The Effect of Phenobarbital on Intestinal Calcium Transport[1] (36471)

EDWARD L. KRAWITT AND PATRICIA A. STUBBERT
(Introduced by B. Forsyth)
Department of Medicine, College of Medicine, University of Vermont, Burlington, Vermont 05401

The mechanisms involved in the absorption of iron and calcium by the small intestine are similar (1, 2). Both ions are transported by a two-step process including uptake at the mucosa and transfer to or toward the serosal surface which involves simple diffusion and carrier mediation and is in part dependent upon oxidative metabolism. In addition, both are more avidly absorbed in the duodenum in comparison to more distal segments of the small intestine, and the absorption of both metals is increased when body stores are deficient.

Since it has recently been shown that phenobarbital enhances the intestinal transport of iron (3, 4), the experiments described in this report were undertaken to determine the effect of phenobarbital administration on the transport of calcium by the rat small intestine.

Methods. Albino male rats weighing approximately 100 g were fed standard laboratory chow (Ralston–Purina Company, vitamin D content 5.31 IU/g diet) and tap water *ad libitum*. Weanling male rats were raised on a vitamin D-free diet (General Biochemicals, rachitogenic diet #2, USP) and demineralized water *ad libitum* for 5 weeks. All animals were housed in hanging cages in a windowless room with automatically controlled temperature and the lighting regulated so as to provide alternating periods of 12 hr of light and dark daily. Animals were injected with 80 mg/kg body wt of Na phenobarbital (USV Pharmaceutical) intraperitoneally daily for 5 days prior to study. Control animals were injected with equal volumes of 0.155 *M* NaCl. When vitamin D_2 was administered, 500 IU were given intragastrically through a blunt needle inserted through the esophagus 40 hr prior to study. Animals were killed by cervical dislocation or decapitation and the intestine was exposed through a midline abdominal incision. The proximal 10–12 cm of intestine was used for the duodenal segment and the distal 15 cm of small intestine for the ileal segment.

Calcium transport was measured by a method based on the *in vitro* everted gut-sac technique of Wilson and Wiseman (5) as adapted by Schachter and Rosen (6). After the bile duct was ligated and transected its point of insertion into the duodenum, the segments of intestine used were rinsed *in situ* with iced 0.119 *M* NaCl, then dissected free, trimmed and everted over chilled glass rods. Sacs of 5 cm in length were filled with 0.5 ml of a medium consisting of 0.125 *M* NaCl, 0.010 *M* fructose, 0.00025 *M* $CaCl_2$, 0.030 *M* Tris–Cl, pH 7.4, and sufficient $^{45}CaCl_2$ to provide approximately 25,000 cpm/ml of medium. Sacs were incubated at 37° with continuous bubbling of 100% O_2 for 90 min in 25-ml Erlenmeyer flasks, which contained 10 ml of an identical medium. At the termination of incubation, the sacs were removed, blotted and drained. Aliquots (0.1 ml) of the medium inside and outside of the sacs were counted in 10 ml of liquid scintillation fluid containing 2,5-diphenyloxazole as a primary scintillator and α-naphthylphenyloxazole as a secondary scintillator in polyethylene vials in a spectrometer (Packard Tri-Carb) using automatic external

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TABLE I. Effect of Phenobarbital Administration on Intestinal Calcium Transport.[a]

Group	No.	Mean wt ± SE (g)	Mean serum calcium ± SE (m*M*)	Mean ^{45}Ca concentration ratio (S/M) ± SE	
				Duodenum	Ileum
Control	9	114 ± 9	2.2 ± .1	4.7 ± 0.6	0.6 ± 0.3
Phenobarbital	9	109 ± 3	2.2 ± .1	5.4 ± 0.6	0.7 ± 0.2
p		> .1	> .1	> .1	> .1

[a] Animals were fed normal laboratory chow and tap water. Phenobarbital (80 mg/kg body wt) was administered ip daily for 5 days prior to study. *S/M*, serosal/mucosal. The *p* values are calculated from a nonpaired *t* test.

standardization. The ratio of the final concentration after 90 min of the tracer inside the sac (serosal medium), over that outside the sac (mucosal medium), written *S/M* was used as the measure of calcium transport.

Serum calcium concentrations were determined by atomic absorption spectrometry.

Results. Results of experiments performed on rats fed standard laboratory chow are summarized in Table I. The administration of phenobarbital did not significantly alter body weight, serum calcium, or *S/M* ratios when comparing the results of the phenobarbital treated rats to those of control animals. The effects of an identical treatment regimen on duodenal transport in rats raised on vitamin D-deficient diet are shown in Table II. Phenobarbital administration resulted in a depression of the *S/M* ratio when compared to vitamin D-deficient controls. The administration of phenobarbital to rachitic rats who received vitamin D 40 hr prior to study resulted in an insignificant increase in the *S/M* ratio when comparing the rates of transport to those in animals who received only vitamin D.

Discussion. In contrast to previous results reported for intestinal iron transport (3, 4), the results of our experiments demonstrate that the transport of calcium by the rat duodenum and ileum are not significantly affected by the administration of phenobarbital in animals raised on a diet adequate in calcium and vitamin D. The administration of phenobarbital to animals raised on a vitamin D-deficient diet, however, resulted in a decrease in duodenal transport although it did not interfere with the expected rise in calcium transport when animals similarly fed were administered vitamin D intragastrically prior

TABLE II. Effect of Phenobarbital on Duodenal Calcium Transport in Rats Raised on a Vitamin D-Deficient Diet.[a]

Group	No.	Mean wt ± SE (g)	*p*	Mean ^{45}Ca concentration ratio (*S/M*) ± SE	*p*
Without vitamin D administration					
D Deficient	14	69 ± 2		2.4 ± 0.2	
			> .1		< .05
D Deficient + phenobarbital	10	67 ± 4		1.7 ± 0.2	
With vitamin D administration					
D Deficient + vitamin D_2	6	70 ± 2		3.7 ± 0.5	
			> .1		> .1
D Deficient + phenobarbital + vitamin D_2	11	66 ± 3		4.2 ± 0.6	

[a] Animals were fed a vitamin D-deficient diet and de-ionized water for 5 weeks. Phenobarbital (80 mg/kg body wt) was administered ip daily for 5 days prior to study. Vitamin D_2 (500 IU) was given intragastrically 40 hr before study. *S/M*, serosal/mucosal. *p* Values are calculated from a nonpaired *t* test.

to study.

The mechanisms underlying this diminished duodenal calcium absorption in the vitamin D-deficient rats are not known. Since the effects were not noted in rats raised on a diet adequate in vitamin D nor in those to whom vitamin D was administered intragastrically prior to study, it appears that the effect is mediated independent of vitamin D and does not represent interference with the action of vitamin D or one of its more active polar metabolites, although Hahn and co-workers (7) have recently demonstrated that phenobarbital administration accelerates the conversion of vitamin D to its metabolites by microsomal liver fractions. This decrease in duodenal calcium transport produced by phenobarbital corresponds in part to that of the cortisone treatment which also diminishes the transport of both calcium and iron in duodenal gut sacs by a mechanism not dependent on vitamin D (8).

The mechanism underlying the previously reported increase in iron transport produced by phenobarbital administration is also unknown, although the hypertrophy of smooth endoplasmic reticulum seen in the duodenum of phenobarbital treated rats (3, 4) suggested that microsomal enzyme induction could be involved at the intestinal level. Nevertheless, these studies indicate that despite the similarities between the duodenal transport mechanisms of calcium and iron, they are affected differently by phenobarbital administration.

Summary. To determine the effect of phenobarbital administration on intestinal calcium transport by the rat intestine, experiments were performed using an *in vitro* everted gut sac technique as a measure of transport. The results demonstrate that duodenal and ileal transport are not affected by phenobarbital administration in rats raised on a diet adequate in calcium and vitamin D. Duodenal absorption, however, is suppressed in animals raised on a vitamin D-deficient diet. The administration of phenobarbital does not interfere with the expected rise in calcium transport when vitamin D is administered to vitamin D-deficient animals. The depression of transport produced by phenobarbital in vitamin D-deficient animals appears to be mediated independent of the vitamin D pathway.

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