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Effects of Experimental Diabetes on Intestinal Strontium Absorption in the Rat[1] (39446)

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The alkaline earth element, strontium, belongs to the same family as magnesium and calcium. All are better absorbed in the proximal than in the distal small intestine (1-3) and show enhancement of transport by vitamin D (2, 4, 5). Calcium and magnesium are important nutrients, whereas strontium is of interest as a contaminant of food and water in the form of its radioactive isotopes. Strontium behaves much like calcium in its transport (1, 2). Duodenal calcium transport is depressed in the rat with alloxan or streptozotocin induced diabetes (6, 7), but magnesium transport is the same as in control rats (8). These effects are present even when duodenal mucosal growth is enhanced in diabetic as compared with control rats. We therefore examined strontium transport and effects of diabetes.

Materials and methods. Male Simonsen albino rats (Simonsen Labs, Gilroy, Calif.) fed commercial rat chow (Wayne Lab Blox, calcium, 1.2%; magnesium, 0.2%; phosphorus, 1.0%; Allied Mills, Libertyville, Ill.) were used. Rats to be made diabetic were given a single intraperitoneal injection of freshly prepared streptozotocin solution (50 mg/ml in citrate buffer at pH 4.5, 110 mg/kg). Weight-matched controls received citrate buffer only. Diabetic rats studied showed glycosuria greater than 2% (Tes-Tape, Eli Lilly Co.) and fasting blood sugar above 250 mg% (Dextrostix, Ames Co.).

Strontium absorption was measured 5 or 12 days after injection in animals fasted overnight but receiving water *ad libitum*. Rats were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg) and placed supine on a warm heating pad. The abdomen was opened by a midline incision and the common bile duct ligated. Cannulas were inserted and tied in place so that duodenum and terminal ileum (15-25 cm proximal to the cecum) could be perfused intraluminally. The perfusion solution contained strontium chloride (2.5 m*M*), sodium chloride (165 m*M*), and nonabsorbed indicator: polyethylene glycol (PEG, 1.0 g/liter), and ^{14}C-PEG (0.6 mCi/g, 0.01 mCi/liter, New England Nuclear, Boston, Mass.). Using a syringe, each segment was flushed with 5-10 ml of normal saline solution followed by an air flush. A 10-ml volume of test solution contained in a plastic centrifuge tube was recirculated through each segment at a constant rate of 2 ml/min (Bowman Pump, Process and Instruments, Brooklyn, N.Y.). To facilitate mixing, the intake tubing from the pump rested at the bottom of the reservoir and the return tubing emptied near the top. During the *in vivo* perfusion, the abdomen was closed, kept moist with saline-dampened gauze, and rectal temperature was maintained at 35-38°. After 2 hr, the test solution was collected in the reservoir and blood was immediately drawn from the aorta for determination of glucose concentrations. Duodenum, terminal ileum, and the intervening midgut were excised, residual contents evacuated by gently pressing the segment flat on absorbent paper, and length and wet weight of segments were measured. To obtain mucosa, each segment was slit lengthwise, spread on a glass plate with mucosa up, and scraped horizontally with a microscope slide (nonbeveled). Tissue was placed in a preweighed crucible, weighed, dried in a vacuum oven at 100° for 18-20 hr, and reweighed to obtain dry weight.

Strontium concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer, Model 303, Norwalk, Conn.) in aliquots of the initial perfusion solution and final reservoir contents. Liquid

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scintillation counting was used to count ^{14}C-PEG: 0.2 ml of sample, 3.8 ml of water, 1 drop of 4% stannous chloride, and 15 ml of Bray's scintillation fluid. Plastic or acid washed (1 *N* HCl) glassware was used throughout.

Net transfer rates for strontium were calculated as follows:

$$\text{Net transfer } (\mu\text{moles/hr}) = \frac{V[C_i - (C_f \times {}^{14}\text{C-PEGR})]}{\text{hr}}$$

where V is initial volume (liter) of solution recirculated, C_i and C_f are the initial and final concentrations of strontium (μM) in the luminal solution, ^{14}C-PEGR is the ratio of initial to final ^{14}C-PEG concentration, and hr is hours recirculated. Net transfer was divided by both mucosal weight (g) and length of segment (cm). Results are expressed as mean values ± one standard error. The Student's t test for unpaired data was used for statistical comparisons, and probability values less than 0.05 were considered significant.

Results and discussion. Table I gives mean data on body weight and small intestinal measurements for control (C) and diabetic (D) rats studied 5 and 12 days after injection. The number of rats used in the study for each group is given at the top of the table and applies to succeeding tables. Mean body weight at time of injection (Initial), just prior to overnight fasting before study (Final), and at time of perfusion (Fasted) are shown at the top of Table I. Body weights did not differ between groups initially. Control rats gained 5–6 g/day in contrast to diabetics who failed to gain weight.

Mean total length and weight of the small intestine was greater in diabetics at both time intervals, but the increase was significant at 12 days only. Weight of small intestine as a percentage of fasted body weight was greater in diabetics at both 5 and 12 days. At 12 days, the percentage for diabetics (4.7%) was double that of controls (2.3%). Total mucosal dry weight in diabetics was 13% greater than in controls at 5 days and 50% greater at 12 days, in contrast to no difference in underlying tissue. Hence, intestinal growth stimulation in diabetic animals is localized primarily to the mucosa.

Mucosal mass expressed as dry weight (mg/cm) is given at the bottom of Table I for the three divisions of the total small intestine. Mucosal mass per centimeter tended to decline progressively from duodenum to ileum for all groups. Mucosal dry weight (mg/cm) of duodenum and midgut was significantly greater in diabetics at both 5 and 12 days, whereas in the ileum, mucosal dry weight was greater only at 12 days.

TABLE I. Body Weight and Intestinal Measurements for Control and Diabetic Rats.

	Mean data			
	5 days		12 days	
	Control (N = 8)	Diabetic (N = 8)	Control (N = 10)	Diabetic (N = 9)
Body weight (g)				
Initial	201	207	200	211
Final	227	196**	269	197**
Fasted	209	175**	246	167**
Total small intestine length (cm)	94	99	94	108*
Total small intestine weight (mg)	5323	5864	5562	7802**
(Weight of small intestine/body weight) × 100	2.5	3.4**	2.3	4.7**
Dry weight (mg)				
Total mucosa	729	822	738	1101**
Total underlying tissue	422	404	518	528
Mucosal dry weight (mg/cm)				
Duodenum	8.1	9.7*	7.8	11.6**
Midgut	8.0	8.4*	8.0	9.9*
Ileum	6.8	6.4	7.4	9.7**

* Diabetic data differ significantly from control data, $p < 0.05$.
** Diabetic data differ significantly from control data, $p < 0.001$.

TABLE II. STRONTIUM ABSORPTION FOR CONTROL AND DIABETIC GROUPS.

	5 days		12 days	
	Control	Diabetic	Control	Diabetic
	(μmoles/hr, mean ± SE)			
per cm × 100				
duodenum	57 ± 3	40 ± 4*	55 ± 4	41 ± 3*
ileum	18 ± 1	16 ± 3	16 ± 2	12 ± 2
per g dry wt mucosa				
duodenum	74 ± 4	44 ± 5**	77 ± 6	37 ± 2**
ileum	28 ± 3	27 ± 5	23 ± 3	13 ± 2*

* Diabetic data differ significantly from control data, $p < 0.05$.
** Diabetic data differ significantly from control data, $p < 0.001$.

Strontium absorption data for duodenum and ileum of both control and diabetic groups at 5 and 12 days are shown in Table II. Data are expressed per centimeter of segment length and per gram of dry weight mucosa. Absorption per centimeter of length defines absorptive capacity of the segment and was lower in diabetics in duodenum at both time intervals. Ileal absorption per centimeter was about one-third that of duodenum for both groups. Although mean ileal absorption per centimeter was slightly lower in diabetics, differences from control values were not significant. Absorption expressed per gram of dry weight of mucosa defines absorptive specific activity of the segment and shows the same relationships as per unit length. Duodenal absorption per gram of mucosa of diabetics was half that of controls. Ileal absorption was much less than duodenal for both groups. Ileal absorption by diabetics differed from controls only at 12 days.

Net luminal water movements in perfused segments were minimal (not shown). In duodenum, mean values ranged from 2% net absorption to 8% net secretion. In ileum, mean values ranged from 0 to 6% net absorption.

The pattern of absorption of strontium in duodenum and ileum of control and diabetic rats (Table II) is similar to that previously observed for calcium (6, 7): duodenal transport is depressed. Ileal transport is less affected, but there is depression of specific absorption in diabetics at 12 days. Thus, diabetes affects strontium and calcium absorption in the same manner. Effects of diabetes on calcium absorption appear to be mediated by abnormal vitamin D metabolism: (a) depressed duodenal calcium absorption in the diabetic rat is restored to the level of controls by 1,25-dihydroxycholecalciferol, the vitamin D metabolite that acts directly on the intestinal mucosa (9); (b) duodenal calcium absorption in the diabetic rats is also corrected by extract of the plant, *Solanum malacoxylon* (10). *Solanum malacoxylon* extract, like 1,25-dihydroxycholecalciferol, also acts directly on the mucosa to stimulate calcium absorption. Since vitamin D shows similar effects on transport of calcium and strontium (2), these findings on strontium absorption in diabetes fit with a defect in vitamin D metabolism and are another example of parallelism between calcium and strontium transport.

Summary. Control and streptozotocin diabetic rats were studied at 5 and 12 days after induction of diabetes. Strontium absorption was measured by *in situ* perfusion of duodenum and ileum. Duodenal absorptive capacity (absorption per unit length) and absorptive specific activity (absorption per gram of dry weight mucosa) were depressed. Depression was present both at 5 days, when mucosal growth is similar in controls and diabetics, and at 12 days, when mucosal growth is 50% greater in diabetics. Effects of diabetes on ileal absorption were minimal in comparison with effects on duodenum. This depression of duodenal strontium absorption in the diabetic rat is analogous to effects of diabetes on calcium absorption and may be mediated by abnormal vitamin D metabolism.

1. Lengemann, F. W., *in* "The Transfer of Calcium and Strontium Across Biological Membranes" (R. H. Wasserman, ed.), p. 85. Academic Press, New York and London (1963).
2. Wasserman, R. H., *in* "The Transfer of Calcium

and Strontium Across Biological Membranes" (R. H. Wasserman, ed.), p. 211. Academic Press, New York and London (1963).
3. Urban, E., and Schedl, H. P., Proc. Soc. Exp. Biol. Med. **132,** 1110 (1969).
4. Urban, E., and Schedl, H. P., Amer. J. Physiol. **217,** 126 (1969).
5. Schachter, D., and Rosen, S. M., Amer. J. Physiol. **196,** 357 (1959).
6. Schneider, L. E., and Schedl, H. P., Amer. J. Physiol. **223,** 1319 (1972).
7. Walker, B., and Schedl, H. P., Clin. Res. **22,** 636A (1974).
8. Miller, D. L., and Schedl, H. P., Clin. Res. **21,** 869A (1973).
9. Schneider, L. E., Omdahl, J., and Schedl, H. P., The Physiologist, **18,** 381 (1975).
10. Schneider, L. E., Wasserman, R., and Schedl, H. P., Endocrinology **97,** 649 (1975).

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