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The absence of an effect of vitamin B_6 deficiency on L-alanine transport across rabbit ileum *in vitro*

The possible role of vitamin B_6 in intestinal absorption of amino acids has been of considerable interest since the discovery by RIGGS *et al.*[1] that pyridoxal increases amino acid accumulation by Ehrlich mouse ascites tumor cells. Although there is evidence implicating pyridoxine and its derivatives in intestinal amino acid transport[2], it is unclear whether there is a direct involvement of these agents in the carrier-mediated transport process or an indirect influence on amino acid transport through their essential role in metabolic processes. Further, *in vivo* studies on the effect of vitamin B_6 deficiency on amino acid absorption cannot exclude the possibility that factors external to the absorptive epithelial cells are responsible for the observed inhibitions (*e.g.*, alterations in intestinal blood flow). Finally, in almost every instance "pyridoxine deficiency" was induced using inhibitory agents such as deoxypyridoxine[3] or L-penicillamine[4]. It is by no means clear that these agents mimic the effects of endogenous B_6 deficiency resulting from dietary restriction on intestinal transport of amino acids.

The purpose of the present communication is to present the results of an exploratory investigation into the effect of vitamin B_6 deficiency, produced by dietary restriction, on L-alanine transport across isolated segments of rabbit ileum.

New Zealand white rabbits were maintained on Purina Laboratory Rabbit Chow (Ralston Purina Co.) *ad libitum* for 1 month. 2 rabbits were then placed on Synthetic Rabbit Stock Colony Diet (General Biochemicals, No. 170970) that contained 20 μg/g pyridoxine and 6 rabbits were placed on the identical diet except that pyridoxine was omitted from the mixture. The assayed pyridoxine content of the pyridoxine-free diet was 0.05 μg/g. All rabbits were treated with a sulfathalidine–streptomycin–neomycin mixture for 5 days after the special diets were initiated to reduce intestinal bacterial flora, and were restrained in collars (kindly supplied by P. H. Derse) to prevent coprophagy.

The rabbits were killed by the intravenous injection of pentobarbital. A section of distal ileum was immediately excised, opened along the mesenteric border and rinsed free of intestinal contents with normal buffer. Transmural fluxes of L-alanine were determined under short-circuit conditions using the methods and apparatus that have been described in detail previously[5,6]. Briefly, a segment of the tissue was clamped as a flat sheet between two identical lucite half-chambers and both surfaces of the tissue were perfused and oxygenated using a water-jacketed gas-lift circulating system that maintained the bathing solutions at 37°. In all experiments the mucosal and serosal bathing solutions were identical and contained 5 mM alanine. Unidirectional alanine fluxes from mucosa to serosa (J_{ms}) and from serosa to mucosa (J_{sm}) were determined simultaneously on adjacent segments of ileum using L-[^{14}C]-alanine (New England Nuclear Corp.). Sampling of the initially unlabeled solution was begun 40 min after the addition of the [^{14}C]alanine to the opposite bathing solution and 6 samples were withdrawn at 15-min intervals. The delay in the initiation of sampling is sufficient to ensure a steady-state transmural flux of [^{14}C]alanine and in all instances the flux remained constant, within experimental error, throughout the experiment.

The composition of the buffer was: NaCl, 140 mM; $KHCO_3$, 10 mM; K_2HPO_4, 1.2 mM; KH_2PO_4, 0.2 mM; $CaCl_2$, 1.2 mM; and $MgCl_2$, 1.2 mM. The gas mixture employed was O_2–CO_2 (95:5, v/v) and the pH of the buffer was between 7.0 and 7.2.

The pyridoxine contents of the diets and blood samples were determined by the Wisconsin Alumni Research Foundation using the microbiological assay described by ATKIN *et al.*[7].

TABLE I

BLOOD B_6 LEVELS AND ALANINE FLUXES

Rabbit No.	*Weight change (g)*	*Blood B_6 (μg/ml)*	J_{ms}	J_{sm}	J_{net}
			($\mu moles/h \cdot cm^2$)		
1	+ 378	0.391	2.2	0.1	2.1
2	+ 425	0.229	2.5	0.2	2.3
3	− 795	0.124	2.1	0.1	2.0
4	− 322	0.096	2.1	0.2	1.9
5	−1413	0.077	3.0	0.2	2.8
6	− 773	0.065	2.5	0.2	2.3
7	− 214	0.041	2.4	0.2	2.2
8	− 560	0.023	2.5	0.3	2.2

The results of these exploratory studies are given in Table I. The two control rabbits, No. 1 and 2, were maintained on the synthetic diet with pyridoxine for 55 days, gained weight and had blood pyridoxine levels of 0.2–0.4 μg/ml whole blood. The values of J_{ms}, J_{sm}, and J_{net} observed for these animals are in good agreement with the many values for transmural L-alanine fluxes that we have determined on isolated rabbit ileum during the course of the past 4 years[6,8] and may be considered typical control values. Rabbits No. 3 and 4 were maintained on the pyridoxine-free diet for 32 and 33 days, respectively. These animals lost weight and their blood pyridoxine levels were markedly reduced compared to the control values. Rabbits No. 5–8 were maintained on the pyridoxine-free diet for periods of up to 54 days. At the time of sacrifice, 3 of these animals were in a moribund condition, had required feeding by nasogastric tube for several weeks, and had blood pyridoxine levels that were 10–25% of the average control values. The unidirectional and net transmural fluxes of L-alanine across segments of distal ileum obtained from the pyridoxine-deficient rabbits are equal to the control values within experimental error.

While it is admittedly hazardous to draw firm conclusions from exploratory studies involving relatively few animals, we feel that several points should be stressed. First, on the basis of weight loss, clinical state and blood pyridoxine assays there is no doubt that the rabbits maintained on the pyridoxine-free diet were in a state of moderate to severe pyridoxine deficiency. Indeed some of these animals would probably not have survived for 54 days were it not for the forced feeding. Second, there is not even the slightest suggestion of an impairment in the ability of *in vitro* segments of distal rabbit ileum to transport L-alanine from the mucosal solution to the serosal solution in the absence of a difference in concentration of the amino acid. We therefore have no reason to believe that a study involving many more animals would alter these findings.

Although it is certainly possible that a more severe vitamin B_6 deficiency would have resulted in impaired L-alanine transport it is doubtful that significantly greater depletion of pyridoxine can be produced in the rabbit by dietary restriction. We are forced to conclude that the ability of *in vitro* segments of rabbit ileum to transport L-alanine is not affected by chronic, severe pyridoxine deficiency.

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An estimation of the sodium and potassium equilibrium potentials in the muscle membrane of the earthworm, *Pheretima hawayana* R.

The South American earthworm, *Pheretima hawayana* R.[1], has been found to have a low resting potential, E_m, across its muscle membrane, maintained by the membrane conductances to more than one monovalent ion[2–4], spontaneous diphasic reversals of the E_m (refs. 2–4) and no electrical excitability[4]. In order to analyze these observations, the membrane behavior in response to the ionic environment, especially to that of Na^+ and K^+, must be studied. This report is limited to the estimation of the theoretical equilibrium potentials caused by the passive distribution of these two