

## Effect of Dietary Magnesium Deficiency on Calcium Transport by the Rat Small Intestine<sup>1</sup> (36824)

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The effect of dietary magnesium deficiency on calcium homeostasis in the rat is complex and is dependent not only on the magnitude and duration of magnesium deprivation but on the maturity of the animal as well as dietary calcium, phosphorus and vitamin D intake (1). Unlike most species, in which magnesium restriction results in hypocalcemia, the rat becomes hypercalcemic (2, 3). If calcium as well as magnesium is restricted, however, hypocalcemia will result (4). Studies of the effect of magnesium deficiency on the intestinal absorption of calcium by the rat have resulted in apparently conflicting results (5–8) which may be accounted for in part by the experimental conditions selected (9). The results of the present investigation, which was undertaken to observe the effects of dietary deprivation on calcium transport by the rat small intestine, indicate that hypercalcemia can develop in the absence of an increased ability of the intestine to absorb calcium. These data suggest that the hypercalcemia may be mediated by a mechanism independent of an adaptive increase in intestinal calcium absorption.

**Methods.** Albino male Sprague-Dawley weanling or 100 g rats (Charles River Breeding Laboratories, Brookline, MA) were fed a diet deficient in magnesium (0.004%) but containing adequate calcium, phosphorus and vitamin D (General Biochemicals, Chagrin Falls, OH) for 10 days. Control animals were fed an identical diet with magnesium added so as to provide 0.053% magnesium. *Ad libitum* control animals were permitted free ac-

cess to the control diet. Pair feeding was accomplished by offering daily an amount of control diet equal to that consumed by experimental animals during the preceding 24 hr. All animals were permitted free access to demineralized water and were housed in hanging cages in a windowless room with automatically controlled temperature and the lighting regulated so as to provide alternating daily periods of 12 hr of light and dark. They were killed by decapitation and blood was collected for determination of serum calcium and magnesium by atomic absorption spectrometry. The intestine was exposed through a midline abdominal incision and the proximal 10–12 cm was used for the duodenal segment. When transport was studied in more distal locations, the segment 20–30 cm distal to the pylorus and the most distal 10–15 cm of small intestine were used for jejunal and ileal segments, respectively.

Calcium transport was measured by the *in vitro* gut sac technique of Wilson and Wiseman (10) as adapted by Martin and DeLuca (11). After the bile duct was ligated and transected at its point of insertion into the duodenum, segments were rinsed *in situ* with iced 0.119 M NaCl, dissected free, trimmed and everted over glass tubes. Sacs of 5 cm in length were filled with 0.5 ml of a medium consisting of 0.125 M NaCl, 0.01 M fructose, 0.00025 M CaCl<sub>2</sub>, 0.030 M Tris-Cl (pH 7.4), and sufficient <sup>45</sup>CaCl<sub>2</sub> to provide approximately 25,000 cpm/ml of medium. Sacs were incubated at 37° with continuous bubbling of 100% oxygen for 90 min in 25 ml Erlenmeyer flasks, which contained 10 ml of an identical medium. At the termination of incubation, sacs were removed, blotted and drained. Aliquots (0.1 ml) of the medium

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TABLE I. Effect of Dietary Magnesium Deficiency on Serum Magnesium and Calcium.<sup>a</sup>

Group	No.	Serum concn (mM)	
		Mg	Ca
Magnesium deficient	20	0.49 ± 0.01	2.55 ± 0.06
Ad lib. controls	10	1.06 ± 0.05 <sup>b</sup>	2.24 ± 0.06 <sup>c</sup>
Pair fed controls	9	0.96 ± 0.03 <sup>b</sup>	2.31 ± 0.07 <sup>b</sup>

<sup>a</sup> Values represent means ± SE. Differences between mean of magnesium deficient group and control group are significant at: <sup>b</sup>  $p < .01$  or <sup>c</sup>  $p < .02$ . Differences between ad libitum and pair fed controls are not significant.  $p$  values are calculated from a nonpaired  $t$  test.

inside and outside of the sacs were counted in 10 ml of liquid scintillation fluid containing 2,5-diphenyloxazole as a primary scintillator and alpha-naphthylphenyloxazole as a secondary scintillator in polyethylene vials in a spectrometer (Packard Tricarb) using automatic external standardization. The transport data are expressed as a ratio of final concentration after incubation of the tracer, inside of the sac (serosal medium) over that outside the sac (mucosal medium), written S/M.

**Results.** Results of determination of serum calcium and magnesium are listed in Table I. Magnesium deprivation resulted in significantly decreased magnesium and increased calcium levels in serum compared to either *ad libitum* or pair fed controls. There was no difference between the two control groups. As illustrated in Table II, in rats weighing approximately 100 g at the onset of magnesium deprivation, magnesium deficiency resulted in an insignificant decrease in the ability of the duodenum to transport calcium as compared to *ad libitum* controls. Because starvation *per se* may result in a decreased level of duodenal calcium transport (12), an experiment was performed utilizing pair fed controls. No significant difference in S/M ratios could be demonstrated (Table II). To examine whether or not magnesium deficiency might produce an increase in ability of more distal segments to transport calcium against concentration gradient weanling rats were fed a magnesium deficient diet for 10 days

and transport ratios were measured in duodenal, jejunal and ileal segments. Although pair fed, the control animals gained more weight than the experimental animals. Duodenal S/M ratios of these immature rats were higher than those of older animals but no change was evident comparing magnesium deficient to control rats in any of the segments studied (Table III).

**Discussion.** Dietary magnesium deficiency in the rat results not only in hypomagnesemia but produces hypercalcemia, hypophosphatemia, and hyperphosphaturia, findings associated with hyperparathyroidism (2, 3). Hypercalcemia and hypophosphatemia are dependent on the presence of intact parathyroid glands while the hyperphosphaturia is independent of parathyroid control (3). Because of this picture resembling hyperparathyroidism, it has been postulated that one of the mechanisms by which the rat develops hypercalcemia, when deprived of dietary magnesium, may be via increased intestinal absorption of calcium mediated by parathyroid hormone. Alternatively, it is possible that parathyroid hormone mediates this effect through the kidney and/or bone.

Studies on the effect of magnesium deficiency on calcium absorption in rat small intestine, however, have produced conflicting results (5-8). In *in vivo* experiments, Alcock and MacIntyre (5) demonstrated a decrease in fecal calcium excretion in magnesium deficient rats. Kessner and Epstein (6), studying

TABLE II. Effect of Dietary Magnesium Deficiency on Duodenal Calcium Transport.<sup>a</sup>

Group	No.	Wt (g)	<sup>45</sup> Ca concn ratio (S/M)
Expt 1			
Magnesium deficient	10	106 ± 2	4.0 ± 0.4
Ad lib. controls	9	133 ± 3	5.4 ± 0.4
$p$		<.001	>.05
Expt 2			
Magnesium deficient	20	112 ± 3	4.2 ± 0.2
Pair fed controls	20	116 ± 5	4.6 ± 0.4
$p$		>.05	>.05

<sup>a</sup> Values represent means ± SE.  $p$  values are calculated from a nonpaired  $t$  test. S/M, serosal/mucosal.

TABLE III. Effect of Dietary Magnesium Deficiency on Intestinal Calcium Transport.<sup>a</sup>

Group	No.	Wt (g)	<sup>45</sup> Ca concn ratio (S/M)		
			Duodenum	Jejunum	Ileum
Magnesium deficient	9	89 ± 3	6.8 ± 0.6	1.2 ± 0.1	1.0 ± 0.1
Pair fed controls	9	101 ± 3	6.5 ± 0.7	1.6 ± 0.2	1.3 ± 0.1
<i>p</i>		<.02	>.05	>.05	>.05

<sup>a</sup> Values represent means ± SE. *p* values are calculated from a nonpaired *t* test. S/M, serosal/mucosal.

the effect of magnesium deficiency in weanling rats, using an *in vitro* everted gut sac technique as a measure of transport, demonstrated an increase in ability of the duodenum to transport calcium compared to pair fed controls. Morehead and Kessner (7) extended those observations using an open-ended gut sac technique and found that the increase in duodenal absorption was abolished by parathyroidectomy. Lifshitz, Harrison and Harrison (8), however, employing everted intestinal loops using different experimental conditions, did not show a difference in calcium transport in rats deprived of magnesium compared with *ad libitum* fed animals. The results of our experiments, using the everted gut sac technique, in weanling rats and in those weighing approximately 100 g at the onset of magnesium deprivation, compared to *ad libitum* or pair fed controls, also failed to demonstrate an increase in small intestinal transport to account for the hypercalcemia produced by dietary magnesium deficiency. This suggests that the hypercalcemia may be mediated independent of an adaptive increase in intestinal calcium transport.

It also should be noted that investigations directed toward elucidation of the mechanism of hypocalcemia, which results from dietary magnesium deficiency in all species studied except the rat, also have produced conflicting results. Recent studies by Dunn (13) in the monkey and Suh, Csima and Fraser (14) in the dog, however, indicate that end organ unresponsiveness to parathyroid hormone or increased calcitonin secretion are not the mechanisms involved in those species. In this regard, it is of interest that in rats made hypocalcemic by simultaneous dietary calci-

um and magnesium restriction, magnesium deficiency apparently does reduce calcium release from bone and inhibits the action of parathyroid hormone on bone resorption (2, 15).

*Summary.* To determine the effect of dietary magnesium deprivation on intestinal calcium transport by the rat intestine, experiments were performed using an *in vitro* everted gut sac technique as a measure of transport. Despite the development of hypercalcemia, no increase in the ability of the intestine to transport calcium was demonstrated. The results suggest that the hypercalcemia may be mediated by a mechanism independent of an adaptive increase in intestinal calcium absorption.

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1. Aikawa, J., "The Relationship of Magnesium to Disease in Domestic Animals and in Humans." 145 pp. Thomas, Springfield, IL (1971).
2. Heaton, F. W., Clin. Sci. **128**, 543 (1965).
3. Gitelman, H. J., Kukolj, S., and Welt, L. G., J. Clin. Invest. **47**, 118 (1968).
4. MacManus, J., and Heaton, F. W., Clin. Sci. **36**, 297 (1969).
5. Alcock, N., and MacIntyre, I., Clin. Sci. **22**, 182 (1962).
6. Kessner, D. M., and Epstein, F. H., Proc. Soc. Exp. Biol. Med. **122**, 72 (1966).
7. Morehead, R. M., Jr, and Kessner, D. M., Amer. J. Physiol. **217**, 1608 (1969).
8. Lifshitz, F., Harrison, H. C., and Harrison, H. E., Proc. Soc. Exp. Biol. Med. **125**, 19 (1967).
9. Wasserman, R. H., and Taylor, A. N., in "Mineral Metabolism" (C. L. Comar and F. Bronner, eds.), Vol. 3, p. 344. Academic Press, New York (1969).
10. Wilson, T. H., and Wiseman, G., J. Physiol.

- (London) 123, 116 (1954).
11. Martin, D. L., and DeLuca, H. F., *Amer. J. Physiol.* 216, 135 (1969).
12. Kessner, D. M., and Epstein, F. H., *Amer. J. Physiol.* 209, 141 (1965).
13. Dunn, M., *Clin. Sci.* 41, 333 (1971).
14. Suh, S. M., Csima, A., and Fraser, D., *J. Clin. Invest.* 50, 2668 (1971).
15. MacManus, J., Heaton, F. W., and Lucas, P. W., *J. Endocrinol.* 49, 253 (1971).

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