amounts, 2) whether cortisone will inhibit calcium absorption in presence of either physiologic or excessive amounts of Vit. D, 3) whether different areas of the intestine differ in ability to absorb calcium physiologically, and whether additional Vit. D and cortisone may affect absorption differently in various intestinal areas.

To eliminate any bone or kidney effects on calcium metabolism, segments of small intestine were studied *in vitro*, using a modification of the gut sac technic of Wilson and Wiseman(10). Similar technics have recently been used by others (11-14) for studying intestinal transport of calcium.

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 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 allowed *ad lib.* tap water and a nutritionally complete diet containing 1.68% calcium, 0.8% phosphorus and 148 units of Vit. D per 100 g of diet. On the 6th day of this regimen the rats were killed by decapitation. A 3 cm segment of small intestine was quickly removed from 3 different areas (duodenum, distal jejunum, distal ileum) and everted. A sac was prepared from each segment by tying both ends, after placing in the lumen 0.35 ml of a solution of the following composition: 0.148 M NaCl, 0.008 M sodium phosphate of pH 7.4, 0.02 M glucose, 4 x 10^{-5} M $CaCl_2$, and 0.02 μc/ml $Ca^{45}Cl_2$ (Oak Ridge Nat. Labs, specific activity $>$ 1000 mc/g). The filled sac was placed in an Erlenmeyer flask containing 2.5 ml of solution identical to that placed in the lumen. Because the mucosal surface was now on the outside and the serosal surface on the inside, any material moving from mucosal to serosal surface was collected inside the gut sac. The flask was aerated for 1 minute with oxygen, stoppered, and placed in a shaking incubator for 2 hours at 37°C. The sac was then removed from the bath, blotted of adherent outside solution, and the contents drained into a small tube. (Volume of fluid recovered in this manner was approximately 85% of that used to fill the sac for the duodenal segments and 95-105% for the jejunal and ileal segments.) Post-incubation Ca^{45} concentration was determined on equal aliquots of inside (serosal) and outside (mucosal) solution by drying on planchets and counting with a thin end window Geiger-

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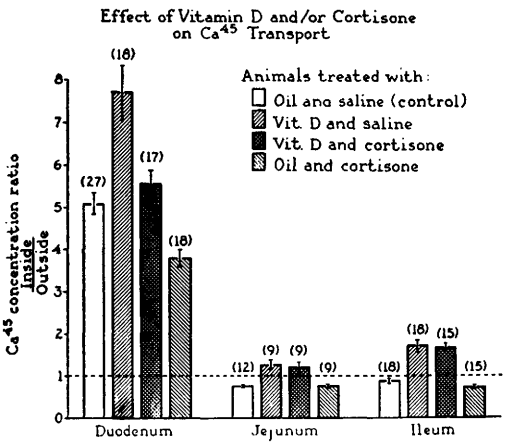

FIG. 1. Effects of vitamin D and/or cortisone on Ca^{45} transport. Dotted line indicates the I/O ratio existing in all segments before incubation. Each bar represents mean post-incubation value, with stand. error, for number of animals studied (indicated in parentheses).

Mueller tube. From these determinations, the Ca^{45} concentration ratio inside solution/outside solution (I/O ratio) was calculated. Since by design, the Ca^{45} I/O ratio was 1 before incubation, this ratio after incubation was used as a measure of transport of Ca^{45} through the intestinal wall during incubation period.

Results. Calcium transport was much greater in the duodenum than in the other 2 intestinal areas (Fig. 1). In the duodenal segment, the I/O ratio was 5.0 for control rats and 7.7 for 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 5.6, and of non-Vit. D-treated rats to 3.8 ($P < 0.001$ and $P < 0.01$ respectively). In the jejunal and ileal segments, there was no calcium transport in control rats, but significant transport ($P < 0.001$) in Vit. D-treated rats. Cortisone did not significantly depress the Vit. D-induced transport of these 2 segments.

Discussion. Since the present study demonstrates movement of calcium through the wall of the gut sac against a concentration gradient, and aerobic conditions are required by this experimental system(11), the observations indicate active calcium transport. This active transport of calcium was not uniform throughout the length of the small intestine. In the presence of physiologic doses of Vit. D, active transport was demonstrable in the duodenal segment only. (The I/O ratio of slightly less than 1 in jejunal and ileal segments of the control rats (Fig. 1) would suggest transport of calcium in the opposite direction. However, the slight dilution of Ca^{45} due to 1) mixing with tissue stable calcium and 2) the slight water absorption in these segments is considered a more likely explanation for the values being slightly less than 1). Large doses of Vit. D increased active calcium transport in the duodenal segment, and initiated active transport in jejunal and ileal segments. Cortisone reduced calcium transport in the duodenum, in the presence of either physiologic or excessive amounts of Vit. D. In contrast, cortisone did not reduce Vit. D-induced calcium transport in the jejunum and ileum.

The present observations do not indicate whether the variation in response of different areas of the intestine to Vit. D and cortisone is due to differences in tissue responsiveness, or to dissimilar mechanisms of action of these substances on different areas of the gut. The mechanism by which cortisone has an effect on calcium transport which is opposite to that of Vit. D is not defined. However, the variable relationship between these opposite effects in the different intestinal segments suggests that competitive inhibition is not an adequate explanation.

The importance of active transport for calcium absorption *in vivo* is not clear. Though calcium concentration of the solution used in the present study was much less than physiological, additional observations by us, and by others(11), indicate that active transport is demonstrable against a calcium concentration equal to that of normal plasma. Therefore, active transport of calcium *in vivo* is at least possible. In the normal intact animal, where calcium concentration of the intestinal contents is generally greater than that of extracellular fluid, absorption could occur by passive diffusion, but other mechanisms may well be involved. The authors suggest that active transport may at

least become an important mechanism when dietary calcium is limited and therefore an adequate gradient for calcium absorption by diffusion may not exist.

Summary. Using an *in vitro* gut sac technic, active transport of calcium through the intestinal wall of the rat has been demonstrated. In the presence of physiologic amounts of Vit. D, active calcium transport is confined to the upper portion of the small intestine. In this area, large amounts of Vit. D increase, and cortisone decreases, calcium transport. In the jejunal and ileal areas, active transport occurs only when large amounts of Vit. D are present, and this transport is not reduced by cortisone.

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Effect of Hydrocortisone on Herpes Virus-Infected HeLa Cells.* (26437)

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Certain of the effects of steroids on cell metabolism and growth of both normal and virus-infected cells have been studied in tissue culture systems(1-7), and the magnitude of effect of steroid on cellular growth has been found to be related to the origin of the cells. Holden and Adams(1) and Grossfeld (2) found a 40-50% reduction of growth rate of fibroblastic cells in tissue culture in presence of hydrocortisone, whereas Grossfeld and Ragan(3) found that similar treatment of cells of epithelial origin had no effect. Franklin and Sinclair(4) found similar inhibition of growth of fibroblastic cells but reported growth stimulation of epithelioid cells treated with prednisolone. The mechanism of growth inhibition of fibroblastic cells would appear to be associated with cytotoxic effects of steroid since Kline *et al.*(5) found that cell lines of connective tissue origin were much more susceptible to the cytotoxic effects of steroid than were cell lines of epithelial origin.

The effect of steroid on cell metabolism has been studied by Grossfeld(6) who found that hydrocortisone increased rate of glycolysis and O_2 uptake of mouse fibroblast cells (strain L). Similarly Fisher and Fisher(7) found that HeLa cells treated with cortisone had a greater rate of glycolysis than did control cells, although control cells utilized slightly more glucose than did the cortisone-treated. Infection of these cells(7) with herpes virus resulted in an increase in rate of

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