Net Movements of Magnesium and Calcium in the Rat Small Intestine *in Vivo*[1] (34376)

ERNEST URBAN[2] (by invitation) AND HAROLD P. SCHEDL
With the technical assistance of Renee Owen

Gastroenterology Research Laboratory, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52240; and Veterans Administration Hospital, Iowa City, Iowa 52240

Magnesium absorption has been studied in the rat small intestine by *in vitro* and *in vivo* techniques (1-4). These studies showed that the absorption rate of magnesium was greater in ileum than duodenojejunum. Specific gut segments have been investigated *in vitro* (1, 2), but *in vivo* the behavior of the entire small intestine was examined by studies of the balance type (3, 4). Since there are no reports of *in vivo* absorption rates per unit weight of segment under controlled conditions we compared duodenojejunal and ileal magnesium adsorption and secretion as well as tissue concentrations.

Methods. Male albino rats (Simonsen strain, wt 300–425 g) fed normal laboratory chow (magnesium 0.2%; calcium 1–2%) served as experimental animals. Food but not water was withheld for 24 hr before study. Four to six rats were studied simultaneously on a given day.

Anesthesia was produced by 0.7 ml/kg intraperitoneal of Dial with urethane solution (Ciba). A midline abdominal incision was made, the common bile duct was ligated and transected and inflow and outflow cannulas were inserted into the proximal 10–12 cm of small intestine. The inflow cannula was positioned just distal to the pyloric sphincter and the outflow cannula was 2–4 cm distal to the ligament of Treitz, thus isolating the lumen of this segment from the remaining intestine. A 10–15-cm segment of the terminal ileum was similarly cannulated with the outflow cannula 1–2 cm proximal to the cecum. These segments will be referred to as duodenum and ileum. After inserting the cannulas the abdominal skin was clamped shut and body temperature of the rat was maintained at 36–38° with a heating pad. The segments were washed with 0.9% NaCl and flushed with air. The lumen of each segment was then perfused by recirculation, at a rate of 2 ml/min for 2 hr from reservoirs containing 12 ml of solution, using a proportioning pump (Technicon). The perfusion solution, adjusted to pH 6.5 with HCl, contained 20 m*M* tris(hydroxymethyl)aminomethane (Tris) (THAM, Sigma Chemical Co.), 0.2% polyethylene glycol (Carbowax 4000) and either 2.5 m*M* or 0.25 m*M* magnesium chloride or zero magnesium. NaCl was added to a calculated total osmolarity of 300. The perfusates, which will be referred to as high Mg, low Mg or zero Mg, contained no calcium prior to perfusion.

After perfusion, the animals were exsanguinated through the inferior vena cava; the segments of intestine were flushed with air, excised, and any remaining content was expressed by gently pressing the tissue flat on absorbent paper. Segments were weighed to 1 mg on a torsion balance, dried in a vacuum oven at 80° for 24 hr, reweighed and then incinerated in a muffle furnace at 500° for 24 hr. The ash was dissolved in 0.5 ml 12 *N* HCl and diluted to 5 ml with distilled water. Magnesium and calcium were determined by atomic absorption spectrometry (Perkin-Elmer model 303) on aliquots of the tissue solution. Aliquots of the perfusate reservoir

[1] This work was supported in part by Training Grant T_1 AM 5390 and Research Grant AM 02354 from the National Institutes of Health.

[2] Present address: Department of Physiology and Internal Medicine, The University of Texas Medical School at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229.

TABLE I. Net Movements of Magnesium and Calcium in the Rat Small Intestine[a] (μmoles/2 hr/g of wet intestine).

Mg conc of perfusate (mM)	Magnesium			Calcium[c]	
	Duodenum	Ileum	$p <$[b]	Duodenum	Ileum
2.5	14.8 ± 0.7	10.0 ± 0.7	0.001	−1.92 ± 0.15	−2.48 ± 0.22
0.25	−0.58[d] ± 0.15	−0.90[d] ± 0.06	NS	−1.94 ± 0.23	−2.34 ± 0.12
0	−1.88 ± 0.14	−1.72 ± 0.05	NS	−1.98 ± 0.18	−2.42 ± 0.12

[a] Groups of 12 rats were studied with each perfusate containing magnesium. The group perfused with zero magnesium comprised 15 animals. Data are mean values ± 1 SE; (+) values denote net absorption from the intestinal lumen; (—) values are net secretion into the lumen.

[b] Comparisons of duodenal and ileal magnesium movement.

[c] The means are not significantly different from each other.

[d] Differs significantly ($p <0.001$) from corresponding zero Mg values.

contents and serum were similarly analyzed. Polyethylene glycol, an indicator of water movement, was analyzed turbidimetrically by an automated modification of the method of Hyden (5). All analyses were performed in duplicate and the average was used for calculations.

"Absorption," designated by positive numbers, is net movement of magnesium out of the gut lumen; "secretion," designated by negative numbers, is net movement of magnesium (or calcium) into the luminal fluid. The following equation was used to calculate absorption and secretion rates:

Net movement (μmoles/g of wet intestine)

$$= \frac{V[X_i - (X_f)P_R]}{W} \text{ for the 2-hr period,}$$

where X_i and X_f are the initial and the final concentrations of magnesium or calcium (μmoles/ml) of the luminal solution; P_R is the ratio of initial to final polyethylene glycol concentration; V is the initial volume of perfusion solution (ml); and W is the wet weight of the perfused segment (g).

Results are given as mean values ± one standard error. The Student's t test, analysis of variance and Tukey's w-procedure were used in the statistical treatment of data (6). Probability values of less than 0.05 were considered to indicate significant differences.

Results. Table I summarizes data on magnesium absorption and secretion and calcium secretion per gram of wet intestine. Data calculated using dry weight of intestine show similar relationships. Perfusion with the high Mg perfusate resulted in absorption of magnesium from the luminal fluid. Absorption was greater in duodenum than ileum during the 2-hr perfusion period. There was secretion of magnesium from the rat into the intestinal lumen with low Mg and zero Mg perfusates. Magnesium secretion was greater with zero Mg than with low Mg perfusate. In contrast to magnesium absorption from the high Mg perfusate, the secretion of magnesium was not significantly different in duodenum and ileum. Net secretion of calcium was unaffected by the magnesium content of the perfusate. The mean calcium secretory rates were lower in duodenum than ileum, but analysis of variance for six means shows that the differences were not significant.

Tissue concentrations of magnesium and calcium in duodenal and ileal segments are shown in Table II. Data from unperfused segments are included for comparison. There was significantly more magnesium in duodenal than ileal segments whether perfused or not. The mean concentration of magnesium in duodenal segments perfused with high Mg perfusate was significantly higher than magnesium concentrations of other duodenal segments both perfused and unperfused. There was no significant difference among magnesium concentrations of the ileal segments.

The tissue concentrations of calcium in duodenum and ileum are the inverse of magnesium: there is less calcium in duodenal than ileal segments and the differences are

TABLE II. Magnesium and Calcium Tissue Concentrations[a] (mg/ml of tissue water).

Mg conc of perfusate (m*M*)	Magnesium			Calcium		
	Duodenum	Ileum	$p <$ [b]	Duodenum	Ileum	$p <$ [b]
2.5	0.249 ± 0.002[c]	0.224 ± 0.008	0.01	0.101 ± 0.005	0.163 ± 0.007	0.001
0.25	0.226 ± 0.004	0.201 ± 0.002	0.001	0.104 ± 0.003	0.148 ± 0.003	0.001
0	0.235 ± 0.006	0.197 ± 0.007	0.01	0.102 ± 0.003	0.153 ± 0.008	0.001
Not perfused	0.233 ± 0.004	0.210 ± 0.005	0.05	0.106 ± 0.003	0.166 ± 0.007	0.001

[a] Groups of 12 rats were studied with each solution and intestinal tissues analyzed after the 2-hr perfusion period. The group not perfused comprised 10 animals. Data are mean values ± 1 SE.

[b] Comparisons of duodenum and ileum.

[c] Differs significantly from other duodenal segments.

greater than with magnesium. Neither magnesium concentration of the perfusate nor perfusion itself affected tissue calcium concentrations. Both perfused and unperfused duodenal and ileal segments have the same calcium content.

Discussion. These studies demonstrate that magnesium absorption is significantly greater in duodenum than terminal ileum of the rat *in vivo*. Using the same experimental model, a similar relationship for calcium was demonstrated (7). Calcium and magnesium do differ, however, when absorption is related to their total serum concentrations. At 2.5 m*M* luminal magnesium concentration, approximately double its concentration in plasma, magnesium is absorbed from the duodenum and ileum (Table I). At 0.25 m*M* about one-sixth the plasma concentration, magnesium is secreted into the lumen. Calcium is absorbed at concentrations of 0.4 m*M* (7), approximately one-sixth the plasma calcium concentration. Thus, with this experimental technique, calcium appears to be better absorbed than magnesium against a concentration gradient.

The following qualitative comparisons between the previous calcium data (7) and the present magnesium data can be made: calcium is also better absorbed than magnesium in duodenum when the luminal concentration exceeds that of plasma. At a luminal calcium concentration of 3.4 m*M* 1.5 times plasma calcium, the duodenal calcium absorption rate of 19.5 μmoles/2 hr/g (7) is greater than the absorption rate of magnesium (14.8 μmoles/2 hr/g; Table I) at almost double its plasma concentration. Under these same conditions, ileal absorption rates were approximately equal: 10 μmoles/hr/g for both calcium and magnesium. Thus, although calcium may be better absorbed than magnesium in duodenum, there is little difference in ileum with luminal concentration greater than plasma level.

Results of the present transport study differ with previous findings (1, 2, 4) demonstrating greater ileal than jejunal magnesium absorption. The disagreement with *in vitro* studies (1, 2) might be simply the difference in technique. In the previous *in vivo* study (4) absorption of magnesium was determined by injecting the radionuclide ^{28}Mg in 0.5 ml of water into the stomach, duodenum, or ileum and measuring fecal isotope excretion during the subsequent 30 hr. These studies (4) delineate the general transport behavior of the entire alimentary canal but do not provide a specific measure of transport per unit weight of a spciefic segment. Thus, with normal transit rate, varying mucosal contact times might cause quite different net absorption rates for the individual segments than we found. It is interesting that absorption capacity per unit weight of intestine when measured under controlled conditions showed the same pattern for magnesium and calcium, *i.e.*, proximal greater than distal. This is in accord with previous studies of similarities in absorption of calcium and magnesium in the rat (3).

Magnesium concentrations in full thickness intestinal tissue are similar to values reported for mucosal scrapings (8, 9). The higher magnesium concentrations in duodenum than ileum are the reverse of the pattern for calcium. [Table II and (7)]. It has been suggested that calcium and magnesium are transported by a common mechanism in the intestine and renal tubule (3). Therefore, it is of interest that the presence of transported magnesium had little influence on tissue calcium (Table II), and that the secretion of calcium from intestinal tissue into lumen was not influenced by magnesium transport.

Summary. Magnesium absorption by the rat small intestine *in vivo* was studied by a perfusion technique under controlled conditions. Net magnesium absorption per unit weight of intestinal segment was significantly greater in duodenum than in ileum. However, comparisons with previous studies of calcium transport using a similar experimental model show that magnesium was less well absorbed than calcium in the duodenum, while ileal absorption rates were almost equal. Tissue concentrations of magnesium were higher in the duodenum than in ileum, the reverse of the pattern for calcium. Neither tissue calcium nor the secretion of calcium into the intestinal lumen was influenced by transported magnesium.

We thank Dr. Paul E. Leaverton for his advice and help with statistical analyses of the data.

1. Ross, D. B., J. Physiol. **160**, 417 (1962).

2. Hendrix, J. Z., Alcock, N. W., and Archibald, R. M., Clin. Chem. **9**, 734 (1963).

3. Alcock, N. and MacIntyre, I., Clin. Sci. **22**, 185 (1962).

4. Chutkow, J. G., J. Lab. Clin. Med. **63**, 71 (1964).

5. Hyden, S. A., Kgl. Lantbruks-Hogskol. Ann. **22**, 139 (1955).

6. Steel, G. D. and Torrie, J. H., "Principles and Procedures of Statistics," p. 81, 109, 112. McGraw-Hill, New York (1960).

7. Krawitt, E. L. and Schedl, H. P., Am. J. Physiol. **214**, 232 (1968).

8. Marcus, C. S. and Wasserman, R. H., J. Appl. Physiol. **21**, 1063 (1966).

9. Nunn, A. S. and Ellert, M. S., Am. J. Physiol. **212**, 711 (1967).

Received July 3, 1969. P.S.E.B.M., 1969, Vol. 132.