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### Research Article

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# Changes in Sucrase, Enterokinase, and Peptide Hydrolase after Intestinal Resection

## THE ASSOCIATION OF CELLULAR HYPERPLASIA AND ADAPTATION

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**ABSTRACT** In a study of changes in digestive enzymes after massive intestinal resection and the mechanisms by which such changes occur, rats were sacrificed 4 wk after removal of the proximal two-thirds of the small intestine. Alterations in the mucosal levels of sucrase, enterokinase, and dipeptide hydrolase (L-leucyl-L-alanine substrate) were examined in the light of associated changes in protein, DNA and wet mucosal weight, measured in standardized gut segments from various regions of intestine.

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of increases in total activity, enzyme activity per milligram DNA fell by over 50% in postanastomotic segments. Subcellular distribution studies showed no change in the percentage of the total activity which was membrane-bound and zymograms confirmed that no new dipeptide hydrolase had appeared after resection.

It is concluded that increases in the segmental totals of various enzymes seen after resection are achieved by disproportionate increases in the number of mucosal cells per segment and that the greatest change in a particular enzyme occurs in the region where the enzyme is normally found in highest concentration.

## INTRODUCTION

From the earliest studies of Senn in 1888 (1) and Flint in 1912 (2) it has been appreciated that massive resection of the small intestine in man and animals is followed by morphological and functional change in the remaining portion. More recently it has emerged that proximal and distal resections have different consequences (3). Resection of the proximal two-thirds of the small intestine causes the greatest morphological change or "compensatory hypertrophy," with little or no nutritional disturbance to the animal (3, 4) while distal resection is associated more with weight loss and malabsorption (4, 5), gastric hypersecretion (6), enhanced gastric emptying (7), and reduced intestinal transit time (5, 7). Whether proximal or distal factors underlie the reported disturbances in ileal blood flow (8), vitamin A transport (9), or mucosal cell kinetics (10-12) is still uncertain.

After proximal resection perfused segments of the remaining small intestine show an increasing capacity to transport glucose in both man (13) and animals (14, 15), such changes being maximal at 4 wk. Conflicting studies have reported that cellular or tissue levels of disaccharidase enzymes are reduced (16) or unchanged (15) in similarly resected animals. The first objective of the present study was to examine the effect of resection on both specific activity and total activity per segment of the disaccharidase sucrase.

In humans, gradual restoration towards normal of the initially elevated nitrogen content of fistulous chyme (17) or feces (18) after resection, occurring during a period when methionine absorption is also returning to normal (19), suggests that there may be adaptive changes in the digestion and absorption of nitrogenous materials. Similar changes in nitrogen excretion in feces have been shown in rats subjected to 75% proximal small bowel resection (4). Tissue levels of enterokinase may be increased postoperatively (20), but the role of peptide hydrolases, the enzymes thought to be most intimately concerned with such functions, has not been examined. Hence, the second objective of this study was to assess the effect of resection on intestinal levels of enterokinase and, more, importantly, on those of a single peptide hydrolase (leu-ala-substrate), representative of a broad class of peptide hydrolases (21). Since dipeptide hydrolase activity is known to be associated mainly with two subcellular fractions, the cytosol (80–90%) and the brush border (5–15%), and since enzymes in these two loci may subservise different physiological functions (21), the second part of the study included an examination of the possibility that resection might either alter the subcellular distribution of the enzyme or result in the appearance of new peptide hydrolases in soluble or membrane-bound forms. A third objective was to document the biochemical changes accompanying tissue hypertrophy (3), in terms of the protein content, DNA content, and mucosal weight of standard segments of intestine and to apply this information to the interpretation of associated changes in enzyme activities.

## METHODS

**Materials.** Diets of S/L "maintenance" chow<sup>1</sup> were fed to the rats. The DNA standard used was polymerized calf thymus DNA.<sup>2</sup> Trypsinogen employed in the enterokinase assays was a nondialyzed preparation crystallized in 50% MgSO<sub>4</sub>.<sup>3</sup> The chromatographic purity of *p*-tosyl-L-arginine-methylester-HCL (L-TAME),<sup>4</sup> the substrate used in tryp-

<sup>1</sup> Simonsen Labs Inc., Gilroy, Calif.

<sup>2</sup> Sigma Chemical Co., St. Louis, Mo.

<sup>3</sup> Worthington Biochemical Corp., Freehold, N. J.

<sup>4</sup> Abbreviations used in this paper: L-TAME, *p*-tosyl-L-arginine-methylester-HCL; S1, supernatant fluid; S2, supernatant.

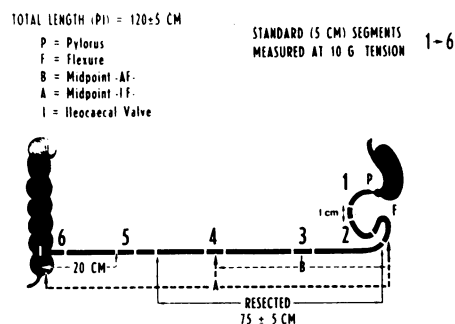


FIGURE 1 Standard segments of rat small intestine used in studies of normal distribution of enzyme activities and of changes in these after resection.

sin assays, has been attested by the manufacturer.<sup>5</sup> The materials used in the preparation of the zymograms have been described in detail previously (21). L-Leucyl-L-alanine was obtained commercially<sup>2</sup> for use in peptide hydrolase assays.

**Animals and diet.** Six male Sprague Dawley rats of Wisconsin strain, of mean weight 385 g at the time of death, served as controls. Five similar rats of mean weight 356 g at the time of final killing and 323 g at the time of operation served as the resected group. Control and resected rats were from the same batch and kept for at least 4 wk before study, during which time weekly weighings and studies of growth rate, food consumption, stool weight, and dietary and fecal nitrogen were performed. The rats were in metabolic cages during collection periods and in free cages at other times. Diet was supplied either in ground powder form during metabolic studies or in pellet form during ordinary feeding. Water was supplied ad lib. at all times. Sucrose (5% wt/vol) was added to the drinking water of resected rats for the first 48 h after surgery. Thereafter rats returned to a standard diet, though generally eating little before the 4th day.

**Operation.** After an overnight fast, without water restriction, rats for resection were anesthetized with diethyl ether or those for sacrifice were stunned by a blow on the head and decapitated. In control rats, the abdomen was opened immediately after decapitation in the cold room and the small intestine, from the pylorus to the ileocecal valve, was removed in one piece. When fat and mesentery had been dissected away, the intestine was washed in ice-cold 0.9% NaCl, suspended vertically in front of a meter stick with a 10 g weight attached to its lower (ileal) end, and its length measured. Keeping the bowel at constant tension, standardized 5 cm long segments of gut were then marked out at six areas along its length and excised. These are shown diagrammatically in Fig. 1. Segments 1 and 2 were duodenal and defined by their distance from the pylorus; segments 5 and 6 were ileal and defined by their distance from the ileocecal valve; segment 4 was 2.5 cm either side of point A, located half-way between the ligament of Treitz (F) and the ileocecal valve (I) and in the rat is comprised of upper ileum; segment 3, 2.5 cm either side of point B, located half-way along its length and excised. These are shown diagrammatically in Fig. 1. Segments 1 and 2 were duodenal and defined by their distance from the pylorus; segments 5 and 6 were ileal and defined by their distance from the ileocecal valve; segment 4 was 2.5 cm either side of point A, located half-way between the ligament of Treitz (F) and the ileocecal valve (I) and in the rat is comprised of upper ileum; segment 3, 2.5 cm either side of point B, located half-way along its length and excised. These are shown diagrammatically in Fig. 1. Segments 1 and 2 were duodenal and defined by their distance from the pylorus; segments 5 and 6 were ileal and defined by their distance from the ileocecal valve; segment 4 was 2.5 cm either side of point A, located half-way between the ligament of Treitz (F) and the ileocecal valve (I) and in the rat is comprised of upper ileum; segment 3, 2.5 cm either side of point B, located half-way along its length and excised. These are shown diagrammatically in Fig. 1.

<sup>5</sup> Mann Research Lab., Inc., New York.

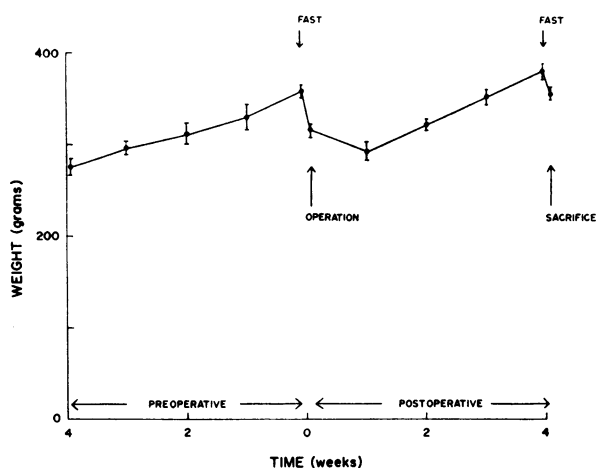


FIGURE 2 Serial changes in mean weights ( $\pm$ SD) of five rats subjected to excision of 70–80 cm of proximal small intestine at time 0 and sacrificed 4 wk later for biochemical studies.

an adequate blood supply to the remaining bowel. End-to-end entero-anastomosis was then carried out by the method of Lambert (22), using 5–0 mersilene sutures inserted with the help of an operating magnifier. 5 mg of streptomycin sulphate and 60,000 U of crystalline procaine penicillin G, dissolved in 0.25 ml of 0.9% saline were introduced into the peritoneal cavity and the abdomen closed in two layers, the first of 00 chromic catgut continuously sutured peritoneum, muscle, and fascia and the second layer, composed of skin alone, was formed with metal clips inserted so that no catgut was accessible to gnawing. Rats were then returned to their cages and allowed to recover. 4 wk later, after sacrifice, the whole intestine was removed from each animal and its length measured in a manner similar to that employed in controls, though on this occasion only standard segments 2, 5, and 6 which remained, were excised and processed for biochemical studies. Segment 5 generally lay 10–20 cm distal to the anastomosis. Also excised were 2-cm segments of gut, immediately adjacent to standard segments 2, 5, and 6 in both normal control and resected animals, for use in the assays of sucrase.

**Preparation of tissues.** After excision the 5-cm segments were washed and gently blotted. Mucosae were scraped on a chilled glass plate, the scrapings weighed and immediately homogenized in 5 ml of 14% glycerol at 4°C with eight strokes of a Potter-Elvehjem homogenizer (Kontes Glass Co., Vineland, N. J.) (clearance 0.004–0.006 cm). Samples were then mixed with a Vortex mixer (Vortex Co., Claremont, Calif.) for 20 s and a 1 ml sample taken for homogenate measurements. The remaining homogenate was spun at 45,000 *g* for 2 h, which yielded a separation of soluble and membrane-bound enzymes similar to that described in previous studies (21). The supernatant fluid (S1) was decanted. The pellet was resuspended in 2.5 ml of 14% glycerol, reagitated for several minutes, until it was fully dispersed, and centrifuged at 45,000 *g* for 1 h. The supernatant (S2) was decanted and added to the first (S1); these two (S1 and S2) combined constituted the “soluble fraction.” The pellet was suspended in 2 ml of 14% glycerol and this suspension constituted the “particulate fraction.” These fractions were used only in studies of dipeptide hydrolase. Glycerol homogenates were used for

determinations of protein, DNA, and the activities of enterokinase and dipeptide hydrolase. The 2-cm segments were similarly measured, washed, blotted, scraped, and weighed. The mucosal scrapings were homogenized as before, 1 ml of 0.9% saline replacing the 5 ml of 14% glycerol, and used for measurements of protein and sucrase activity.

**Protein.** Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (23) using a standard of bovine serum albumin.

**DNA.** DNA was rendered free of RNA by serial precipitations in cold 0.2 N perchloric acid and alkaline hydrolysis by the method of Schmidt and Tannhauser, modified as suggested by Munro and Fleck (24). DNA was measured by the method of Cerriotti (25), omitting lipid extraction until the end of color development, when samples were thrice extracted with an equal volume of chloroform. Polymerized calf thymus DNA was used as a standard.

**Enterokinase.** Enterokinase activity in homogenates was measured by the method of Hadorn, Tarlow, Lloyd, and Wolff (26) with the modification that Tris-maleate buffer was replaced in the activation mixture (ionic strength 0.125 M) by 0.0625 M citric acid/citrate and 0.0625 M maleic acid/maleate buffer at pH 5.8. Preliminary studies in this laboratory<sup>6</sup> suggested that this was a more suitable buffer system. When enterokinase was omitted, the trypsinogen did not auto-activate under the conditions of the assay. Trypsin produced under the action of enterokinase was assayed by measuring the rate of hydrolysis of L-TAME (0.01 M) at pH 8.1 in 0.046 M Tris-HCl buffer and 0.046 M CaCl<sub>2</sub>, from recordings of the rate of change of absorbance at 247 nm in a recording spectrophotometer at 25°C. 1 Unit of trypsin is defined as the amount of the enzyme which hydrolyses 1  $\mu$ mol of L-TAME per minute. 1 U of enterokinase is that which activates this much trypsin per minute from trypsinogen.

**Peptide hydrolase.** Activity of the dipeptide hydrolase responsible for the hydrolysis of L-leucyl-L-alanine was measured by a ninhydrin method, the modification of the Yemm and Cocking procedure proposed by Matheson and Tattie (27). Full details of this assay have been published previously (21). Zymograms were carried out by the methods described in detail elsewhere (21).

**Sucrase.** Activity of sucrase in saline homogenates was measured by the method of Dahlqvist (28).

**Statistics.** Comparisons between data from control and resected animals were performed by means of Student's *t* test. Differences in all figures are significant at the level of  $P < 0.001$  unless otherwise indicated; NS indicates that differences were not significant.

## RESULTS

**Effect of operation on growth.** The effect of massive proximal intestinal resection on the mean weights of five rats is shown in Fig. 2. Weekly weighings showed a progressive weight gain in the 4 preoperative wk; this pattern was very similar in controls. It can be appreciated that apart from the week immediately after operation, when animals suffered a mean weight loss of 25.2 g, in addition to a mean loss of 43.6 g sustained during the preoperative fast, their growth rates were not impaired by the operation and in fact were some-

<sup>6</sup> D. M. McCarthy and Y. S. Kim. Manuscript in preparation.

what increased in the 2nd and 3rd postoperative wk when compared to controls or to their own preoperative growth rates. The magnitude of this difference may be judged from the data in Table I. An inspection of Fig. 2 reveals that controls may be matched either for weight or age but not both simultaneously, as the effect of operation is to retard growth by the equivalent of 3–4 wk. The data presented on enzyme activities are in respect of weight-matched animals but similar conclusions were reached when data from resected animals were compared with data from independently studied age-matched animals, 60 g heavier in weight. In the present studies the normal growth pattern had been reestablished for at least 3 wk before sacrifice in resected animals and weights at this time were close to those of fully grown rats ( $410 \pm 10$  g).

**Metabolic data.** Pertinent metabolic data, illustrating the key features of the effect of operation, are shown in Table I. Values for control rats varied little from week to week until rat weights reached about 400 g, when a plateau effect was seen. Rats varied greatly in their immediate response to operation, especially in terms of stool output and food intake and no meaningful metabolic data were obtained in the 1st postoperative wk. In the 2nd postoperative wk there were significant increases in stool weight and fecal nitrogen though these did not correlate, the change in nitrogen excretion being relatively much greater. There was also an increase in growth rate. By the 4th wk stool weight and growth rate did not differ significantly from controls and only fecal nitrogen was still significantly increased though the value was much closer to normal.

TABLE I  
Metabolic Data\* From Control and Resected Animals

| Parameter            | Control‡ (6) | Resected (5)                     |  |
|----------------------|--------------|----------------------------------|--|
|                      |              | 2nd wk postoperation             | 4th wk postoperation                             |
| Growth rate (g/wk)   | 22 ± 11.4    | 30.6 ± 5.3<br><i>P</i> < 0.01§   | 26.8 ± 4.2<br><i>P</i> < 0.10                    |
| Stool weight (g/wk)  | 54.4 ± 5.1   | 66 ± 7.7<br><i>P</i> < 0.001     | 55 ± 8.9<br><i>P</i> = NS<br>( <i>P</i> < 0.005) |
| Food intake (g/wk)   | 147.5 ± 8.0  | 139.2 ± 16.3<br><i>P</i> < 0.005 | 155.3 ± 7.7<br><i>P</i> < 0.01                   |
| Fecal nitrogen × 100 | 23.9 ± 2.2   | 32.8 ± 1.9<br><i>P</i> < 0.001   | 27.2 ± 2.0<br><i>P</i> < 0.001                   |
| Dietary nitrogen     |              |                                  | ( <i>P</i> < 0.001)                              |

\* Means ± SD: data applies to 1 wk totals. The number of animals in each group is shown in parenthesis.

‡ 1 wk total is mean value over 4 wk.

§ "P" values (unbracketed) apply to differences between either resected group and normal controls. "P" values (bracketed) in column 4 apply to differences between the 2nd and 4th wk in resected animals.

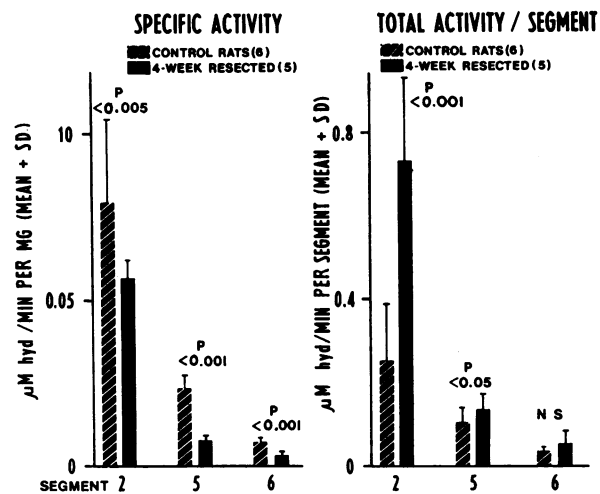


FIGURE 3 The effect of resection on sucrase activity in segmental homogenates. The number of animals in each group is shown in parenthesis.

**Sucrase.** Results of sucrase assays are shown in Fig. 3. In controls, enzyme specific activity was high in proximal and low in distal segments. In resected rats the specific activity was reduced after operation, the greatest reduction (60%) occurring in the segment just distal to the anastomosis. A lesser (30%) but statistically more significant fall occurred proximally in segment 2. Means of total protein in each segment increased considerably in all segments, contributing markedly to the fall in specific activity. Thus when enzyme activity was expressed as the total per segment shown on the right of Fig. 3, a different pattern emerged. Activity increased 2.9-fold in segment 2, 1.2-fold in segment 5 and was unchanged in segment 6.

**Enterokinase.** In control animals enterokinase could be detected only in the first three segments, diminishing sharply with caudad progression. Activity fell almost

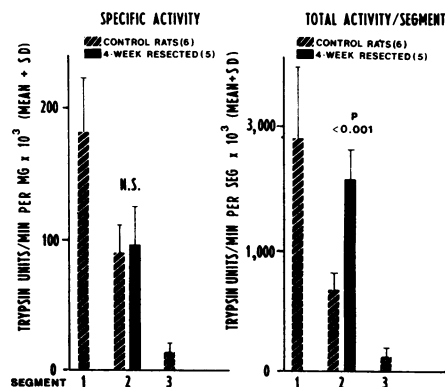


FIGURE 4 The effect of resection on enterokinase activity in segmental homogenates. Enzyme activity could not be detected in ileal segments.

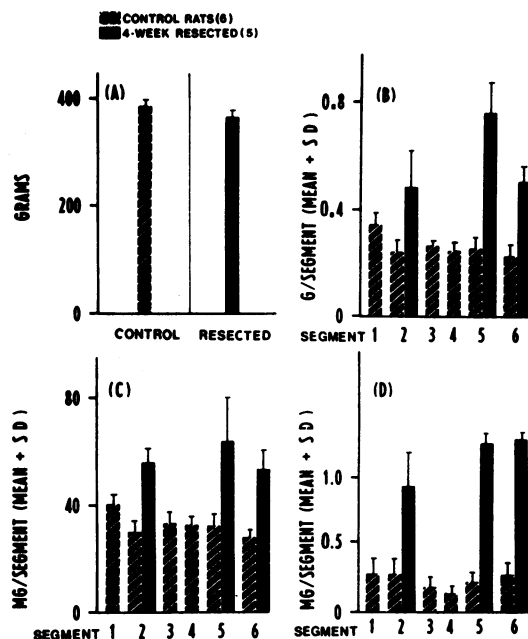


FIGURE 5 Comparison of control and proximally resected rats with respect to body weight (A) and mean totals per standard intestinal segment of wet weight of mucosa (B), protein (C), and DNA (D).

50% between the first and second duodenal segments in fasted animals; in no rat, either control or experimental, was activity detected in ileal homogenates. Of the excised segments from resected rats only segment 2 could be used for comparison with controls. These results are summarized in Fig. 4. While there was no significant change in specific activity of the enzyme, total activity per segment increased two- to threefold in segment 2.

*General tissue parameters.* Data compiled from 5 cm segments are summarized in Fig. 5, which shows the considerable effect of resection on wet mucosal weights,

TABLE II  
Mean Values and Ratios of Means for a Number of General Tissue Parameters, in Control and Resected Animals

|                            | Segment 2 | Segment 5 | Segment 6 |
|----------------------------|-----------|-----------|-----------|
| <b>Mg DNA/g wet wt</b>     |           |           |           |
| Control (C)                | 1.22      | 0.84      | 1.21      |
| Resected (R)               | 1.93      | 2.01      | 2.19      |
| Ratio (R/C)                | 1.57      | 2.38      | 1.80      |
| <b>Mg protein/g wet wt</b> |           |           |           |
| Control (C)                | 128.26    | 122.21    | 119.41    |
| Resected (R)               | 116.04    | 102.92    | 91.44     |
| Ratio (R/C)                | 0.90      | 0.84      | 0.77      |
| <b>Mg protein/mg DNA</b>   |           |           |           |
| Control (C)                | 104.69    | 144.95    | 98.34     |
| Resected (R)               | 60.37     | 51.21     | 41.70     |
| Ratio (R/C)                | 0.58      | 0.35      | 0.42      |

total protein, and total DNA contents of standard segments in animals of similar body weight (Fig. 5A). All are significantly increased in resected animals. The greatest changes occurred in segment 5, just distal to the anastomosis. The parameter showing the greatest increase relative to controls was DNA content, which increased three- to fivefold. Controls were deliberately chosen at a slightly heavier body weight, to offset the effects of any correlation between body mass and mucosal weight per unit length of intestine.

Additional data, showing changes in wet weight of intestinal mucosa and relative to each other, are shown in Table II. Again there is a marked increase in DNA per gram of tissue accompanied by a slight fall in protein per gram of tissue and a marked reduction in the protein/DNA ratio.

### Peptide hydrolase studies

*Homogenate.* The results of assays of dipeptide hydrolase activity in homogenates from control and resected animals are shown in Fig. 6. The greatest differences between the two groups are seen when the data are expressed per segment, and are especially marked in the segments just distal to the anastomosis. As shown in Fig. 5 there was a marked increase in mucosal weight per standard segment after resection. Peptide hydrolase activity per gram wet weight of mucosa (Fig. 6), apart from a slight though significant increase in duodenum, was not altered to any significant extent. The specific activity was increased in all segments by

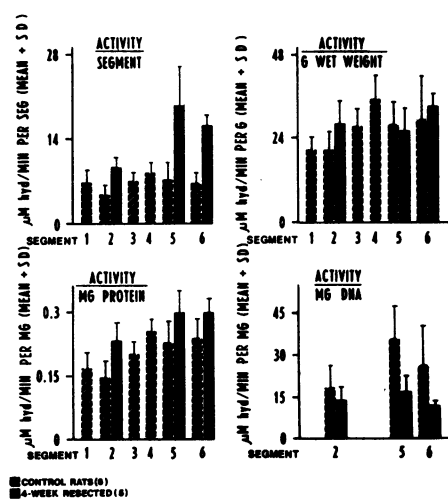


FIGURE 6 Changes in dipeptide hydrolase activity (L-leucyl-L-alanine substrate) in intestinal homogenates after small bowel resection. Changes are significant at the level of  $P < 0.001$ , except for the changes in activity/gram wet weight in segments 5 and 6 and activity/milligram DNA in segment 2, which failed to reach significance.

comparable amounts though a somewhat greater increase was seen in the more proximal segment 2. By contrast, when enzyme activity was expressed per milligram DNA a very different pattern emerged, with over 50% reductions in the postanastomotic segments 5 and 6. Proximally, the mean value was also reduced but the difference was not statistically significant.

**Particulate fraction.** The effect of resection on the particulate fraction as depicted in Fig. 7, shows that there is a significant regional variation in both total and specific activities of the particulate enzyme. These range from low values in duodenum to a maximum in the proximal ileum and fall again in the terminal ileum. This distribution was consistently observed. In resected animals, specific activity was significantly increased throughout the intestine; the greatest increase (twofold) again was seen in the duodenum.

Relatively greater changes were seen in the total enzyme activity per segment and large increases (about threefold) were seen in the segments distal to the anastomosis. In controls, the pattern of regional variation of total activity per segment was slightly different from that observed for specific activity but apart from a more proximally located peak activity, it was broadly similar in that high levels were seen in the mid gut and ileum. Zymograms of both particulate and soluble fractions showed no evidence of cross-contamination.

**Soluble fraction.** Examination of Fig. 8 shows that, after resection, levels of soluble enzyme rose proximally and distally in a manner similar to that of the particulate fraction, though there was relatively much wider variation among animals in the level of soluble enzyme. Specific activity again showed the largest increase in the duodenum, with greater changes in total activity being seen in the distal segments.

**Particulate/soluble distribution.** The relative changes in particulate and soluble enzyme activities are illus-

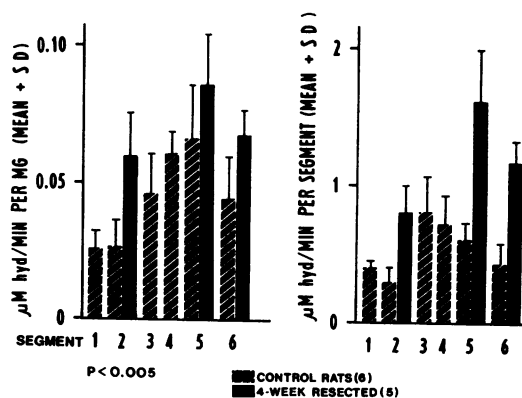


FIGURE 7 Effect of resection on dipeptide hydrolase activity in particulate fractions of various segments of intestine, 1-6.

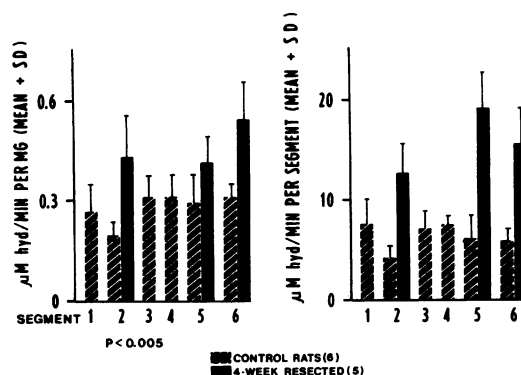


FIGURE 8 Effect of resection on dipeptide hydrolase activity in soluble fractions of various segments of intestine, 1-6.

trated in Fig. 9, which shows data from two representative areas of change, i.e., duodenum (segment 2) and postanastomotic ileum (segment 5), in both control and resected animals. In proximal segments of the controls, the particulate enzyme activity amounted to about 6% of the total activity whether expressed as a percentage of the total activity in the homogenate or of the sum of the activities in particulate and soluble fractions (as in Fig. 9). This figure reached 8% in ileum, segment 5. After resection the total activity in soluble and particulate fractions per standard segment of intestine rose considerably but the percentage of the total activity associated with the particulate fraction (shown under the abscissa) remained unchanged.

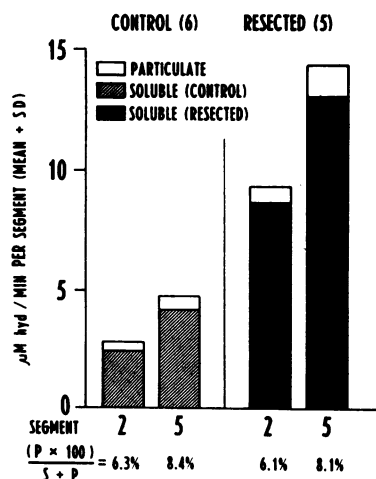


FIGURE 9 Comparison of totals of dipeptide hydrolase activity in soluble and particulate fractions from two regions of intestine in control and resected rats: on the bottom line is given the percentage of the total activity found in the particulate fraction. Abbreviations: S, soluble; P, particulate.

*Zymograms.* Zymograms confirmed the previously described (21) differences between soluble and particulate fractions in control animals. In the resected group corresponding bands were all present and no new bands were seen. In the absence of solubilization (21) the particulate fraction was devoid of enzyme activity.

## DISCUSSION

The significant falls in fecal nitrogen and stool weight in rats, between the 2nd and 4th postoperative wk are in agreement with earlier observations (4, 17, 18) and suggest intestinal adaptation in protein assimilation after resection, although the possibility that simple transection of the gut may make some contribution to the observed changes cannot be entirely excluded. Any change due to transection is likely to be insignificant in comparison to the effect of the massive resection, since it has been shown (4) that resections of 25% of the proximal small bowel were not followed by increased nitrogen excretion or abnormal growth curves: on the other hand resection of 75% of the proximal small intestine (4) was associated with postoperative elevation of fecal nitrogen at 6–11 days, similar to that observed in the present study, which returned to normal gradually over a 9 mo period after operation.

Associated elevations in tissue levels of enterokinase were documented but applied only to the proximal small bowel. This latter effect might favor more complete or more rapid activation of trypsinogen, with consequent increases in the luminal concentrations of peptones and oligopeptides derived from dietary or endogenous protein. However, without increases in the activities of peptide hydrolase enzymes, similar to that found in this study, facilitated activation of trypsinogen might have little direct effect on nitrogen assimilation.

The activity of peptide hydrolases in intestinal mucosa may be influenced by many factors, e.g. diet (29), fasting (30), chronic malnutrition (31), steroid administration (32), and the type of substrate used to measure them (21, 33). Hence there is a need to ensure careful matching of control and resected animals particularly with regard to type of diet, weight, and length of preoperative fast, and also to ensure that animals return to normal growth and nutrition after resection, there being many who fail to do so because of strictures, abscesses and other complications of surgery. Similar reservations apply to measurements of the activity of sucrase, also known to be an adaptive enzyme (34). Simple transection of the intestine may lead to minor changes within 2 or 3 cm of either side of the anastomosis (35): for this reason a gap of at least 10 cm was left between the anastomosis and segment 5, in the present studies. Regional variations in mucosal enzyme concentrations also highlight the need for careful

standardization of the segments being studied. In the present experiments all of these factors were matched in control and resected animals and therefore it seems reasonable to attribute the observed effects to the resection per se; however the possibility remains that endogenously synthesized steroid or other hormones played a part in the production of the observed effects (11, 36, 37), especially since the known changes in cell kinetics also occur in the unresected members of paired parabiotic rats (36).

In the present study, changes in enzyme activities have been examined in various ways, related to changes in other tissue parameters also affected by resection. The increases per standard segment in wet weight, DNA, and protein are consistent with the cellular hyperplasia described by morphologists (2) and confirm that increases in tissue in segments distal to the anastomosis are much greater than those in the proximal remnant (3).

The fall seen in the specific activity of sucrase, a largely membrane-bound enzyme, confirms the finding of Weser and Hernandez (16). However, the total activity per segment was greatly increased proximally and correlates well with data showing enhanced carbohydrate absorption by such segments. The occurrence of the greatest increase proximally accords with the recent observation that adaptive changes in glycolytic enzymes, in response to dietary sucrose variations, are also most marked in the proximal segment (38). Since the diet is presumably the only source of luminal sucrose these distributions are not surprising. The measurements of sucrase activity, in separate saline homogenates, were undertaken because of difficulties in assaying sucrase in the presence of glycerol (21, 39); other homogenates were prepared in glycerol in order to stabilize the labile peptide hydrolases present (21).

The distribution of sucrase both regionally and subcellularly probably is similar to that of enterokinase which also serves a proximal function (40). While some workers (40) have alleged that enterokinase activity is found in rat ileal homogenates we have been unable to confirm this finding. The present results agree with those of studies on the guinea pig, which also indicate a lack of ileal activity (41).

Unlike sucrase or enterokinase, which are largely membrane-bound enzymes and normally present in greater amounts in proximal intestine, dipeptide hydrolases are largely soluble enzymes and normally achieve higher levels in the ileum. Induction of new forms or other qualitative change in this enzyme complex was excluded by zymograms of the soluble and particulate fractions, which revealed no new bands appearing during hyperplasia. Quantitatively the most significant changes in total activity of these enzymes after resection



occurred in ileum although both specific activity and total activity were also increased significantly in proximal segments. Thus the greatest change in total enzyme activity seems to occur in the region where the enzyme is normally found in highest concentration.

A greater change distally was seen not only for peptide hydrolase activity but also, as discussed above, for mucosal weight, protein and DNA per segment, and protein and DNA per gram wet weight of tissue. Such observations correspond with the results of studies of labeled villi which show that after resection cell migration rates are increased markedly in distal segments but are little changed in proximal intestine (10). Similarly, others have shown that postoperatively increases in the heights of ileal villi greatly exceed those of jejunal villi (13). By contrast, studies of crypt cell kinetics after resection show almost identical responses proximally and distally (11, 12). This suggests that cell turnover in the crypts is controlled by mechanisms different from those regulating the villi. Related to this proximal-distal difference it was of interest that increases in the specific activity of peptide hydrolase were greatest and falls in enzyme activity per milligram of DNA were least in proximal segments, where DNA and presumably cell population changed least. The reasons underlying these regional differences in cellular response remain obscure but are of considerable importance to the understanding of intestinal physiology. Whether they are nutritional (14) or related to the presence in the lumen of "villus promoting" factors (42), in some way associated with the presence of pancreatic juice, awaits elucidation. Alternatively there may be differences in the ability of villus cells to achieve maturity with regard to enzyme production when cell turnover rates are increased (43). Indeed some evidence indicates that after resection the mucosa acquires metabolic characteristics normally associated with immature, less differentiated tissue (9, 44) and epithelial cells isolated from such segments show diminished amounts of disaccharidase activity per cell (16).

While some data are available on the effects of resection on a number of digestive enzymes, results have been somewhat confusing. Histochemical studies have failed to show changes. Biochemical studies presenting specific activity data have shown significant reductions (16) or no change (15) in disaccharidase activity. Enzyme concentrations in mucosa were observed to be elevated two- to threefold in the case of enterokinase and unchanged in the case of alkaline phosphatase (20). A study on the activities of lipid reesterifying enzymes, in microsomal fractions from four regions of rat intestine, provided some important insight into these problems (45). It was shown that, after resection, enzyme changes expressed as total ac-

tivity per segment greatly exceeded changes in specific activity (per milligram protein) because of local hypertrophy in gut segments. These relatively greater changes in total activity were similar to the findings in the present study with respect to enterokinase, sucrase, and peptide hydrolase. The fact that enzyme levels expressed in this way show a uniform upward trend in resected segments offers more insight into the adaptive response than the somewhat misleading conclusions drawn solely from measurements of specific activity.

The changes recorded in DNA content, whether expressed per segment (Fig. 5D) or per gram weight of tissue (Table II), suggest that there is a three- to fivefold increase in the number of cells per segment, i.e., a true hyperplasia of the tissue. Sucrase, enterokinase, and peptide hydrolase are all found in villus cells and not in crypts (41, 46). An increase in the number of cells per segment, as documented morphologically and supported by DNA determinations, is associated with an increase in enzyme activity per segment. But since enzyme activity per milligram DNA falls in resected rats there must also be a marked increase in cells devoid of or low in enzyme activity, these appearing either as crypt cells (11, 12) or immature cells on villi (43). The increase in villus height is only of the order of 40% at maximum (14) and frequently much less, but a large increase in the crypt cell population has also been described in several studies (2, 11, 12, 47): one of these (47) reported that the crypt cell layer had increased by 70% at 4 wk postresection.

Protein content per gram of tissue fell slightly while DNA increased about twofold. The 42-65% fall in protein/DNA ratio suggests that in addition to hyperplasia there may be less protein per cell, or a change in the mean ratio of nuclear to cytoplasmic materials in the tissue but, since the cell population in each segment is heterogeneous and nonuniform, no firm conclusions can be drawn. Changes in cell size have never been observed in morphological studies.

The observed changes in several brush border enzymes raised the possibility of ultrastructural change. An observation that resection was followed by marked increases in the thickness of lateral cell membranes and microvillus tubules (48) has not been substantiated from two other laboratories (49, 50). While this merits further examination, the data in the present study, showing the relative constancy of the percentage of total activity associated with the particulate fraction, are in keeping with the conclusion that microvillus membranes are morphologically unchanged in the hyperplastic tissues. The terms hyperplasia or regenerative endomorphosis (47) devoid of mechanistic implications, seem best suited to describe the change, the control of the

process being far from clear, though humoral factors appear to play an important role (36).

These data support the general hypothesis that segmental increases in enzyme activity in the intestine of animals who have undergone massive resection are achieved largely by an increase in the cell population. Furthermore the greatest increase in the level of a particular enzyme seems to occur in the region of the intestine in which the highest amount of the enzyme is normally found.

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