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Divalent Cation Transport by Rat Cecum and Colon in Calcium and Magnesium Deficiency[1] (39778)

M. MICHAEL PETITH,[2,3] AND H. P. SCHEDL

Gastroenterology Research Laboratories, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

Although gastrointestinal transport and metabolism of calcium and magnesium are interrelated, these relationships have not been precisely defined and mechanisms involved are poorly understood. In the rat, the large intestine responds to dietary calcium restriction by markedly enhanced calcium absorption (1) and is also the major site of magnesium absorption (2). Since the large intestine may be important in calcium and magnesium homeostasis, we sought to clarify interrelationships between these two cations with respect to their transport by large intestine. We measured effects of magnesium deficiency on calcium transport and of calcium deficiency on magnesium transport in cecum and colon of the rat.

Materials and methods. Magnesium deficiency study. Each of several shipments of male albino rats (Sprague-Dawley derived, Simonsen Laboratories, Gilroy, Calif.) weighing 95-170 g was randomized into two weight-comparable groups which were housed individually in metabolic cages and pair-fed a 0.002% magnesium (low Mg) diet or a 0.07% (normal Mg) diet. These diets were otherwise identical, containing adequate amounts of calcium (0.6%), phosphorus (0.5%), and vitamin D (6000 IU/kg) with dextrose as carbohydrate source (No. 170490, General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio).

Calcium deficiency study. Simonsen rats weighing 110-185 g were randomized into two weight-comparable groups which were caged separately in sets of up to six rats and allowed to eat *ad libitum*. Pilot studies done in conjunction with previous experiments on calcium transport by small (3) and large (1) intestine showed no difference in *ad libitum* consumption of normal Ca and low Ca diets. These diets (TD-73361 and TD-73362, General Biochemicals) were identical [0.9% phosphorus, 0.2% magnesium, vitamin D_2 (3000 IU/kg), 65% sucrose as carbohydrate source] except for calcium (0.02%, low Ca diet; 1.2%, normal Ca diet).

Perfusion technique. After consuming the respective diets and demineralized water for about 3 weeks, animals were fasted for 16-20 hr with free access to water prior to study and were then anesthetized by intraperitoneal injections of Nembutal (50 mg/kg/rat), supplemented as needed. The abdominal cavity was entered through a longitudinal incision and the ileocecal valve was ligated. Three incisions were made: into the tip of the cecum, 1-1.5 cm beyond the ileocecal valve, and 2-2.5 cm above the anus; a proximal and a distal segment were thus made accessible for study and termed cecum and colon, respectively. Contents were expressed gently and segments were flushed with saline and then air. Into the open ends of each of the two segments, an inlet and an outlet cannula were inserted and tied in, which were connected by polyvinyl tubing to a reservoir containing an initial volume of 10 ml of perfusate. Reservoirs were recirculated for 2 hr at 4 ml/min by use of a proportioning pump (Model 1, Technicon Instruments Corp., Chauncey, N.Y.). In both studies, two to four rats from the same shipment and including both diet groups were perfused on each day of study with one of several solutions. Perfusion solutions for the

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[3] Send reprint requests to M. Michael Petith, M.D., Department of Internal Medicine, University of Iowa Hospitals, Iowa City, Iowa 52242.

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magnesium deficiency study had initial calcium concentrations of 0.4, 0.8, and 1.2 m*M*; in the calcium deficiency study, perfusion solutions had initial magnesium concentrations of 0, 0.25, or 2.5 m*M* magnesium. All solutions were isotonic with saline and contained phenol red (50 mg/liter; magnesium deficiency study) or [^{14}C]polyethyleneglycol (PEG; calcium deficiency study) as indicator of net water movements.

Processing and analysis of samples. After perfusion, animals were killed by transecting both diaphragms. Perfused segments were cut at the inlet and outlet cannulas and the ileocecal valve and gently stripped from the mesentery. Segments were cut longitudinally and, in the case of the cecum, also incised radially and spread onto a 5 × 7 index card; from the outline, each surface area was measured at least twice by planimetry (compensating polar planimeter, No. 620005, Keuffel and Esser Co.). Full thickness wet weights and, after a 24-hr drying period in a vacuum oven (Model 19, Precision Thelco) at 100°, full thickness dry weights were obtained to 0.0001 g on a Sartorius (Type 2258, Westbury, N.Y.) balance. Test solutions and perfusates were processed for determination of ^{40}Ca by atomic absorption spectrometry (Model 303, Perkin-Elmer), for determination of phenol red by spectrophotometry (4) (Beckman DU with Gilford modification "Model 222-G" with rapid sampler), or for determination [^{14}C]PEG by liquid scintillation counting (Beckman LS-250). Net calcium movements in percentages and per hour were calculated from the difference between initial and final concentrations of ^{40}Ca and were adjusted for net water movements based on initial and final indicator concentrations. In each animal, a blood sample was obtained from the tail after induction of anesthesia and from the inferior vena cava after termination of perfusion; the mean serum ^{40}Ca and magnesium values were calculated from both samples. All samples were done in duplicate. For statistical analysis, the unpaired Student *t* test was used for all comparisons; when comparing data from cecum and colon in the same animals, the paired Student *t* test was applied.

Results and discussion. Magnesium deficiency–Calcium absorption study. Rats taking the low Mg diet developed hypomagnesemia and hypercalcemica, and, in spite of pair-feeding, did not gain as much weight as rats taking a normal Mg diet. Except for colonic wet weight, corresponding gut parameters do not differ significantly between diet groups (Table I). The rates of net calcium movements shown in Fig. 1 are based on full-thickness dry weight. In groups perfused with 0.4 and 0.8 m*M* calcium, net secretion is seen, being always higher in colon than in cecum, and lower at 0.8 than at 0.4 m*M* in the colon. Segment specificity is also found when 1.2 m*M* calcium is perfused: In control rats, absorption is higher in cecum than in colon, and in magnesium deficient rats, net absorption is noted in the cecum and net secretion in the colon. With the 0.4 and 0.8 m*M* Ca perfusate, mean cecal and colonic secretory rates were consistently higher in magnesium-deficient than in control animals, but differences were not statistically significant. Similarly, mean cecal absorption from 1.2 m*M* was slightly lower in deficient than in control rats; in colon, controls showed minimal absorption, and deficient rats secretion ($P > 0.05$). The minimal changes of net calcium movements in response to magnesium deficiency in the *in situ* model might be related to the altered chemical gradient between serum and lumen due to hypercalcemia (Table I). However, our observations are similar to *in vitro* data, where equal initial ^{40}Ca concentrations with tracer ^{45}Ca were present in mucosal and serosal media (5). In this study, serosal/mucosal ^{45}Ca concentration ratios after incubation of everted sacs of jejunum and ileum for 90 min were slightly lower in rats fed a low magnesium diet for 10 days as compared with those in rats fed a control diet, but differences were not significant for either segment. In contrast, other investigators studying rats after the same period of magnesium restriction, i.e., 10 days, with Ussing-type chamber preparations (6), observed higher mucosa-to-serosal calcium transport in the duodenum of magnesium-deficient rats compared with controls; they reported return to normal of calcium transport rates in rats deprived of magnesium for 19 days, but an age-matched

TABLE I. ANIMAL, SERUM, AND LARGE INTESTINAL DATA.[a]

	Low Mg diet	Normal Mg diet	Low Ca diet	Normal Ca diet
Body weight, unfasted (g)				
Initial	121.7 ± 2.5	119.3 ± 2.4	150.0 ± 4.5	144.9 ± 4.8
Final	188.9 ± 2.5[b]	234.2 ± 3.1	262.0 ± 8.2	269.5 ± 7.4
Serum (mmole/liter)				
Calcium	2.45 ± 0.02[b]	2.28 ± 0.01	2.15 ± 0.03[c]	2.25 ± 0.03
Magnesium	0.52 ± 0.01[b]	1.01 ± 0.02	1.36 ± 0.05	1.23 ± 0.04
Cecum				
Surface area (cm^2)	11.0 ± 0.6[d]	10.7 ± 0.4	13.8 ± 0.7[e,f]	12.0 ± 0.5[g]
Full-thickness weight (g)				
Wet	0.62 ± 0.04[g]	0.56 ± 0.02[g]	0.88 ± 0.06[e,g]	0.72 ± 0.04[g]
Dry	0.129 ± 0.007[d]	0.124± 0.004[g]	0.181 ± 0.010[g]	0.166 ± 0.009[g]
Colon				
Surface area (cm^2)	12.6 ± 0.7	10.9 ± 0.6	10.9 ± 0.7	10.1 ± 0.4
Full-thickness weight (g)				
Wet	0.48 ± 0.03[h]	0.40 ± 0.01	0.48 ± 0.03	0.49 ± 0.03
Dry	0.099 ± 0.006	0.088 ± 0.004	0.115 ± 0.006	0.123 ± 0.007

[a] Mean values ± SE in growing rats taking low Mg diet (0.002% magnesium), normal Mg diet (0.06% magnesium), low Ca diet (0.02% calcium), or normal Ca diet (1.2% calcium) for 3 weeks. (n = 26–30 for animals taking the low or normal Mg diet; n = 21–24 for animals taking the low or normal Ca diet).

[b] $P < 0.001$ compared with normal Mg diet.

[c] $P < 0.02$ compared with normal Ca diet.

[d] $P < 0.005$ compared with colon.

[e] $P < 0.05$ compared with normal Ca diet.

[f] $P < 0.01$ compared with colon.

[g] $P < 0.001$ compared with colon.

[h] $P < 0.01$ compared with normal Mg diet.

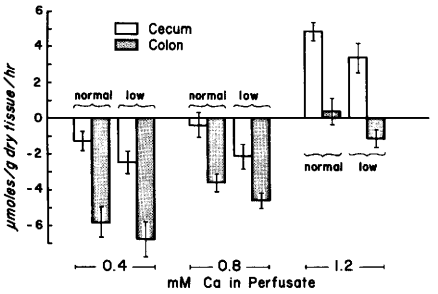

FIG. 1. Net cecal and colonic calcium movements (mean ± SE; n for each mean = 6–10) in growing rats taking a 0.002% (low Mg) or 0.07% (normal Mg) magnesium diet and perfused *in vivo* with 0.4, 0.8, or 1.2 m*M* calcium in saline. In all groups, rates are segment specific ($P < 0.05$ for difference between cecum and colon). When comparing rats taking a low with those taking a normal magnesium diet, differences are not significant.

control group was apparently not studied. Large intestinal adaptation was not evaluated in either paper on small intestinal transport. In the latter study (6), magnesium content of the control diet was much higher (0.24%) than in the current experiments (0.07%) as well as in the previous everted sac study (0.05%) (5). Therefore, dietary magnesium well in excess of the nutrient requirements of the rat (0.04%) (7) may in part account for the differences observed. In the current experiments, serum calcium was elevated in magnesium-deficient rats compared with rats taking the 0.07% magnesium diet (Table I); thus, calcium metabolism was clearly disturbed. Balance studies showing decreased fecal calcium excretion of rats after up to 3 weeks of magnesium restriction suggest altered intestinal calcium transport, but do not localize the site of adaptation (8). These findings disagree with the lack of adaptation of either large (current study) or small intestine (6) after a similar length of magnesium restriction; however, comparison with either study may not be valid because of the different magnesium content of the control diet (0.17%) used in the balance experiments.

Calcium deficiency—Magnesium absorption study. Rats taking low calcium diet grew as well as those taking the normal calcium diet (Table I). In comparison with control rats, calcium-deficient rats had a lower serum calcium content but the difference in serum magnesium was not signifi-

cant, and gut parameters were generally not different (Table I). Rates of net magnesium movement in Fig. 2 and of net calcium movement in Fig. 3 are based on full-thickness dry weight. Net magnesium secretion is noted when a magnesium-free solution (0 m*M*) or 0.25 m*M* magnesium are perfused; net absorption is seen from the 2.5 m*M* magnesium perfusate. In contrast to net calcium movements (Fig. 1), net magnesium movements did not differ between cecum and colon for individual perfusates (except in rats taking the low Mg diet, when 0.25 m*M* magnesium is perfused), and thus are generally not segment specific in our model. Thus, cecal and colonic magnesium transport does not respond to dietary calcium deficiency. Therefore, results of balance

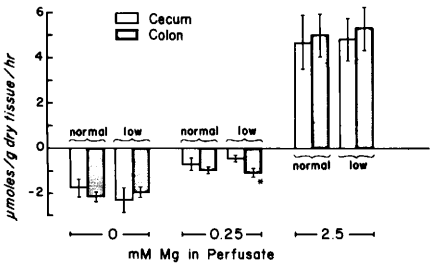

FIG. 2. Net cecal and colonic magnesium movements (mean ± SE; *n* for each mean = 5–7) in growing rats taking a 0.02% (low Ca) or 1.2% (normal Ca) calcium diet and perfused *in vivo* with 0, 0.25, or 2.5 m*M* magnesium in saline. With the 0.25 m*M* magnesium perfusate, net secretion in colon is greater than in cecum of rats taking a low calcium diet as compared with controls ($P < 0.05$); otherwise there is no segment specificity and rats do not adapt significantly to low calcium diet.

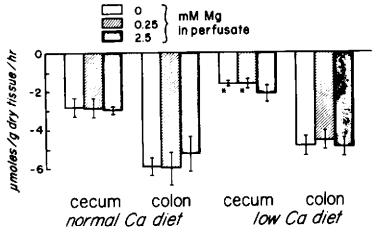

FIG. 3. Net cecal and colonic calcium secretion (mean rates ± SE; *n* for each mean = 5–8) in growing rats taking a 0.02% (low Ca) or 1.2% (normal Ca) calcium diet and perfused *in vivo* with 0, 0.25, or 2.5 m*M* magnesium. Net calcium secretion is lower in rats taking the low calcium diet for colon perfused with 0 and 0.25 m*M* Mg (asterisk; $P < 0.05$), but is not influenced by the intraluminal magnesium concentration of the perfusate in either segment.

studies showing decreased fecal magnesium excretion in calcium deficiency (8) cannot be explained on the basis of large intestinal adaptation, although the large bowel has been reported to be the major site of magnesium absorption in the rat (2). Possibly, changes of small intestinal magnesium transport may account for the previous findings, but such data do not appear to be available at this time. Net calcium secretion into the lumen of the large intestine (Fig. 3) was higher in colon than cecum in all groups and with all perfusates, but was not significantly affected by intraluminal magnesium concentration. In the cecum, calcium secretion into the 0 or 0.25 m*M* Mg perfusate was lower in calcium-deficient than in control rats, whereas differences seen with the 2.5 m*M* Mg perfusate and in the colon with any perfusate were not significant.

The current studies examined calcium and magnesium transport by the large intestine *in vivo*, evaluating the transport of each divalent cation under conditions of depletion and sufficiency of the other. In this model, we are unable to document an effect of magnesium deficiency on calcium transport or of calcium deficiency on magnesium transport. These findings are of interest because of the reported importance of large intestinal magnesium transport in the rat (2), the response of cecal and colonic calcium transport to dietary calcium restriction (1), and the dependence of colonic calcium transport on vitamin D (9). Vitamin D dependence of cecal calcium transport has not thus far been examined. However, while our data are basic for delineating the pattern of divalent cation transport by large intestine, further experiments systematically examining both small and large intestine under the same conditions are needed to localize effects of dietary restriction of one cation on the intestinal transport of the other and correlate transport data with balance studies.

Summary. The effects of calcium and magnesium deficiency on net divalent cation movements of large intestine were studied in growing rats by *in vivo* perfusion of cecum and colon. Magnesium-deficient rats and controls were perfused with 0.4, 0.8, and 1.2 m*M* calcium in saline; large intestinal calcium movements were similar in both diet groups despite hypercalcemia in

magnesium-deficient animals. In a separate study, calcium-deficient rats and controls were perfused with 0, 0.25, and 2.5 m*M* magnesium in saline; large intestinal magnesium movements were the same in both diet groups. Thus, large intestinal calcium transport in the rat, which adapts to calcium or vitamin D deficiency, does not respond to magnesium restriction, and large intestinal magnesium transport is not affected by calcium deficiency. These findings suggest that effects of magnesium and calcium restriction on fecal excretion of divalent cations in balance studies are not mediated by large intestinal adaptation.

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