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Intestinal Calcium Loss in Infant Rats[1] (40492)

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The permeability of the small intestine to water and electrolytes (1), including calcium (2, 3), appears to be greater in infant than adult rats. During osmotic diarrhea loss of sodium and chloride into the intestinal lumen was greater in the infant than in the adolescent rat (1). Thus it would be expected that under similar conditions loss of calcium would also be greater in the intestine of infants than adults. Since, to our knowledge, there is no information regarding the possibility of excess loss of calcium in the small intestine of infants during osmotic diarrhea, we undertook these experiments. Calcium secretion was compared in the jejunum and ileum in suckling (2-week-old) and adolescent (6-week-old) rats during in vivo perfusion of isotonic and hypertonic solutions free of calcium.

Materials and methods. Secretion of calcium was compared in segments of the jejunum and of the ileum in 2- (14- to 15-day-old) and 6- (42-day-old) week-old male albino rats purchased from Biolabs (Madison, Wisconsin). The experimental procedures used were similar to those described previously (1, 2). At the time of study, rats were anesthetized with a solution containing a mixture of ethyl urea (K & K Laboratories, Plainview, New York) and sodium pentobarbital (6:1). About 15–20 centimeter long segments of the jejunum (just distal to the ligament of Treitz) and of the ileum (just proximal to the ileocecal junction) were rinsed with 10 ml of an isotonic sodium chloride solution, flushed with 50 ml of air and cannulated. The cannulated segments were inserted back into the abdominal cavity and perfused *in situ*, at a rate of 0.3 ml/min with a solution containing per liter (double distilled water): 120 mmoles of sodium chloride, 5 mmoles of potassium chloride, 25 mmoles of sodium bicarbonate, 20 mg of Phenol Red and mannitol (Fisher Scientific Company, Fair Lawn, NJ) in quantities sufficient to achieve osmolalities of 300 and 500 mOsm/kg. The glassware and tubings used for the studies were rinsed with 1N hydrochloric acid to remove adherent calcium. For study of the effect of each solution with different osmolality, groups of eight to nine rats at each age were used. During the perfusion period, the rat's body temperature was maintained at 36–37°. The solution perfused through the cannulated segments over the first 60 min of perfusion was discarded, while that over the next 60 min was collected in three consecutive 20-min periods. The concentrations of calcium and Phenol Red were determined in the initial perfusion solutions and in each of the collected solutions (perfusates). The perfused jejunal and ileal segments were stripped from the mesentary and their length measured. The wet weight of the segments was determined after gently pressing out the luminal contents with the forefingers. The mucosa was scraped with the edge of a glass slide and the wet weight of the mucosa and underlying tissue were determined. The tissues were then dried in a vacuum oven at 90–95° for 24 hr and their dry weight determined. Calcium content of the perfusion fluid was determined immediately after perfusion using a Perkin Elmer atomic absorption spectrometer and Phenol Red as described by Schedl and Clifton (4).

Net water transport was determined from change in concentration of Phenol Red as follows:

$$\text{New water transport, ml/20 min.} = V_i(1 - PR_i/PR_f)$$

where V_i depicts volume of fluid perfused ml/20 min, and PR the concentration of Phenol Red in μg/ml, in the perfusion fluids. The subscripts i and f refer to initial and final values in the perfusion solutions before perfusion (i), and in the perfusates collected (f).

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Net transport of calcium was calculated using the following formula: Net transport, μmoles/20 min = $V_i(C_i - C_f PR_i/PR_f)$, where C represents concentration (μmoles/ml) of calcium in perfusion fluid. During the 60-min collection period, steady state conditions had been achieved since net transport of water and calcium during each of the three 20-min collection periods did not vary from each other by more than 20%. The amounts of water and of calcium transported during these three periods were added to obtain amount transported in 1 hr. To be able to compare transport rates of water and calcium in the segments of the rats at the different age groups, the amounts transported were normalized for the marked differences in size (weight) of the perfused segments.

Statistical analysis: The unpaired "t" test was used to compare mean values between corresponding segments of rats at the two age periods, and the paired "t" test to compare mean values between the segments in each group of rats. A value of $p < 0.05$ was taken as indicating a statistically significant difference between the corresponding mean values (5).

Results. Body weight and measurements of the perfused intestinal segments are depicted in Table 1. Wet weight and weight/cm length of the full thickness wall and of the mucosal scrapings were several fold greater in segments of the 6-week-old than in corresponding segments of the 2-week-old rats. Mucosal wet weight (absorbing mass) formed a greater percentage of full thickness segment wet weight in the 6-week-old (about 75%) than in the 2-week-old (about 50%) rats ($p < 0.01$).

All perfusion solutions contained trace amounts of calcium, the mean value was 5 μmoles/liter. During perfusion concentration of calcium in the perfusion solutions increased to around 50–120 μmoles/l, indicating that there was net secretion of calcium into the intestinal lumen.

Rates of net secretion of calcium in the perfused segments are shown in Table II. In the jejunal segments, during perfusion of the isotonic and hypertonic solutions, rates of net secretion of calcium were several fold greater in the 2- than in the 6-week-old rats, regardless of the parameter on which rate of secretion was expressed. In the ileal segments, based on length of segments, net secretion of calcium was not significantly different in the 2- and 6-week-old rats. However, based on weight of the segments or that of the scraped mucosa, secretion rates were several fold greater in the 2- than in the 6-week-old rats.

During perfusion of the isotonic solutions in the 2-week-old rats, net secretion of calcium was similar in the jejunal and the ileal segments, however, in the 6-week-old rats, rates of net secretion were twofold or more greater in the ileal than in the jejunal segments. Perfusion of the hypertonic solution seemed to have significantly increased rate of net secretion of calcium in the jejunal segments of the 2- and of the 6-week-old rats, regardless of the basis of expressing net secretion rate. In the ileal segments rate of net secretion was unchanged by perfusion of the hypertonic solutions.

During perfusion of the hypertonic solutions, rates of secretion were greater in the jejunal than in the ileal segment of the 2-week-old rats, however, in the 6-week-old rats, rates of net secretion were not significantly different in the jejunal and the ileal segments.

TABLE I. AGE, BODY WEIGHT, AND MEASUREMENTS OF THE INTESTINAL SEGMENTS IN THE RATS STUDIED.[a]

Age of rats (days)	14–15				42			
Body weight (g)	36 ± 1				197 ± 2			
Perfusion solution osmolality, (mosmol/kg)	300		500		300		500	
Number of rats used	8		8		9		8	
Segment perfused	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Length	12.4 ± 1.5	16.8 ± 2.2	15.2 ± 3.0	14.4 ± 2.4	18.6 ± 3.1	16.5 ± 1.1	16.7 ± 1.3	16.3 ± 1.3
Wet weight of:								
Full thickness wall								
(mg)	245 ± 18	303 ± 43	256 ± 57	209 ± 35	1370 ± 195	1036 ± 42	1034 ± 62	928 ± 96
(mg/cm)	20.5 ± 2.3	18.0 ± 0.8	17.2 ± 3.1	14.8 ± 1.0	74.6 ± 2.2	63.5 ± 3.0	62.5 ± 1.8	56.7 ± 3.6
Mucosal Scrapings (mg)	146 ± 23	145 ± 26	112 ± 48	104 ± 25	1053 ± 155	674 ± 30	770 ± 39	666 ± 58
Water Content (mg/100 mg)	78.3 ± 0.4	79.6 ± 0.2	78.1 ± 1.5	79.6 ± 1.7	80.3 ± 0.1	81.0 ± 0.4	78.8 ± 0.2	78.5 ± 0.3

[a] Values are means ± SE.

TABLE II. NET CALCIUM AND WATER TRANSPORT DURING PERFUSION OF THE ISOTONIC AND THE HYPERTONIC SOLUTIONS IN THE INTESTINAL SEGMENTS STUDIED.[a]

Age of rats (days)	14–15				42			
Perfusion solution osmolality (mosmol/kg)	300		500		300		500	
Segment perfused	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Net calcium secretion (hr/ μmoles/cm)	0.12 ± 0.01	0.11 ± 0.01	0.15 ± 0.01*	0.08 ± 0.01	0.04 ± 0.01**	0.08 ± 0.01***	0.06 ± 0.01**	0.09 ± 0.01
μmoles/g wet weight of:								
Full thickness wall	6.8 ± 0.9	5.9 ± 0.6	11.5 ± 1.2*	5.4 ± 0.8***	0.5 ± 0.1**	1.2 ± 0.1** ***	1.2 ± 0.2* **	1.6 ± 0.1**
Mucosal scrapings	12.8 ± 2.3	13.9 ± 3.1	22.7 ± 1.9*	14.4 ± 3.5***	0.6 ± 0.1**	1.9 ± 0.1** ***	1.8 ± 0.3* **	2.6 ± 0.4**
μmoles/g dry weight of:								
Mucosal scrapings	60.0 ± 10.9	68.6 ± 15.3	131.0 ± 9.9*	70.3 ± 17.7***	3.2 ± 0.7**	9.8 ± 0.5** ***	9.5 ± 2.3* **	10.4 ± 1.0**
Net Water Secretion[b] (hr/ ml/g wet weight) of:								
Full thickness wall	0.2 ± 0.2	−0.2 ± 0.1	9.9 ± 0.9*	4.4 ± 1.2* ***	−1.2 ± 0.3**	−4.1 ± 0.6** ***	6.6 ± 0.6* **	6.5 ± 1.9*

[a] Values are means ± SE.

[b] Negative values indicate net absorption from the lumen into the body.

* Mean value in segments perfused with the hypertonic solution significantly different than the corresponding mean value in segments perfused with the isotonic solution, $p < 0.05$–0.001.

** Mean value in 42-day-old rats significantly different than corresponding mean value in 14- to 15-day old rats, $p < 0.005$.

*** Mean value in ileal segments significantly different than corresponding mean in jejunal segments, $p < 0.01$–0.005.

Discussion. The mechanism(s) by which calcium is transported across the intestinal mucosa during early life is not known. In our previous studies transport of calcium appeared to be predominately by a diffusive process in suckling rats and predominately by an active process in adolescent rats (3). The findings in the adolescent rats were similar to those reported for adult rats (6–9). Using a perfusion technique similar to the one used in the present study, from a luminal solution containing 3.4 mmoles/l of calcium, the lumen-to-mucosa flux and the estimated mucosa-to-lumen flux of calcium were both several fold greater in the intestine of the 2- than of the 6-week-old rats (2). Because of the greater bidirectional movement of calcium across the intestinal mucosa in infant rats, it would be expected that if the intestinal lumen was perfused with a solution free of calcium, calcium would move into the lumen and the magnitude of net secretion would be greater in infant than in older rats. The present studies tested the validity of this hypothesis. Previously we noted that perfusion with hypertonic solutions caused greater losses of water and electrolytes into the intestinal lumen of infant than adult rats (1). The present studies also determined the effect of osmotically induced diarrhea on net secretion of calcium.

The results of the present studies confirmed the above hypothesis. Indeed during perfusion of the isotonic solution, free of calcium, loss of calcium into the lumen was several fold greater in segments of the 2- than of the 6-week-old rats. Perfusion of the hypertonic solution caused a 2 fold increase in rate of net secretion of calcium in the jejunal segments of both the 2- and 6-week-old rats, but not in the ileal segments. The latter finding suggested that the ileal segment at 2 weeks of age has developed the ability to somehow conserve calcium similar to that in the adolescent rats. However, because of the diffusive nature of calcium transport in the 2-week-old rats relatively large amounts of calcium were still lost into the lumen.

In adolescent rats the finding of greater rate of net secretion of calcium in the ileal than jejunal segment was similar to observations in adult rats (6, 10), and suggested that the jejunal segments could conserve calcium

more efficiently than the ileal segment when lumen osmolality was isotonic. This was not true in the 2-week-old rats where loss of calcium was of equal magnitude in the jejunal and ileal segments.

The magnitude of the loss of calcium in relation to body weight and serum calcium content can be determined if the total amount of calcium lost through the small intestine is known. Total amount of calcium that could be lost from the entire small intestine was calculated from the data available and with the following assumptions: (a) Loss of calcium in the unperfused segment of the small intestine was at a rate similar to the average noted for the proximal and distal intestine; and (b) the total length of the small intestine in 2 week old 35–40 g rats was about 50 cm and in 190–210 g rats about 85 cm. These values were based on previous experience in a large number of rats. The weight of the unperfused small intestine was calculated from its estimated length and the mean of the weight/cm ratio of the corresponding jejunal and ileal segments. The total amount of calcium lost, as μmoles/hr from the entire small intestine was calculated as the sum of those measured for the jejunum and ileum and that derived for the unperfused segment.

The calculated loss of calcium from the entire small intestine (μmoles/hr) was 5.9 and 6.6 respectively in the 2-week-old rats perfused with the isotonic and hypertonic solutions. In the 6-week-old rats the corresponding values were 5.0 and 7.1. In relation to body weight the net loss as μmoles/hr/100 g body were: 15.1 and 19.4 respectively in the 2-week-old rats perfused with the isotonic and hypertonic solutions. The corresponding values in the 6 week old rats were 2.5 and 3.6. Although net amounts of calcium secreted into the lumen were similar in the 2- and 6-week-old rats, the rates of secretion and the net loss of calcium corrected for body weight were two- to sevenfold greater in the suckling than adolescent rats. The magnitude of the loss of calcium into the intestinal lumen would predispose infant animals to lose relatively larger proportions of body calcium through the intestinal tract, especially during periods of osmotic diarrhea, when oral intake of calcium is restricted. Although hypocalcemia is not a common finding in diarrheal states, it is possible that the relatively large losses of calcium from the body are compensated by mobilization from bones into the serum.

Summary. Segments of the jejunum and ileum of 14-15-day-old and 42-day-old rats were perfused in situ with isotonic or hypertonic calcium free solutions. There was net secretion of calcium in all segments perfused. Rates of net secretion of calcium were several fold and significantly greater in segments of the 14- to 15-day-old than in corresponding segments of the 42-day-old rats. In the 14- to 15-day-old rats net secretion of calcium was similar in jejunal and ileal segments, while in the 42-day-old rats net secretion was twofold greater in the ileal than jejunal segments. Perfusion of the hypertonic solution almost doubled net secretion of calcium in the jejunal segments at both ages, but had no effect on rate secretion of calcium in the ileal segments. These findings suggest that infants are more prone to lose calcium into the lumen of the intestine than adults, especially during episodes of osmotic diarrhea when oral intake is restricted acutely.

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