PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 553–557 (1980)

Intestinal Resection and Calcium Absorption in the Rat (40813)

WILLIAM C. EASTIN*, HELEN D. WILSON†, AND HAROLD P. SCHEDL†

Patuxent Wildlife Research Center, U.S. Fish and Wildlife Service, Laurel, Maryland 20811 and †Gastroenterology Research Laboratories, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

Despite the clinical importance of bowel resection and its potential effect on calcium transport by the gut, direct measurements of calcium absorption in animal models of resection are limited. Prior studies examined older animals after considerable lapse of time postresection (1). We examined the effects of 70% resection of mid small intestine on duodenal and ileal calcium transport in younger growing rats and report our results.

Materials and methods. Surgery. Male albino rats, Sprague–Dawley derived, weighing 200 g, were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts, and housed in the animal care facility. The rats were divided randomly into two groups: one group for transection and one group for resection. Rats were anesthetized with ether, and the small bowel exposed by a midline abdominal incision. The intestine from the ligament of Treitz to the ileocecal valve was delivered onto a warm saline-moistened gauze sponge. By placing a thread along the antimesenteric border, the combined length of jejunum and ileum was estimated. Seventy percent of the total jejunum and ileum was removed leaving 20 cm of distal ileum intact. The remaining jejunal and ileal segments were anastomosed using 6-0 silk and tapered curved needles (Ethicon, Inc., Somerville, N.J.). The intestine was then returned to the peritoneal cavity, and peritoneum and muscle were closed with 4-0 silk thread. The skin was closed with wound clips. Animals for transection were treated identically except that the intestine was divided and reanastomosed at the midpoint between the ligament of Treitz and ileocecal junction. After surgery, animals were allowed to recover, fed 5% glucose solution for 24 hr and then fed laboratory rat chow and water *ad libitum*. The animals from the two experimental groups were not pair fed. At 7–9 days after surgery, transected and resected animals were examined for effects on calcium transport.

Absorption measurements. Rats were anesthetized with sodium pentobarbital (40 mg/kg body wt), the abdomen opened by midline incision, and the duodenum and ileum isolated, washed with saline, and flushed with air. The duodenal inlet cannula was at the pylorus and the exit cannula was placed just beyond the ligament of Treitz. The distal ileum was also cannulated and recirculated at the same time. Segments were recirculated with 15 ml of 3.4 m*M* calcium solution containing 159 m*M* NaCl and 30 mg/liter phenol red as nonabsorbed indicator of water movement. ^{45}Ca (25 μCi/liter 20.3 μCi/μg, New England Nuclear) was added for measurement of flux out of the lumen. After 2 hr of recirculation at 2 ml/min, the segments were pumped empty to recover the solutions. The circulated segments were removed, placed on absorbent paper, and the contents expressed. Length and full thickness wet weight were obtained. The segments were then slit open lengthwise on a glass plate, mucosal side up, and the mucosa was scraped from the underlying tissue with a glass microscope slide. Wet weight of mucosa and underlying tissue was measured and dry weight was obtained after drying at 100°C and 20 psi vacuum.

Analyses. Initial (uncirculated) and final solutions were analyzed in duplicate for ^{40}Ca by atomic absorption spectrophotometry (Perkin Elmer 303), for phenol red spectrophotometrically (2). ^{45}Ca was measured by liquid scintillation counting

0037-9727/80/040553-05$01.00/0

(Beckman LS 250) using 1 ml of solution and 10 ml aqueous counting scintillant (ACS, Amersham).

Calculations (3). Net Ca absorption, micromoles/gram per hour

$$= \frac{V_i[(^{40}Ca_i) - (^{40}Ca_f \cdot PRR)]}{W \cdot t}.$$

Flux out of the lumen, micromoles/gram per hour

$$= \frac{V_i[(^{45}Ca_i) - (^{45}Ca_f \cdot PRR)]}{\bar{x}\ SA \cdot W \cdot t}.$$

Flux into the lumen, micromoles/gram per hour = (Flux out of the lumen, μmoles/g per h) − (net absorption, μmoles/g per h), where V refers to volume of perfusate in ml; subscripts i and f = initial and final luminal values; ^{40}Ca = calcium concentration, micromoles/milliter, ^{45}Ca = radioactive calcium content in cpm/ml; PRR = ratio of initial to final phenol red concentrations; $\bar{x}$ SA = mean specific activity of initial and final luminal fluid in cpm/μmole; W = mucosal scrapings dry weight in g; t = recirculation time, h. The unpaired Student's t test compared treatment groups.

Results and discussion. Body weight and intestinal measurements for the transected (control) and resected groups are shown in Table I. Although mean body weight after the 7- to 9-day postoperative period was about 10% greater in transected than in resected animals, the difference was not significant. Segments perfused were slightly longer in resected than transected animals. When adjusted for differences in segment length (g/cm data), mean weights per unit length were greater in resected than in transected animals: (i) mucosal weight per unit length was significantly greater in both duodenum and ileum; (ii) full thickness wet weight was significantly greater only for ileum. Most of this intestinal growth is in mucosa and is much greater in the distal than in the proximal residual segment as observed by previous investigators (4–6).

Net calcium absorption and secretion and flux out of the lumen data are shown in Figs. 1 and 2 for the transected (control) and resected groups. Transport data based on mucosal dry weight (Fig. 1a) show duodenal net calcium absorption in the resected group to be depressed to half the level in the transected group ($P < 0.02$). This depression is the consequence of decreased flux out of the lumen per gram duodenal mucosa in the resected group ($P < 0.02$) (Fig. 1b). Flux into the lumen calculated as the difference between flux out of the lumen and net absorption was the same for transected and resected groups whether calculated as micromoles per gram dry weight mucosa per hour (17.6 ± 3.2 vs

TABLE I. BODY WEIGHT AND INTESTINAL MEASUREMENTS[a]

	Group		
	Transected	Resected	Significance[b]
Animals/group	9	11	
Body weight (g)			
Initial	198 ± 13	208 ± 13	NS
Final	234 ± 12	210 ± 8	NS
Intestinal segments			
Full thickness length (cm)			
Duodenum	9.6 ± 0.4	12.0 ± 0.8	<0.005
Ileum	10.0 ± 1.0	12.8 ± 1.0	NS
Wet weight (g/cm)			
Duodenum	0.092 ± 0.008	0.110 ± 0.007	NS
Ileum	0.074 ± 0.007	0.132 ± 0.018	<0.02
Mucosa dry weight (g/cm)			
Duodenum	0.011 ± 0.001	0.015 ± 0.001	<0.005
Ileum	0.008 ± 0.001	0.015 ± 0.001	<0.001

[a] Data are means ± SE.
[b] P values calculated by unpaired Student's t test. NS = not significant.

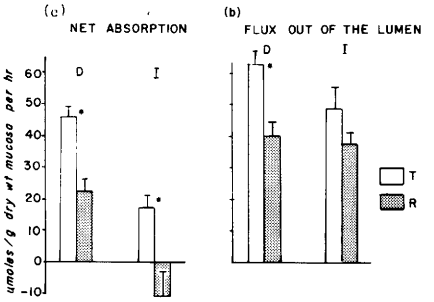

FIG. 1. Net movements (a) and flux out of the lumen (b) of calcium per gram mucosal dry weight. Transected (control) and resected groups were luminally perfused with 3.4 m*M* calcium in duodenum and ileum. *Data from resected groups differ from transected group ($P < 0.02$). Net absorption is shown by + values; net secretion by − values. In duodenum of the resected group, net absorption per unit weight of mucosa is decreased to half that of the transected controls. In ileum, resected animals show net secretion in contrast to net absorption in the transected group.

17.0 ± 1.7, respectively) or per centimeter per hour (0.19 ± 0.03 vs 0.26 ± 0.03). Effects in ileum are even more striking. The ileum of resected animals shows net calcium secretion. Transected animals show net absorption at approximately one-third the corresponding duodenal rate, comparable to our previous data in unoperated control animals (7, 8). Fluxes out of the lumen per gram mucosa are about 25% higher in ileum of transected as compared with the resected group (differences not significant).

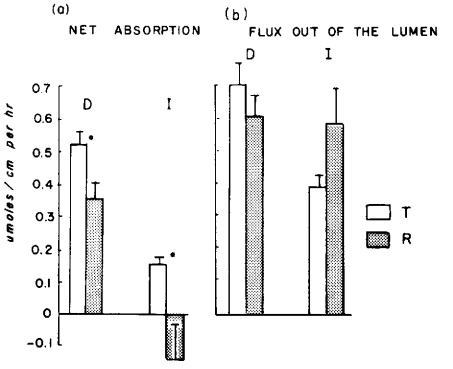

FIG. 2. Net calcium movements (a) and flux out of the lumen (b) of calcium per centimeter segment length. See caption to Fig. 1. Mucosal growth in resected animals increases net movements and fluxes per unit segment length, as compared with data based on mucosal weight shown in Fig. 1.

This suggests that the major effect of resection in ileum is to increase flux into the lumen, thus causing net secretion. This is supported by data on flux into the lumen. Expressed as micromoles/centimeter per hour, ileal flux into the lumen in resected rats is significantly increased compared to transected rats (0.74 ± 0.22 vs 0.25 ± 0.04). Flux into the lumen micromoles per gram dry weight mucosa in the resected group was 48.6 ± 11 compared to 30.2 ± 5.4. However, this difference is not significant, because of the large scatter from calculating the flux as a difference.

Data summarizing net calcium movements and fluxes out of the lumen per segment, i.e. per centimeter segment length, are shown in Fig. 2. Absorption is decreased in duodenum of resected animals ($P < 0.02$), despite a mucosal mass per unit length in this segment which is 50% greater than that of the transected group (Table I). In ileum, transected animals showed net absorption, whereas in resected animals, net secretion occurred. Fluxes out of the lumen per centimeter were similar in duodenum of transected and resected groups. The larger mean flux out of the lumen per centimeter in the ileum of the resected than the transected group (difference not significant), results from the near doubling of mucosa per centimeter in the resected as compared with the transected group. Per unit mucosal dry weight, mean flux out of the lumen in ileum is slightly lower in the resected than in the transected group (Fig. 1b).

The abnormalities in calcium absorption we find early after resection in the growing rat are much greater than those observed late after resection in older animals (1). The prior study involved either proximal or distal resection of 50 cm (about 50%) of small intestine and absorption studies after 6–7 weeks when animals weighed 400 g. The proximal resection is most comparable to our study, since it was accompanied by mucosal growth. Duodenal absorption per gram mucosa was depressed, but was compensated for by mucosal growth, since absorption per centimeter was the same in resected and control groups. There was no effect on ileal calcium absorption. Distal re-

section in this prior study resulted in no mucosal growth: duodenal transport fell and ileal transport rose. We found the defect in duodenal calcium absorption to be only partly overcome by increased mucosal growth. The ileal defect, i.e., calcium secretion, was augmented by mucosal growth in the resected animals. Thus an adaptive response later in the course of resection is suggested by the prior work.

The cause for the marked early depression in duodenal calcium absorption (50% of control value) and the defect in ileal calcium transport (secretion instead of absorption) in resected animals is unknown. The findings are inconsistent with a vitamin D-mediated effect. The duodenum is the small intestinal site most sensitive to vitamin D deficiency (9–14), and the ileum is relatively insensitive. In addition, these animals ingested a diet adequate in vitamin D. The massive mucosal growth response to resection, particularly in the ileum, suggests immaturity of rapidly proliferating cells as the cause for the defect. Crypt cell proliferation is doubled at the time of our studies of calcium absorption (6) as well as at the later time used in previous studies (1). At the later time interval, cell maturity is normal in resected animals, i.e., normal crypt-to-villus transit (5) and ileal cell desquamation (15). Cell life span has not been examined at the time of our study, hence the hypothesis of immaturity of mucosal absorptive cells must be tested.

Consistent with the defect in calcium transport in resected animals is the finding of osteopenia (16) in animals subjected to a similar resection procedure. Multiple clinical studies of calcium metabolism in patients with resection are also consistent with calcium malabsorption. Resection of the small intestine in man produces malabsorption of virtually all nutrients, including calcium (17). Calcium malabsorption and bone disease are most prominent after distal resection, which causes loss of bile salts and steatorrhea and leads to malabsorption of vitamin D and vitamin D deficiency. This long-term process is unlike the rapidly developing defect we observed. It is clear from our data that intestinal calcium transport problems are to be anticipated in patients with resection. This study provides direct evidence for major defects in calcium transport at specific sites. Prior work (1) taken in conjunction with our studies shows an evolution of this lesion with time. The mechanism and treatment of the defect remain to be defined.

Summary. Calcium transport was measured in duodenum and ileum of young rats 7–9 days post 70% resection of mid-small intestine. Duodenal calcium absorption per unit weight of mucosa was depressed to half that of transected controls because of decreased flux out of the lumen. Since absorption per cm segment length of duodenum was also depressed in resected as compared with transected animals, the mucosal hyperplasia secondary to resection did not compensate for decreased absorption per unit weight of mucosa. Ileum of resected animals showed net calcium secretion in comparison with net absorption in transected controls. Possible causes for depression of calcium absorption include abnormal vitamin D metabolism with decreased enterohepatic cycling of polar vitamin D metabolites or immaturity of rapidly proliferating mucosal cells.

We thank Fred Kirchner, Radiation Research Laboratory, University of Iowa, for advice regarding the intestinal resections.

1. Urban, E., and Pena, M., Amer. J. Physiol. 226, 1304 (1974).
2. Schedl, H. P., and Clifton, J. A., Gastroenterology 41, 491 (1961).
3. Petith, M. M., Proc. Soc. Exp. Biol. Med. 160, 28 (1979).
4. Dowling, R. H., and Booth, C. C., Clin. Sci. 32, 139 (1967).
5. Hanson, W. R., and Osborne, J. W., Gastroenterology 60, 1087 (1971).
6. Hanson, W. R., Osborne, J. W., and Sharp, J. G., Gastroenterology 72, 701 (1977).
7. Petith, M. M., and Schedl, H. P., Amer. J. Physiol. 231, 865 (1976).
8. Younoszai, M. K., and Schedl, H. P., Gastroenterology 62, 565 (1972).
9. Urban, E., and Schedl, H. P., Amer. J. Physiol. 217, 126 (1969).
10. Urban, E., and Schedl, H. P., Amer. J. Physiol. 219, 944 (1970).

11. Younoszai, M. K., Urban, E., and Schedl, H. P., Amer. J. Physiol. **225**, 287, (1973).
12. Petith, M. M., Wenger, J. R., and Schedl, H. P., Amer. J. Dig. Dis. **23**, 943 (1978).
13. Petith, M. M., and Schedl, H. P., Amer. J. Physiol. **231**, 865 (1976).
14. Walker, B. E., and Schedl, H. P., Proc. Soc. Exp. Biol. Med. **161**, 149 (1979).
15. Weser, E., and Tawil, T., Gastroenterology **71**, 412 (1976).
16. Simmons, D. J., Hyland, G., Lesker, P., Cohen, M., Stein, T., and Wise, L., Surgery **78**, 460 (1975).
17. Trier, J. S., *in* "Gastrointestinal Disease," (M. H. Sleisinger and J. S. Fordran, eds.), p. 1138. Saunders, Philadelphia (1978).

Received October 4, 1979. P.S.E.B.M. 1980, Vol. 163.