# Vitamin D Deficiency and Neutral Amino Acid Absorption in the Rat Intestine[1] (34835)

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Although vitamin D deficiency is characterized by impaired intestinal absorption of calcium, renal tubular reabsorption of neutral amino acids (1, 2) is also decreased. Intestinal and renal amino acid transport are similar in being (1) concentrative, saturable, and competitive; (2) showing optical and chemical specificity; and (3) dependant on sodium ion and energy (3). Certain congenital diseases are characterized by combined renal and intestinal amino acid transport defects (4, 5). These lines of evidence, in particular, the renal defect in amino acid transport in vitamin D-deficient rats, caused us to seek a similar defect in the small intestine. In addition, in a preliminary report (6) the findings were interpreted as showing decreased L-histidine absorption in everted sacs prepared from vitamin D-deficient rabbits. We investigated this problem *in vivo* using the rat, since data on the renal transport defect had been obtained by this type of study. To eliminate effects due to possible differences in amino acid metabolism, we used α-aminoisobutyric acid (AIB). AIB is a nonmetabolized neutral amino acid that participates in transport processes.

*Materials and Methods.* Vitamin D-deficient male albino weanling rats (Snell-Supplee strain) were obtained from Animal Resources, Baltimore, Maryland. They were depleted of vitamin D by feeding a rachitogenic diet (Rachitogenic Test Diet U.S.P., General Biochemicals, Chagrin Falls, Ohio) for 6 weeks. They were housed in a dark room and feed was handled with gloves. Vitamin D repletion was carried out 48 hr before the study. One of a pair of rats matched for weight was injected intramuscularly with 500 IU of vitamin $D_2$ (Cal Biochem, Los Angeles, Calif.) and the other rat received the same amount (0.25 ml) of the carrier solvent (1:5, alcohol:propylene glycol). The depleted and repleted rats were placed in separate cages and fasted, with water *ad libitum* for 24 hr before study. All rats were anesthetized with Dial-Urethane (0.07 ml/100 g body weight). The abdominal cavity was opened with a longitudinal incision. The duodenum was picked up and the bile duct was cut between double ligatures. The duodenal inlet cannula was inserted near the pylorus and the exit cannula about 15 cm distal to the ligament of Treitz. The intestine just proximal and distal to the cannulated segment was tied off. After flushing the segment with 50 ml of isotonic saline and 100 ml of air, it was replaced in the abdominal cavity. The edges of the abdominal wall were clamped together and covered with a gauze pad soaked with saline. Rats were perfused while lying on their backs on a heating pad. Rectal temperature was maintained between 36.5 and 37.5°. To measure α-aminoisobutyric acid (AIB) absorption a solution containing 159 m*M* sodium chloride, 9.30 g/liter; phenol red, 20 mg/liter; 5 m*M* nonlabeled AIB, 0.515 g/liter; and $^{14}$C-AIB (New England Nuclear, Boston, Mass., specific activity 4 mCi/mmole) 40 μCi/liter, was perfused through the cannulated gut segments. Two perfusion techniques were used: (1) single-pass continuous perfusion at a constant rate ranging between 0.16 and 0.18 ml/min for 1.5 hr using an Autoanalyzer pump (Model

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#206). In each rat individual effluent samples were collected at 30, 45, 60, 75, and 90 min after the start of perfusion. Phenol red analysis showed minimal (±5%) net fluid movement during perfusion. The loss in radioactivity from the perfusate during the last hour of perfusion was relatively constant and did not vary by more than 15% as measured from the $^{14}$C-AIB counts in the effluents obtained during that time. The 60- and 90-min effluents were taken as representative of the whole perfusion period, and the calculated AIB absorptions during these periods were added and the results were expressed in terms of μmoles per half hour. (2) In the second technique, a 10-ml volume of the above solution was recirculated through the segment at 1.5 ml/min for 1.5 hr (Bowman pump). After the perfusion blood was obtained from the inferior vena cava, the perfused intestinal segments were stripped from the mesentery and cut at the inlet and exit cannulae. Length of the perfused and unperfused small intestine was measured by placing the segments straight and under no tension on absorbant paper. The index fingers were placed over the center of the segment and gently pressed toward opposite ends to expel the contents. The perfused segment was rolled on the paper to remove the surface blood and weighed on a Torsion (Model B) balance to within 1 mg. It was then cut longitudinally and flattened on a glass plate with the mucosal surface upward. The mucosa was scraped off using the edge of an unbeveled microscope slide and transferred into preweighed porcelain crucibles and weighed as rapidly as possible. The underlying tissue was weighed in the same way. After drying in a vacuum oven for 24 hr at 85–90°, the tissues were weighed, dissolved in 3 ml of 1 *N* NaOH, neutralized with concentrated HCl, and diluted to 10 ml with water. Radioactivity was counted in a liquid-scintillation spectrometer (Beckman LS-250). Fifty-lambda aliquots of the initial solution and perfusates and 0.2 ml of plasma were added to plastic counting vials containing 1 ml of 0.05 *N* HCl. The diluted tissue solutions, 0.5 ml, were added to 0.5 ml of 0.05 *N* HCl in similar vials. A modified Bray's solution (7), 10 ml, was added to all vials. Counting efficiency was 85% for all samples. Phenol red was analyzed colorimetrically after alkalinization with a borate buffer (9). AIB absorption was calculated as follows:

$$\text{AIB absorption, cpm} = \quad (1)$$
$$V_i\left[(\text{cpm/ml})_i - (\text{cpm/ml})_f\left(\frac{PR_i}{PR_f}\right)\right]$$

$$\text{AIB absorption } \mu\text{moles} = \quad (2)$$
$$\frac{\text{AIB absorption, cpm}}{\text{initial specific activity}}$$

where: *cpm* is counts/min; AIB absorption, cpm is the total counts/min lost from the lumen; *V* is volume; *PR* is phenol red concentrations; the subscripts i and f refer to initial and final values; and initial perfusate specific activity is cpm/initial AIB concentration, m*M*.

*Results.* The means of body weights of all groups of rats were about 80 g or ⅓ that of normal rats of the same age. The means of the lengths of the perfused intestinal segments were about 20 cm and similar in all groups. Perfused segments of corresponding depleted and repleted groups were similar with respect to water content (about 80%) and wet weight/cm of the segment (38–40 mg/cm) and of the mucosal and underlying tissue fractions. AIB absorption rates and tissue concentrations in the perfused segments are shown in Table I. There were no statistically significant differences between the mean values of AIB absorption in the depleted and repleted rats in either perfusion or recirculation experiments. Absorption was about 30% lower with the single-pass perfusion. AIB tissue concentrations were the same in the depleted and repleted rats with both techniques. Blood AIB concentrations were about one-tenth that of the intestinal mucosal concentrations and the same in the depleted and repleted rats. Final AIB concentrations in the perfusing solutions were also not different in the depleted and repleted rats in either perfusion or recirculation experiments. The concentration gradient of AIB between perfusate, mucosa, underlying tissue, and plasma was downhill in the single-pass perfusion ex-

TABLE I. AIB Absorption and Tissue Concentrations in the Proximal Small Intestine. (mean ± SE)

| Study technique | Perfusion | | Recirculation | |
|---|---|---|---|---|
| Groups[a] (number of rats) | de (8) | de + D (8) | de (8) | de + D (8) |
| AIB absorbed during study period:[b] | | | | |
| μmole/g wet weight of segment | 7.9 ± 1.0 | 5.6 ± 0.5 | 30.9 ± 2.1 | 32.8 ± 2.9 |
| μmole/g wet weight of mucosa | 13.9 ± 0.2 | 10.5 ± 0.9 | 60.2 ± 4.8 | 66.2 ± 6.9 |
| μmole/cm segment length | 0.27± 0.04 | 0.23± 0.02 | 1.47± 0.14 | 1.46± 0.13 |
| AIB tissue concentration[c] | | | | |
| μmole/g mucosal wet weight | 3.3 ± 0.4 | 2.9 ± 0.3 | 3.4 ± 0.4 | 2.6 ± 0.3 |
| μmole/g underlying tissue wet weight | 2.0 ± 0.2 | 1.9 ± 0.2 | 2.0 ± 0.1 | 2.0 ± 0.2 |

[a] All rats were depleted of vitamin D (de), after which some were given vitamin D (de + D).
[b] Absorption is per 30 min for perfusion and per 90 min for recirculation.
[c] AIB tissue concentrations were measured at the end of the perfusion periods which lasted for 90 min in both experiments.

periment. In the recirculation experiment the concentration gradient was slightly uphill between perfusing fluid and the intestinal mucosa and downhill between intestinal mucosa, underlying tissue, and plasma.

*Discussion.* There are similarities in transport behavior of amino acids in the gastrointestinal tract and kidneys (3). For this reason in states of impaired renal amino acid transport there may be similar derangements in the intestinal tract. This occurs in certain inherited diseases such as cystinuria and Hartnup disease (4, 8). Hyperaminoaciduria due to defective tubular function has been noted in human rickets (1) as well as in experimentally induced vitamin D deficiency in rats (2) and rabbits (6). The present experiments were designed to determine whether there is a similar defect in the intestinal tract. This could explain in part the growth retardation in rachitic animals. In previous studies the absorption of L-histidine has been compared using everted sacs from vitamin D-deficient rabbits, normal rabbits, and normal rabbits treated with vitamin D (6). The analysis of transport kinetics showed an increased $K_m$ and decreased $V_{max}$ for L-histidine absorption in the intestinal sacs from rachitic animals as compared to normal controls. The rachitic rabbits also showed hyperaminoaciduria. However, the validity of comparing absorption by intestinal sacs of normal to those of rachitic rabbits is open to question. There are differences in growth and amino acid metabolism.

This is the first report of an *in vivo* study of amino acid absorption in vitamin D-deficient rats. Repleted rats were treated at the same time in exactly the same way except that they received vitamin D 48 hr previously. The age, body weight, and gut size were similar in the depleted and repleted rats. AIB absorption expressed as μmoles per gram segment wet weight or per cm length was not significantly changed by vitamin D (Table I). Tissue water content was similar for comparable groups, and when transport was calculated on the basis of dry weight, the data also showed no effect of vitamin D. Thus, under the experimental conditions vitamin D deficiency had no effect on AIB absorption.

We have previously studied intestinal AIB absorption in normal rats by the same techniques (10). During single-pass perfusion (0.2–0.3 ml/min) of an isotonic mannitol solution containing 5 mmoles AIB, the proximal 10–20% of the small intestine in normal rats absorbed 11–14 μmoles/hr/g of sement. In the present study the proximal intestine of the rachitic rat abosrbed 11–15 μmoles/hr/g of segment (Table I). In recirculation experiments, the absorption rates slightly exceed those in normal studies (10).

The current view of transport against an electrochemical gradient involves a carrier

protein molecule that is part of the membrane and is linked to energy sources of the cell. Calcium transport stimulation by a vitamin D metabolite appears to be via synthesis of protein(s) involved in transport (11, 12). Data of the present study are against a similar role for vitamin D in neutral amino acid transport.

*Summary.* Intestinal neutral amino acid absorption was studied in vitamin D-depleted and repleted rats using α-aminoisobutyric acid. Vitamin D had no effect on absorption or concentration of α-aminoisobutyric acid in intestinal tissue or plasma when two *in vivo* techniques, recirculation and perfusion, were used.

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