Effect of Magnesium Deficiency on Gastrointestinal Transfer of Calcium.* (31236)

DAVID M. KESSNER† AND FRANKLIN H. EPSTEIN‡
Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Experimental magnesium deficiency usually results in characteristic disturbances in calcium metabolism manifested by hypercalcemia, nephrocalcinosis and generalized metastatic calcification(1,2). The mechanism of the hypercalcemia is obscure, though there is some physiological evidence of a similarity in transport and binding between the two divalent cations, calcium and magnesium.

The present experiments suggest that increased absorption of calcium from the intestine contributes to the calcium accumulation of magnesium deficiency. Studies of calcium transport by everted sacs of rat intestine show that when animals are depleted of magnesium, the capacity of the intestine to transport calcium against a concentration gradient from mucosa to serosa is augmented.

Methods. Weanling male CD conventional§ rats were used in all experiments. The animals were housed individually, allowed free access to water and fed a synthetic diet (3) devoid of magnesium but containing optimal concentrations of calcium, phosphorus, and vitamin D_2. Control animals were pair-fed the same diet containing magnesium (8 mg/100 g diet). Intestinal transport of calcium and magnesium was measured employing the everted gut sac(4,5). Animals were killed by decapitation and blood was collected in heparinized tubes. A segment of proximal duodenum 3-4 cm long was rapidly excised, everted, and washed thoroughly in a solution containing 0.9% NaCl and 0.3% glucose. The tissue was bathed in cold 0.146 M NaCl, 0.004 M KCl solution while being handled. The inside of the sac (serosal surface) was filled with .4 to .6 ml of the following solution: NaCl 0.151 M, fructose 0.02 M, $CaCl_2$ 0.001 M, Ca^{45} .02 μc/ml solution (Ca^{45} (P-3) Oak Ridge National Laboratory) and tris (hydroxyl methyl) aminomethane .004 M, with the final pH of the solution adjusted to pH 7.4 with 1 N HCl. Within 7-9 minutes after excision, the filled sac was placed in a flask containing 2.5 ml of the same solution used to fill the sac and incubated for 3 hours in the Warburg apparatus gassed with 100% O_2. Oxygen consumption was measured after equilibration and at the end of the incubation period the content of stable and radioactive calcium or Mg^{28} was determined in the inside and the outside fluids.

Ca^{45} was counted using a Packard Tri-Carb Liquid Scintillation spectrometer with a naphthalene-dioxane-PPO liquid scintillator (6). Stable calcium was measured by a modification of the EDTA titration technique of Fales(7,8). When magnesium transport was determined .001 M $MgCl_2$ and 1 μc/ml of Mg^{28} (Brookhaven National Laboratory) was added to the ambient fluid used for calcium transport studies. Mg^{28} was counted in a Picker Well Scintillation Counter with a Spectroscaler III A. At all times the ratio of counts/background was greater than 10:1.

Stable Mg was measured in intestinal tissue by digesting 25-50 mg of tissue in 2 ml of concentrated HNO_3 and determining the magnesium content of a diluted aliquot by atomic absorption spectrophotometry, using a Perkin-Elmer model #214(9).

In this system, as pointed out by Schachter and co-workers(5), calcium is accumulated on the serosal side of the intestinal sac so that a concentration gradient for ionized Ca^{++} is created between serosal (S) and mucosal (M) fluids. The process by which the gradient is formed involves a rapid uptake of Ca^{++} from the mucosal side by intestinal tissue and a slower release of Ca^{++}

* Aided by grants from U.S.P.H.S. (AM-06397, HE-00834, AM-5015) and Connecticut and New Haven Heart Assns.

† During the tenure of a U.S.P.H.S. post-doctoral fellowship.

‡ Assisted by Career Research Award 1-K6-AM-21,578, U.S.P.H.S.

§ Charles River Breeding Laboratories, Brookline, Mass.

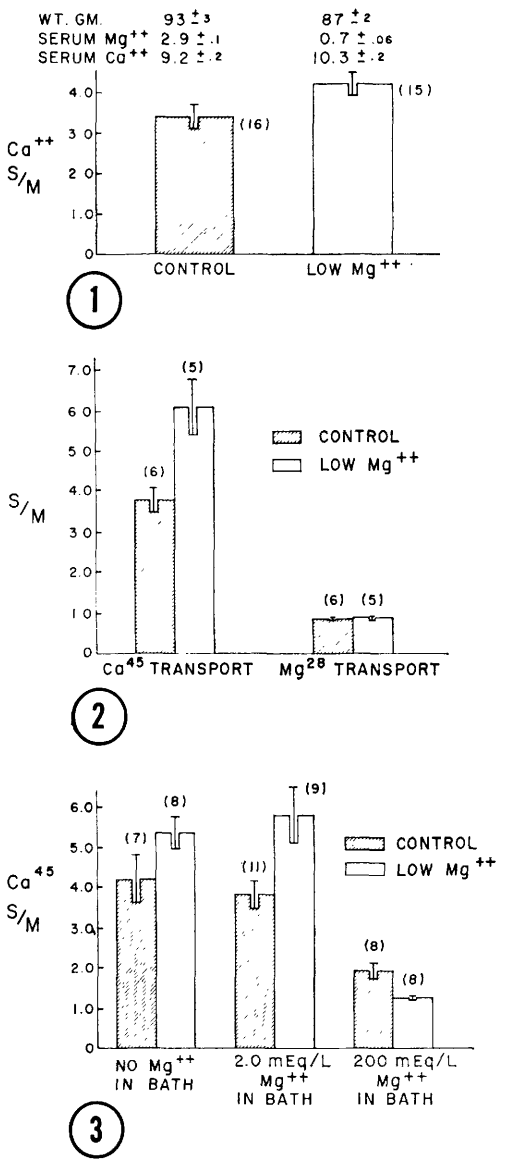

FIG. 1. Calcium transport by duodenal gut sacs of control and magnesium-deficient rats. Results are expressed as the ratio of concentration of Ca^{++} in the serosal (S) fluid to concentration in the mucosal (M) fluid. Number of animals in each group indicated in parenthesis. The bar represents standard error (SE) of mean. Wt refers to weight of the rats (mean ± S.E.). Total serum Mg and serum Ca expressed in mg per 100 ml. S/M Ca^{++} control = 3.4 ± .3, low Mg^{++} = 4.2 ± 3, P = <.05.

FIG. 2. Effect of dietary restriction of magnesium on transport of Ca^{45} and Mg^{28} in the everted duodenal sac. Number of animals indicated in parenthesis. S/M indicates concentration in serosal/mucosal fluid. The bar represents SE of mean. Magnesium deficiency increased Ca^{45} S/M but did not change S/M for Mg^{28}. S/M Ca^{45} control = 3.8 ± .3, low Mg^{++} = 6.1 ± .7, P = <.025.

FIG. 3. Effect of added magnesium on calcium transport by everted duodenal sacs of control and magnesium-deficient rats. Number of animals in each group indicated in parenthesis. Addition of 2 meq/l Mg^{++} did not change S/M for Ca^{45}, though 200 meq/l Mg^{++} depressed calcium transport.

to the serosal medium(5,10). The volume of fluid within the sac (serosal surface) tends to increase slightly in the course of the incubation, but in the experiments to be reported these changes were small (10-15%) and did not differ from one experimental group of animals to another. With equal initial concentrations of Ca^{45} on both sides of the intestinal wall, therefore, a final S/M ratio greater than 1.0 indicates net accumulation against a concentration gradient from the mucosal surface to the serosal surface. Changes in this gradient have been taken, in the discussion to follow, to indicate changes in the process by which calcium is translocated across the intestinal wall and concentrated on the serosal side. Because S/M ratios for calcium vary with age and total dietary intake(4,5), experimental groups must be compared, as in the present experiments, with pair-fed controls of the same age.

Results. Calcium transport in magnesium-deficient rats. Weanling rats fed a magnesium-free diet ate poorly and did not grow at a normal rate. They developed hyperemia between the 4th and 6th day of magnesium deficiency, hyperirritability and trophic skin ulcers between the 7th and 9th day and 10% of the animals had convulsions and died by the end of 2 weeks. After 10 days of magnesium deficiency the animals demonstrated striking hypomagnesemia, and a mild increase in serum calcium (Fig. 1). Despite these changes the magnesium content of the intestine itself was unaltered (Table I). Oxygen consumption of the everted gut sacs was unchanged by magnesium deficiency (Table II).

The serosal/mucosal (S/M) concentration ratio for calcium[45] and stable calcium was consistently increased in everted intestinal sacs from magnesium-depleted rats when compared to pair-fed controls (Fig. 1-3, Table I and II). The increase varied from 24-60%

TABLE I. Serum Mg, Mg Content of Duodenal Gut Sacs and Ca^{++} S/M Ratios in Control and Mg-Deficient Rats.

	No. of rats	Serum Mg, mg/100 ml	Mg content of intestinal tissue, mg/100 g dry wt	Ca^{++} S/M ratio
Control	8	2.9 ± .1 *	83.3 ± 3.3	3.4 ± .15
Low magnesium	8	.7 ± .06	87.6 ± 2.7	4.5 ± .34
P		<.01	N.S.	<.02

* Values are mean ± S.E.

TABLE II. Effect of Magnesium Deficiency on Ca^{++} S/M Ratio, Oxygen Consumption, and Bulk Transfer of Calcium by Everted Duodenal Gut Sacs.

Group	No. of rats	Ca^{++} S/M	O_2 consumption, ml O_2/hr/mg dry wt	Δ Inside Ca^{++},† μeq Ca^{++}/g dry wt
Control	16	3.4 ± .27*	12.8 ± 1.1	29.9 ± 3.5
P		<.05	N.S.	<.05
Low magnesium	15	4.2 ± .27	12.6 ± .9	40.2 ± 2.3

* Mean ± S.E.

† Calculated as follows: Final sac vol (ml) × final sac conc $\left(\frac{\mu eq\ Ca^{++}}{ml}\right)$ — Initial sac vol (ml) × initial sac conc $\left(\frac{\mu eq}{ml}\right)$ = Δ Inside Ca^{++} (μeq).

of the control S/M ratio with P values of less than .05 in each experiment. Net accumulation of Ca^{++}, measured by the increase in amount of stable calcium in the fluid within the sac per gram dry weight was similarly increased by magnesium deficiency (Table II).

Magnesium transport. The accumulation of magnesium by everted gut sacs was studied in 6 control and 5 magnesium-depleted rats, by measuring the S/M ratio of Mg^{28}. Simultaneous measurements were made of the concentration ratio of Ca^{45}. Neither normal nor magnesium-deficient animals were able to maintain a concentration ratio of Mg^{28} greater than 1.0 (average S/M ratio = 0.85), and there was no evidence of net bulk transfer of Mg^{28} from mucosa to serosa (Fig. 2). Although magnesium deficiency considerably increased the S/M ratio of Ca^{45}, it did not change the concentration ratio of Mg^{28}.

Effect of magnesium added to the bathing medium on calcium transport in vitro. Magnesium chloride was added to a final concentration of 2 meq/l to the serosal and mucosal fluids of gut sacs from normal and magnesium-depleted rats, in order to approximate the concentration of magnesium in normal serum. The addition of magnesium did not reduce the elevated Ca^{45} S/M ratio of the magnesium-deficient groups; in fact, the Ca^{45} ratio was affected in neither control nor magnesium-depleted animals (Fig. 3). When the concentration of magnesium bathing the sacs was increased 100-fold to 200 meq/l, on the other hand, marked inhibition of calcium accumulation was noted in both control rats and those depleted of magnesium. A similar effect of high concentrations of Mg^{++} has been reported by others(5,11).

Discussion. In 1909 Mendel and Benedict suggested a relationship between calcium and magnesium metabolism(12) after showing that infusions of calcium or magnesium augmented the urinary excretion of the other cation. Early studies of the effect of alterations in magnesium content of the diet also demonstrated that rats deprived of magnesium excreted less calcium in the stool and had an increased carcass content of calcium(13). These findings were confirmed by Tufts and Greenberg(2) and observed in dogs by Kruse, Schmidt and McCollum(14). Further studies of the effect of magnesium deprivation on calcium metabolism were carried out by MacIntyre and Davidsson who observed that magnesium-deficient rats are hypercalcemic, and develop nephrocalcinosis(1). This is associated with decreased excretion of calcium in

the feces and the urine(15,16).

The present experiments demonstrate clearly that magnesium deficiency increases the ability of isolated intestinal sacs to concentrate calcium against a serosal/mucosal gradient. The increased Ca^{45} S/M ratio produced by magnesium deficiency was not the result of a decreased intake of food, since control animals were pair-fed with the experimental ones. Furthermore, restriction of growth *per se*, whether produced by dietary restriction or by aging, appears to diminish, rather than augment, calcium absorption by the intestine (4,5).

Enhanced transport of calcium in magnesium deficiency might conceivably result from a direct interaction between these 2 divalent ions and an enzyme system responsible for their transfer across cell membranes. Certain microsomal and mitochondrial ATPases are stimulated by calcium as well as magnesium (15,16) but there is no evidence that these enzymes are involved in transcellular movement of the cations. Another possibility is that the effects of dietary magnesium deficiency are due to changes in intestinal enzyme transport systems resulting from the prolonged treatment. Such changes in enzyme quantity or function in the transport system of the gut wall could be of sufficient magnitude to prevent recovery when Mg^{++} was again provided *in vitro*.

Still another possible explanation for an effect of magnesium deficiency on intestinal transport of calcium involves the action of parathyroid hormone. Magnesium deprivation in the rat appears to stimulate reabsorption of calcium by the renal tubule as well as the intestine(17,18), to increase the ionized calcium of serum(19) and to increase the renal clearance of phosphate(18,20-22). All these consequences of magnesium deficiency can be simulated by infusions of parathyroid hormone. Administration of parathyroid hormone to rats has been reported to enhance the transport of calcium by their isolated gut sacs(23). Finally, the hypercalcemia of magnesium deficiency is dependent on the presence of intact parathyroid glands(24,25). It is thus conceivable that the increased intestinal absorption of calcium in magnesium deficiency, exemplified in the present experiments, is the result of an increase in the secretion of parathyroid hormone.

Summary. Experimental magnesium deficiency in rats is associated with an increased ability of the isolated everted intestine to transfer calcium against a concentration gradient. The augmented calcium accumulation of magnesium deficiency is not altered by replacement of magnesium in the bath. Although a direct effect of magnesium depletion on cellular transport mechanisms is not ruled out, the results are consistent with a humoral influence, like that of parathyroid hormone, on the gut in magnesium deficiency.

The authors gratefully acknowledge the technical assistance of Mrs. Lorraine Pozika.

1. MacIntyre, I., Davidsson, D., Biochem. J., 1958, v70, 456.
2. Tufts, E. V., Greenberg, D. M., J. Biol. Chem., 1937, v122, 693.
3. Manitius, A., Epstein, F. H., J. Clin. Invest., 1963, v42, 208.
4. Kessner, D. M., Epstein, F. H., Am. J. Physiol., 1965, v209, 141.
5. Schachter, D., Dowdle, E. B., Schenker, H., ibid., 1960, v198, 263.
6. Bray, G., Analyt. Chem., 1960, v1, 279.
7. Copp, D. H., J. Lab. Clin. Med., 1963, v61, 1029.
8. Fales, F. W., J. Biol. Chem., 1953, v204, 577.
9. Zettner, A., Seligson, D., Clinical Research, 1963, v9, 406.
10. Schachter, D., Dowdle, E. B., Schenker, H., Am. J. Physiol., 1960, v198, 275.
11. Hendrix, J. A., Alcock, N. W., Archibald, R. M., Clin. Chem., 1963, v9, 734.
12. Mendel, L. B., Benedict, S. R., Am. J. Physiol., 1909, v25, 1.
13. Medes, G., J. Biol. Chem., 1926, v68, 295.
14. Kruse, H. D., Schmidt, M. M., McCollum, E. V., ibid., 1934, v106, 573.
15. Taylor, C. B., Biochim. Biophys. Acta, 1962, v60, 437.
16. Carafoli, E., Rossi, C. S., Lehninger, A. L., J. Biol. Chem., 1964, v239, 3055.
17. Alcock, N., MacIntyre, I., Clin. Sci., 1962, v22, 457.
18. Welt, L. G., Yale J. Biol. Med., 1964, v36, 324.
19. Welt, L. G., Gitelman, H. Disease-a-Month, May 1965, p9-10.
20. MacIntyre, I., Boss, S., Troughton, V. A., Nature, 1963, v198, 1058.

21. Smith, W. O., Baxter, D. H., Lindmer, A., Ginn, H. E., J. Lab. Clin. Med., 1962, v59, 211.
22. Ko, K .W., Fellers, F. X., Craig, J. M., Lab. Invest., 1962, v11, 294.
23. Rasmussen, H., Endocrinology, 1959, v65, 517.
24. Heaton, F. W., Clin. Sci., 1965, v28, 543.
25. Gitelman, H. J., Kukolf, S., Welt, L. G., Clin. Res., 1966, vXIV, 63.

Received February 25, 1966. P.S.E.B.M., 1966, v122.

Mercaptoethanol-Sensitive Neutralizing-Antibody in Natural Infection With Coxsackievirus B5.*† (31237)

REI OHTA-HATANO AND YORIO HINUMA (Introduced by E. A. Mirand)
Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan

Many investigations have shown an early appearance of 19 S antibody in animals including man either artificially immunized or naturally infected with viruses(1-7). In most studies the 19 S antibody proved sensitive to treatment with 2-mercaptoethanol (2-ME). The early appearance of 2-ME sensitive antibody seemed to provide a valuable indicator for detecting recent infection with certain viruses. However, there have been only few reports on appearance of 2-ME sensitive antibody during natural virus-infections in man (2,6).

The present communication describes the development of 2-ME sensitive and resistant neutralizing-antibody in sera obtained from children and infants infected with Coxsackievirus B5 and discusses the diagnostic significance of detecting the 2-ME sensitive antibody at an early stage of infection.

Materials and methods. Sera. Serum specimens were obtained from patients of aseptic meningitis caused by Coxsackievirus B5 in the 1961 epidemic in Aomori City(8,9). The patients from whom sera was obtained had been determined as Coxsackievirus B5-infected by virus isolation and/or serodiagnosis. Sera at acute and convalescent phases, together with sera obtained 2 years after the illness(10) were examined. All sera were stored at —20°C and then inactivated at 56°C for 30 minutes on the day of use.

Determination of neutralizing antibody. The virus used was the AM-L-69-63 strain which was a representative strain of Coxsackievirus B5 isolated in the 1961 epidemic (8). Neutralization tests were performed by the conventional tube test method employing HeLa (S_3) cell monolayers. Two-fold serial dilutions of serum or fractionated serum in 0.01 M phosphate buffered saline of pH 7.2 (PBS) were mixed with equal volume of 100 TCD_{50}/0.1 ml of virus and then incubated at 4°C overnight. After incubation, 0.2 ml of the mixture was inoculated into duplicate tubes and incubated at 37°C for 4 days. Titer of neutralizing antibody was expressed as reciprocal of highest serum dilution which protected cells completely.

Treatment with 2-mercaptoethanol (2-ME). One to two dilutions of whole serum in PBS or fractionated serum was mixed with equal volume of 0.2 M 2-ME made in PBS. The mixtures were incubated at 37°C for 2 hours and then dialyzed against PBS at 4°C for 2 days in order to remove 2-ME. Both untreated and treated samples of single serum were tested simultaneously.

Sucrose gradient centrifugation. Zonal density ultracentrifugation was accomplished in a 40P Hitachi Centrifuge employing a swinging bucket rotor and a preformed gradient of sucrose ranging from 10 to 37%. Sera (about 0.5 ml) were layered over the gradient. Centrifugation was carried out for 15 hours at 35,000 r.p.m. About 20 fractions of approximately 0.25 ml were obtained by a puncture

* Aided in part by a grant from the Ministry of Education of Japan.

† Based on a portion of a dissertation submitted by R. Ohta-Hatano to the Graduate Division of Tohoku University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medical Science.