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Intestinal Brush Border Alkaline Phosphatase in the Rat after Proximal Small Bowel Resection[1] (39753)

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Extensive resection of small bowel leads to morphological and functional adaptations of remaining intestine (1-3). Intestinal calcium transport in the rat also undergoes changes after gut resection, the most significant transport modifications occurring after proximal small bowel resection (4). Calcium binding activity of rat intestinal mucosa undergoes changes after intestinal resection but its functional significance in altered postresection calcium transport remains in doubt (5). Intestinal mucosal brush border alkaline phosphatase may play a functional role in calcium transport of the rat (6-8) and chicken (9, 10). The studies described in this report were undertaken in rats 6-7 weeks after extensive proximal small bowel resection to investigate effects of gut resection on intestinal mucosal brush border alkaline phosphatase in segments of remaining duodenum, midgut, and ileum. Comparisons were made with similar segments from a sham-operated control group.

Materials and methods. Male albino rats (Sprague-Dawley strain) weighing 100-140 g were allowed *ad libitum* access to regular commercial rat laboratory chow (Wayne Lab Blox, Allied Mills, Libertyville, Ill.) and water. Food but not water was withheld for 24 hr before and 24 hr after intestinal resection or sham operation and again for 24 hr before sacrifice 6-7 weeks later.

Intestinal resection. Under intraperitoneal sodium pentobarbital anesthesia (40 mg/kg), 50 cm of proximal intestine was resected and continuity of the intestine was restored by end-to-end anastomosis (Fig. 1). Sham-operated animals underwent simple midintestinal transection and reanastomosis without removal of intestinal tissues. Details of operative procedures have been fully described previously (4).

Preparation of purified mucosal brush borders. Six to seven weeks after either proximal resection or sham operation, purified brush borders were prepared by the method of Forstner *et al.* (11) as detailed by Reddy (12) from segments of duodenum, midgut, and ileum in each of the two groups of animals (Fig. 1). In the sham-operated animals the midgut segment was located approximately at mid small intestine. In the resected rats the midgut segment was intestine that had been located at mid small intestine before resection. However, as a result of proximal intestinal resection and reanastomosis, the midgut segment was cephalad compared to the sham group (Fig. 1). For duodenum three 10-cm segments from each of three rats were combined as a tissue pool and used for the preparation of purified duodenal brush borders. For midgut and ileum corresponding 15-cm segments from each of three animals were combined for tissue pools in the preparation of purified midgut and ileal brush borders. Protein content of the purified brush border suspensions was determined by the Lowry method *et al.* (13) using bovine serum albumin as standard. Protein content was calculated as milligrams of protein/gram of wet mucosa. The quality of the final brush border preparations was additionally monitored by electron microscopy.

Enzyme assay: Alkaline phosphatase activities of the mucosal brush border preparations were assayed using *p*-nitrophenyl phosphate as substrate exactly as described by Reddy (12). Incubation was at 37° and *p*-nitrophenol liberated by the reaction was measured at 410 nm in a Beckman DG-GT

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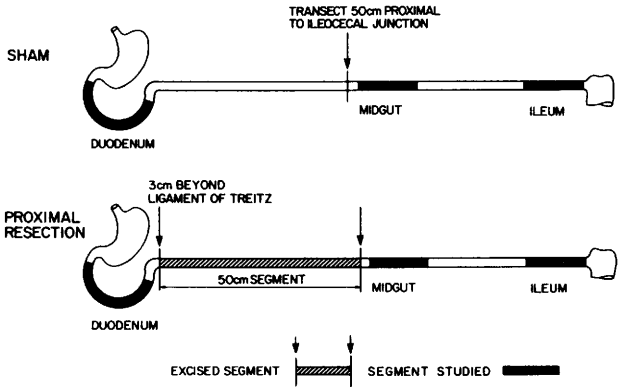

FIG. 1. Diagram showing the locations of intestinal transection for sham-operated rats and excised segment of the resected rats. Length of small intestine from pyloric sphincter to ileocecal junction was approximately 100 cm before resection. Also shown are the locations of segments utilized in tissue pools for the preparation of mucosal brush borders 6–7 weeks later.

recording spectrophotometer. Brush border alkaline phosphatase activity was expressed in two ways: (i) specific activity of alkaline phosphatase, defined as activity per gram of wet mucosa; and (ii) total alkaline phosphatase activity, defined as activity per centimeter of segment length.

Skeletal calcium: In each experimental animal after sacrifice a femur was excised, cleaned of adherent tissues, dried overnight in a vacuum oven at 80°, and weighed. The bone was ashed in a muffle furnace at 500° for 16 hr. The ash was weighed, then extracted with 5 ml of 1 *N* HCl, ash and acid being gently agitated for 30 min. An aliquot of the liquid extract was assayed for calcium by atomic absorption spectrometry. Bone data were tabulated as dry weight of bone, bone ash, organic matrix (dry bone weight minus bone ash), concentration of calcium in bone, and total calcium content of the bone.

All experimental results are given as mean values ± 1 SEM. Corresponding data from the sham-operated and proximally resected groups of animals were analyzed statistically by the student *t* test. *P* values less than 0.05 were considered to indicate statistically significant differences.

Results. Postoperative mortality after intestinal resection was 20%. This was similar to our prior experience (4, 5). Table I shows data of the survivors. Neither initial nor final body weights of the sham-operated and resected groups of animals differed. Assay of the femur, a representative sample of rat skeleton, also showed the same bone weight, bone ash, organic matrix, concentration of calcium, and total calcium content in sham-operated and resected groups of rats at sacrifice. Table I also shows the effects of proximal resection on duodenal, midgut, and ileal mucosal mass. After proximal resection significant increases occurred in wet mucosal mass of duodenum ($P < 0.02$) and midgut ($P < 0.02$) but the increased mucosal mass of ileum failed to reach statistical significance.

Brush border studies: Table II summarizes data obtained from the purified brush borders in the sham-operated and proximally resected groups of animals. In each group, the protein content of the brush border suspensions differed slightly between segments. However, protein concentrations of brush border preparations from corresponding duodenal, midgut, and ileal segment pools of the two experimental groups did not differ ($P > 0.05$). This indicates similar degrees of purity of the brush border suspensions in the corresponding segment pools of sham-operated and resected animals.

In both sham-operated and resected rats enzyme specific activity per gram of wet mucosa and total enzyme activity per centimeter of gut length exhibited a proximal-to-distal gradient with the duodenum being

TABLE I. GROUPS STUDIED: CONTROL AND RESECTED RATS.[a]

Parameters measured	Sham operated (n = 21)	Proximal resection (n = 21)
Body weight (g)		
Initial	122 ± 20	129 ± 13
Final	355 ± 28	331 ± 37
Bone (femur)		
Weight of dry bone (g)	0.465 ± 0.015	0.443 ± 0.021
Bone ash (g)	0.257 ± 0.010	0.253 ± 0.012
Organic matrix (g)	0.210 ± 0.007	0.195 ± 0.010
Ca concentration (mg/g of dry bone)	264 ± 6.0	255 ± 6.3
Total Ca in bone (mg)	122 ± 5.1	113 ± 4.5
Mucosal mass (mg wet wt/cm gut length)		
Duodenum	25.2 ± 1.5	35.2 ± 3.6*
Mudgut	20.3 ± 2.2	28.7 ± 2.8*
Ileum	13.1 ± 1.3	17.2 ± 1.8

[a] Values are means ± 1 SEM; n is the number of animals studied. Initial body weights were at operation; other data were obtained at sacrifice 6–7 weeks later.

* Indicates significant difference from corresponding data of the sham-operated group.

TABLE II. BRUSH BORDER PREPARATION PROTEIN CONTENT AND ALKALINE PHOSPHATASE ACTIVITIES.[a]

Parameters measured	Sham operated	Proximal resection
Brush border protein (mg/g of wet mucosa)		
Duodenum	2.52 ± 0.30 (6)	3.57 ± 0.85 (7)
Midgut	1.76 ± 0.30 (6)	2.56 ± 0.82 (7)
Ileum	1.92 ± 0.39 (7)	1.67 ± 0.59 (7)
Alkaline phosphatase[b] specific activity/g of wet mucosa		
Duodenum	37.7 ± 4.3 (6)	23.3 ± 3.1* (7)
Midgut	0.20 ± 0.01 (6)	1.09 ± 0.36* (7)
Ileum	0.15 ± 0.04 (7)	0.06 ± 0.03 (7)
Alkaline phosphatase[b] (total activity/cm gut length)		
Duodenum	0.922 ± 0.168 (6)	0.980 ± 0.166 (7)
Midgut	0.006 ± 0.001 (6)	0.034 ± 0.014* (7)
Ileum	0.002 ± 0.001 (7)	0.001 ± 0.001 (7)

[a] Values are means ± 1 SEM; numbers in parentheses are the number of tissue pools upon which the determinations were made. Each tissue pool was derived from three animals.

[b] Expressed as μmole of p-nitrophenol released/min.

* Indicates significant difference from corresponding data of the sham-operated group.

greater than midgut or ileum ($P < 0.001$), although in the sham-operated group there were no significant differences between mean midgut and ileal alkaline phosphatase specific and total activities: The midgut segment of this study was essentially proximal ileum rather than jejunum (Fig. 1). The greater proximal enzyme activity is consistent with prior descriptions in unoperated rats (6–8). However, in resected animals duodenal alkaline phosphatase specific activity was 38% less than in the sham-operated group ($P < 0.01$), whereas total activity of duodenal alkaline phosphatase remained unchanged. Duodenal mucosal mass increased 40% after resection (Table I). The increased duodenal mass thus effectively compensated for the decreased duodenal enzyme specific activity. In contrast to duodenum, in the midgut both alkaline phosphatase specific activity and total activity increased more than fivefold after resection ($P < 0.001$). Midgut mucosal mass increased after resection (Table I) but this increase was small (41%) compared to the fivefold increases in specific and total enzyme activities.

Discussions. This is the first study of enterocyte brush border alkaline phosphatase associated with extensive proximal small

bowel resection. The effects of resection varied with the segment of intestine. Midgut, and not duodenum, was the site of maximal adaptive enzyme change. The intestinal resections performed in this study approximated 50% small intestine (4, 14). As much as 67% intestine may be resected in rats without long-term loss of body weight (1). The resected animal of this study achieved the same body weight as the sham operated by 6–7 weeks, consistent with our prior experience (4, 5) and the experience of others (2, 15).

In this study wet mucosal mass after resection increased in duodenum and midgut. It was not possible to prepare mucosal brush borders and simultaneously determine mucosal tissue water content in the same animals. Thus we could not directly substantiate unchanged tissue water. However, in prior experiments with similar resections we have shown an increase of dry mucosal mass without alteration of percentage of tissue water in three similar segments, ileum having the smallest mass increase (4). Others have also well documented hyperplasia of remaining small bowel mucosa in animals after extensive intestinal resections (3, 15–18).

Bone weights and bone mineralization were utilized as measures of skeletal maturation and adequacy of intestinal absorption of dietary calcium 6–7 weeks after resection (19). There were no differences between the measured parameters in the skeleton of sham-operated and resected rats. These data imply that after 50% proximal small bowel resection the animals achieved not only overall nutritional adequacy as shown by similar body weights, but in addition the post resection compensatory changes of intestinal calcium absorption (4) permitted normal skeletal development. Similar reasoning implies that it is also unlikely that the resected animals were vitamin D-deficient at sacrifice.

Vitamin D-repleted compared to vitamin D-deficient rats have increased intestinal alkaline phosphatase activity. An early study has reported the major effect in jejunum (6). A more recent study restricts the vitamin D effect on brush border alkaline phosphatase activity to duodenum. More caudal intestinal sites remained unaffected by vitamin repletion (7). Vitamin D repletion has been shown to increase *in vivo* duodenal but not ileal calcium transport (20). Similarly, dietary calcium restriction in the rat also increased duodenal brush border alkaline phosphatase activity and there were no effects in more distal small bowel. However, calcium transport increased not only in duodenum but also in more distal small intestine (8). Thus relationships between brush border alkaline phosphatase and calcium transport appear to hold only for duodenum.

A similar relationship between duodenal brush border alkaline phosphatase activity and calcium transport seems to hold after intestinal resection. Because it was not possible to prepare brush border suspensions and to perform calcium transport studies simultaneously in the same animals, comparisons are made using transport data from separate but similar animals that had undergone identical sham operations and proximal small bowel resections. The two studies were performed by the same personnel (4). The transport studies showed that after proximal resection mucosal transport specific activity (transport per gram of mucosa) decreased 37% in duodenum, increased maximally in midgut (67%), and remained unchanged in ileum. Duodenal mucosal mass increased 47%, effectively compensating so that the duodenal segment transport capacity (transport per centimeter of intestinal length) was the same in resected as in sham-operated animals. Midgut was the site of maximally increased segment transport capacity (117%) where increases of both mucosal transport specific activity and mucosal mass occurred together. Ileal transport specific activity remained unaltered and segment transport capacity increased in parallel with a small increase of ileal mucosal mass (4).

It will be readily apparent that the directional changes of brush border enzyme specific activities in this study parallel the altered calcium transport specific activities in all three intestinal segments. It is striking in duodenum how closely the decline of post-resection microvillus alkaline phosphatase specific activity mirrors the decreased duo-

denal transport specific activity, while in both studies increased duodenal mucosal mass compensated completely so that total duodenal enzyme activity and segment transport capacity remained unaffected. Consequently it is tempting to postulate that enterocyte brush border alkaline phosphatase plays an important role in the postresection adaptation of duodenal calcium transport. However similarities between duodenal calcium transport and duodenal mucosal brush border alkaline phosphatase do not automatically signify functional involvement and could be fortuitous. In midgut the postresection changes of alkaline phosphatase and calcium transport are parallel only in direction but not in magnitude of change. The more than fivefold increased enzyme specific activity and total enzyme activity far outweigh the 67% rise of transport specific activity and 117% increase of transport capacity in midgut (4). Thus while midgut was the site of maximum adaptation in both studies there must remain considerably more doubt about a functional involvement of midgut brush border alkaline phosphatase with calcium transport. In ileum the unaltered postresection enzyme specific activity and transport-specific activity allow for no speculations about interrelationships.

If microvillus enterocyte alkaline phosphatase does play a role in postresection duodenal calcium transport adaptations, then it is logical for alkaline phosphatase to participate in the uptake of calcium at the luminal cell surface, perhaps by facilitating diffusion into the enterocyte. A nonenergy-dependent carrier-mediated calcium transport process acting on the luminal side of the mucosal cell has been demonstrated in the rat (21, 22). A recent study has shown increased calcium uptake by isolated duodenal brush border vesicles after dietary calcium restriction (23). Two brush border proteins with alkaline phosphatase activity have now been described in chick duodenal mucosa. However only one of these proteins had calcium binding activity. Its appearance after vitamin D repletion correlated closely with onset of increased calcium transport (24).

Summary. In rats 50 cm of proximal intestine were resected with preservation of duodenum and ileum. Mucosal brush border alkaline phosphatase was measured 6–7 weeks later in segments of duodenum, ileum, and a midgut segment located preresection at mid small intestine. Sham-operated animals served as controls. After resection there was significant mucosal growth in duodenum and midgut. In duodenum brush border alkaline phosphatase specific activity (activity per gram of wet mucosa) decreased, but increased mucosal mass compensated so total enzyme activity (activity per centimeter of intestinal length) did not change. In midgut both enzyme specific activity and total activity increased maximally while in ileum neither enzyme specific activity nor total activity changed. These studies show that brush border alkaline phosphatase undergoes postresection changes but alterations vary with the location of the intestinal segment studied. Comparisons with an earlier study of calcium transport suggest that duodenal brush border alkaline phosphatase may play a role in postresection transport adaptation.

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