Neurotensin Augments Intestinal Regeneration After Small Bowel Resection in Rats

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Massive small bowel resection (SBR) is characterized by increased proliferation of residual gut mucosa and pancreas. Neurotensin (NT), a gut tridecapeptide, stimulates growth of normal gut mucosa and pancreas. This study examined whether NT affected growth of the small intestine and the pancreas after either distal or proximal SBR. Male Fischer 344 rats were divided into four groups. Group 1 underwent ileal transection with reanastomosis (SHAM) and group 2 underwent 70% distal SBR. Group 3 underwent SHAM operation (jejunal transection), and group 4 underwent 70% proximal SBR. After operation, each group was further subdivided to receive either saline (control) or NT (300 μg/kg) subcutaneously in gelatin every 8 hours for 7 days. At death, the pancreas and proximal jejunum (from groups 1 and 2) or distal ileum (from groups 3 and 4) were removed, weighed, and analyzed for DNA, RNA, and protein content. Both proximal and distal SBR significantly increased mucosal growth in the remnant intestine; a more pronounced effect was noted with proximal SBR. Administration of NT significantly augmented the adaptive changes in both groups of rats by mechanisms involving increases in both cell size (hypertrophy) and cell number (hyperplasia). Pancreatic growth was stimulated by distal (but not proximal) SBR; NT did not augment this response. The authors conclude that NT augments intestinal growth after SBR by mechanisms involving an increase in overall mucosal cellularity. Administration of NT may be therapeutically useful to enhance mucosal regeneration during the early period of adaptive hyperplasia after SBR.

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RESECTION OF THE small bowel in rats induces compensatory hyperplasia of the remaining gut mucosa.[1-3] These changes, beginning as early as 48 hours after resection, include an increase in the production rate of crypt cells, villus enlargement, and enhanced absorption.[1-5] The specific signals controlling this adaptive response are thought to include such factors as luminal nutrients,[6] pancreaticobiliary secretions,[7] and humoral factors.[8-10] In addition to the adaptive changes noted in the remnant of intestinal mucosa, small bowel resection is also associated with a stimulation of pancreatic growth that appears to be due, in part, to elevated levels of cholecystokinin, a potent pancreatic trophic factor.[11,12]

Neurotensin (NT), a tridecapeptide, is localized mainly in the central nervous system and in endocrine cells (N cells) of the gut mucosa in the jejunum and ileum.[13] The functions of NT in the gut include stimulation of pancreatic and biliary secretions[14] and inhibition of small bowel and gastric motility.[15] In addition, NT is trophic for several tissues in the gastrointestinal tract, including pancreas,[16,17] colon,[18] and small bowel.[19-21] Administration of NT stimulates mucosal growth in rats fed normal laboratory chow,[19] and prevents mucosal hypoplasia in rats fed an elemental diet.[20,21] Also, NT may be involved in the early stages of intestinal regeneration after small bowel resection.[22-24] Collectively, these data suggest an important role for NT as a potent enterotrophic factor and as a contributing factor to the growth of other gastrointestinal tissues.

The purpose of this study was to determine whether NT could affect intestinal regeneration and pancreatic growth after resection of either the proximal or distal small bowel.

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Materials and Methods

Experimental Design

Ninety-six male Fischer 344 rats (3 to 4 months of age, 170 to 280 g, National Institute of Aging, Bethesda, MD) were housed at a constant temperature (22 C) and humidity with 12-hour light and dark cycles. During this period, all rats were fed standard laboratory chow (Formulab Chow, Purina Mills, Inc., St. Louis, MO) *ad libitum.*

After at least a 7-day period of acclimation, rats were fasted overnight, weighed, and divided into four groups. Rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg body weight). The abdomen was opened by a midline incision, and the small intestine was carefully measured along the antimesenteric border. Sham-operated transection controls underwent either ileal transection 5 cm proximal to the cecum (group 1) or jejunal transection 5 cm distal to the ligament of Treitz (group 3), without any intestine removed, followed by reanastomosis. Rats in group 2 underwent a 70% distal small bowel resection (DSBR) (approximately 65 cm of small bowel), beginning 5 cm proximal to the cecum. Rats in group 4 received a 70% proximal small bowel resection (PSBR), beginning 5 cm distal to the ligament of Treitz. In all groups, intestinal continuity was restored by end-to-end enteroenterostomy with 7–0 interrupted silk sutures. The abdomen was closed in one layer with 3–0 silk sutures. After operation, all rats were given 10 mL saline subcutaneously and allowed free access to water beginning 24 hours after operation. Sham-treated rats were pair-fed to the resected groups starting on postoperative day 3.

Beginning the morning of postoperative day 2, rats in each of the four study groups were further subdivided to receive subcutaneous injections of either saline (control) or NT (300 μg/kg, Bachem Inc., Torrance, CA) every 8 hours for 7 days.

Peptide Preparation

A stock solution of NT was prepared by first dissolving the amount of NT needed for the study in 1 mL of sterile water containing 0.1% (wt/vol) bovine serum albumin (Calbiochem-Behring, La Jolla, CA) and then diluted to the required concentration with saline containing 0.1% bovine serum albumin. Equal portions of this solution, sufficient for a single injection of all animals of a given group, were stored in glass vials at −20 C. Saline containing 0.1% bovine serum albumin (control) was likewise divided into equal aliquots and stored at −20 C. To prolong absorption, saline or NT was mixed 1:4 (vol/vol) with 15% (wt/vol) hydrolyzed gelatin (Sigma Chemical Co., St. Louis, MO) before administration.

Tissue Collection

The last injections were given at midnight on the eighth postoperative day. After an overnight fast, rats were weighed and then killed by decapitation beginning at 8:00 A.M. on postoperative day 9. The abdomen was opened and the proximal jejunum (20 cm) from groups 1 and 2, or distal ileum (20 cm) from groups 3 and 4, was removed. All segments were suspended vertically with a 15-g weight to ensure constant lengths. The mesentery was trimmed and luminal contents were removed by flushing with cold saline and gentle manual stripping. Each segment was then blotted dry, weighed, and the mucosa carefully scraped from the underlying seromuscular layer on a chilled platform, using a glass slide. In addition, the pancreas was removed from all rats and weighed. All specimens were immediately frozen at −70 C until assayed for DNA, RNA, and protein content.

DNA, RNA, and Protein Determination

Tissues were thawed and homogenized. The DNA content was measured by the Burton[25] modification of the diphenylamine procedure with calf thymus DNA used as the standard. Ribonucleic acid content was measured by means of the orcinol procedure with yeast RNA as the standard.[26] Protein content was determined by the method of Lowry and colleagues,[27] with bovine serum albumin as the standard.

Statistical Analysis

Mucosal and pancreatic weights and DNA, RNA, and protein contents were normalized to kilograms of body weight and values expressed as mean ± standard error of the mean. The data were analyzed by the two-way classification analysis of variance. The two classifications were defined as operation (SHAM and small bowel resection) and injection (saline and NT). The least significant procedure was used for mean separation. In all instances, a p value < 0.05 was considered significant.

Results

Body Weight

None of the groups of rats developed diarrhea; however, four rats died after DSBR and six rats died after PSBR because of anastomotic leakage. There was an approximately 5% to 6% weight decrease in both groups of rats that had small bowel resection compared with corresponding SHAM groups. There were no differences in body weights of NT and saline-treated rats within groups.

Effect of NT on Proximal Small Bowel Mucosa After DSBR

Distal small bowel resection alone significantly stimulated mucosal growth in the proximal jejunum (Fig. 1). Mucosal weight was increased by 34%, DNA content by 24%, RNA content by 32%, and protein content by 37% compared with the comparable sham group given saline injections. Neurotensin injections, administered to rats after DSBR, significantly augmented the response of the gut mucosa to resection alone. Indices of gut mucosal growth were all increased (weight by 23%, DNA content by 12%, RNA content by 22%, and protein content by 24%) compared with the values obtained in rats treated with DSBR and saline. As expected, NT given to rats after sham operation significantly stimulated gut mucosal growth.

Effect of NT on Distal Small Bowel Mucosa After PSBR

Proximal small bowel resection provided a stronger stimulus to intestinal adaptation than did distal resection (Fig. 2). Values of weight, DNA, RNA, and protein content were increased 50% to 70% over corresponding values in sham rats given saline injections. Even though PSBR produced a more pronounced increase in mucosal growth than DSBR, NT administration significantly augmented this response. Mucosal weight was increased by 23%, DNA content by 20%, RNA content by 21%, and protein content by 26% compared with rats treated with PSBR and saline injections.

Figure 3 summarizes and compares the effects of bowel resection either alone or combined with NT on mucosal growth in the residual gut.

Effect of NT on Pancreatic Growth After DSBR

Resection of the distal 70% of the small bowel stimulated increases of pancreatic weight (17%), DNA (15%) and RNA (28%) compared with sham operation (Fig. 4). In contrast to the small bowel mucosa, administration of NT did not augment pancreatic growth. Administration of NT to sham-treated rats resulted in significant increases of pancreatic weight (18%), RNA (14%), and protein (19%) compared with sham rats treated with saline.

Effect of NT on Pancreatic Growth After PSBR

Resection of the proximal small bowel resulted in a small (8%), but significant, increase in pancreatic DNA content (Fig. 5). Values of weight, RNA, and protein were not affected. Neurotensin treatment to sham and PSBR rats resulted in significant increases of growth measurements.

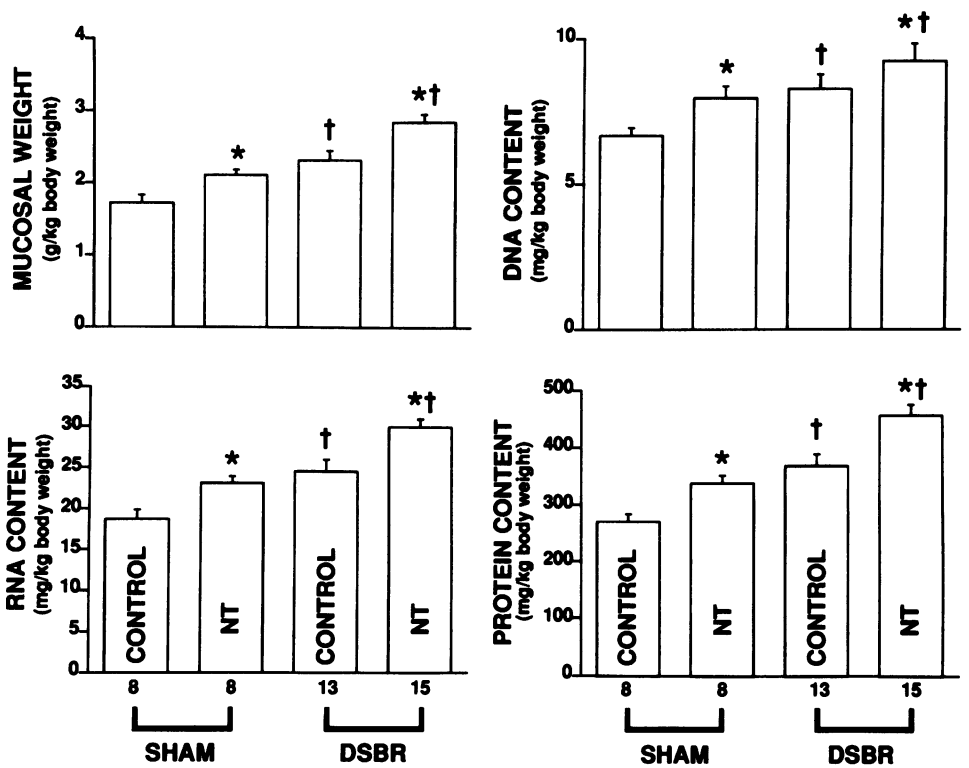

FIG. 1. Mucosal weight, DNA, RNA, and protein content of proximal jejunum (20 cm) from rats after either sham operation or 70% distal small bowel resection (DSBR). Rats were further subdivided to receive injections of saline (control) or NT (300 μg/kg). *p < 0.05 (NT vs. control); †p < 0.05 (DSBR vs. sham control).

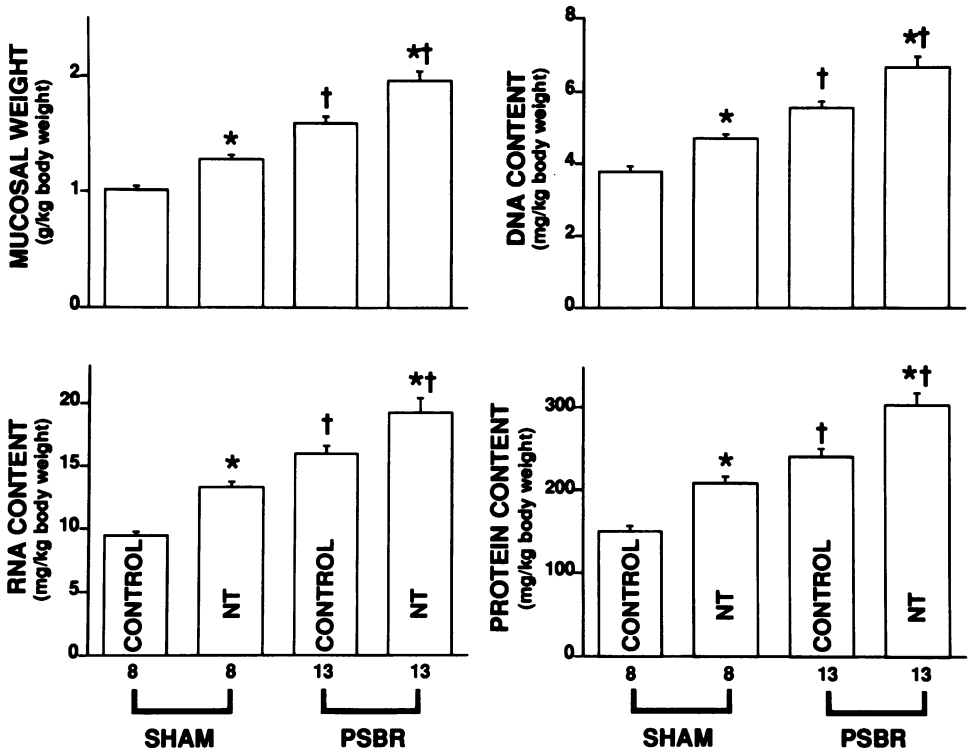

FIG. 2. Mucosal weight, DNA, RNA, and protein content of distal ileum (20 cm) from rats after either sham operation or 70% proximal small bowel resection (PSBR). Rats were further subdivided to receive injections of saline (control) or NT (300 μg/kg) *p < 0.05 (NT vs. control); †p < 0.05 (PSBR vs. sham control).

Discussion

Our findings demonstrate that NT (300 μg/kg) can augment gut adaptive hyperplasia that occurs after either proximal or distal small bowel resection. Neurotensin significantly increased mucosal weight, protein, and RNA content, indicators of cellular hypertrophy, and in addition, stimulated actual cellular proliferation, as demonstrated by the increases of DNA content.

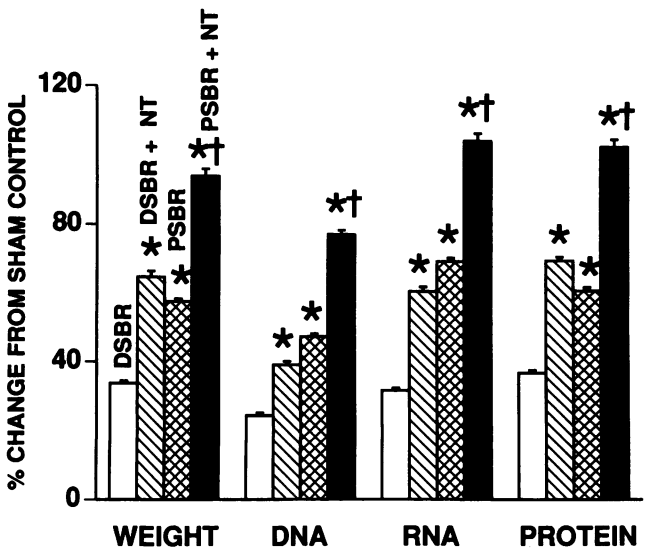

FIG. 3. Summary of changes of mucosal weight, DNA, RNA, and protein content after either distal small bowel resection (DSBR, open bar) or proximal small bowel resection (PSBR, double-hatched bar) alone or combined with NT injections. *p < 0.05 vs. DSBR; †p < 0.05 vs. PSBR.

Adaptive hyperplasia of intestinal mucosa occurs after extensive small bowel resection.[1-5,28] The residual intestine undergoes adaptive changes, both structural and functional, which include small bowel dilation, epithelial cell hyperplasia, and enhanced absorption.[1-10,28] Increases in DNA and RNA content are noted as early as 2 days after resection.[4,5] In our current study, we found significant increases in weight, DNA, RNA, and protein content in the remaining intestinal mucosa 9 days after either proximal or distal intestinal resection. Consistent with other reports,[1,29] we found a differential trophic response of the residual jejunum and ileum. Mucosal growth was increased 50% to 70% in the ileum after proximal small bowel resection compared with 25% to 35% increases of mucosal growth in the jejunum after distal small bowel resection. The reason for this differential effect is not known, but may be due, in part, to a greater supply of nutrients delivered to the remaining ileum. Because NT is found in highest concentration in the distal ileum, another possibility to explain why the proximal mucosa is less responsive may be that most of NT-containing gut is removed by a distal resection.

Signals controlling the compensatory hyperplasia of gut

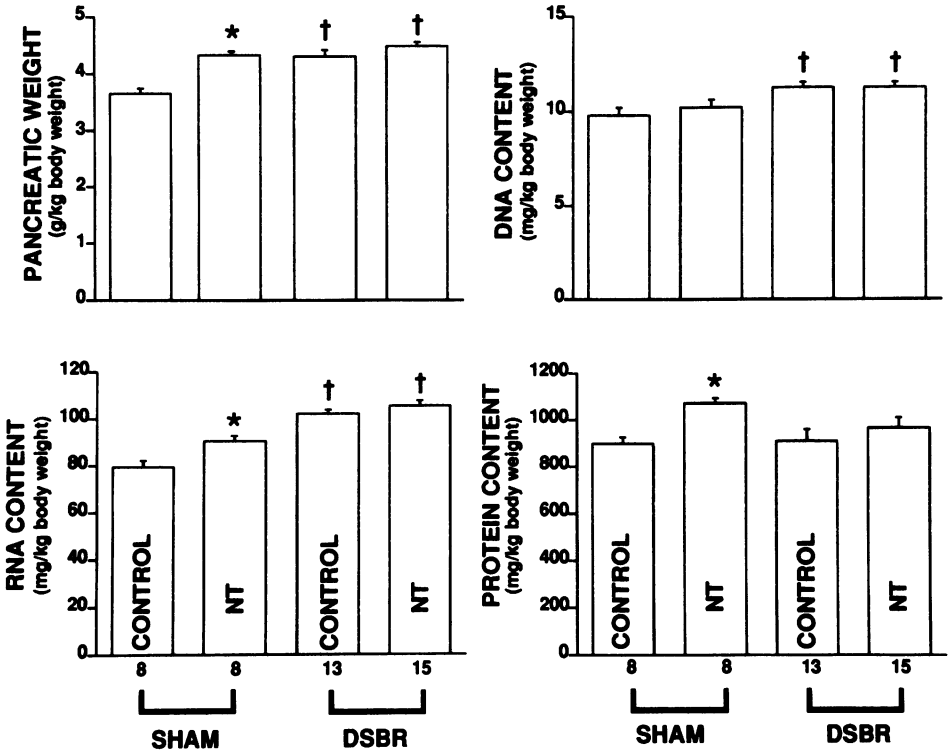

FIG. 4. Pancreatic weight, DNA, RNA, and protein content from the same animals shown in Fig. 1. *p < 0.05 (NT vs. control); †p < 0.05 (DSBR vs. sham control).

mucosa are complex; factors that appear to be important for a maximal trophic response include intraluminal factors (for example, luminal nutrients and pancreaticobiliary secretions).[6,7] Humoral factors also appear to play an important role in small bowel adaptation, a role that has been demonstrated dramatically by Williamson and colleagues[8] in parabiotic rats. Several gastrointestinal hormones, including gastrin and enteroglucagon, have been considered as potential candidates in the response to resection, but the evidence is largely inferential.[10,30,31]

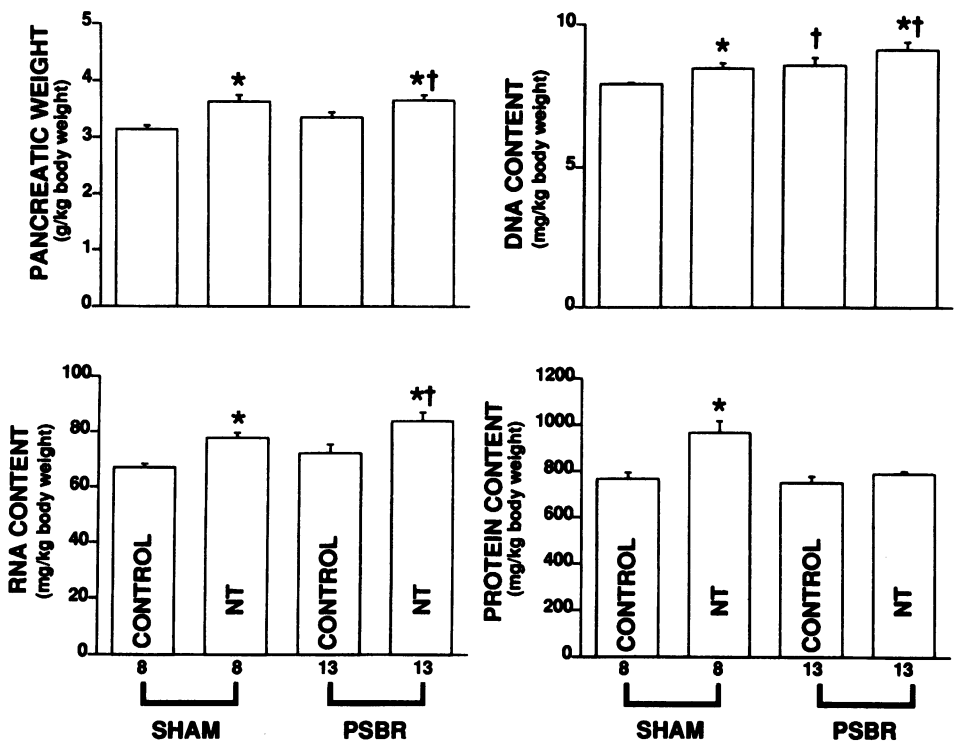

FIG. 5. Pancreatic weight, DNA, RNA, and protein content from the same animals shown in Fig. 5. *p < 0.05 (NT vs. control); †p < 0.05 (PSBR vs. sham control).

Another potential candidate for the humoral stimulation of gut mucosal growth after resection is NT. Neurotensin stimulates growth of both normal and atrophic gut mucosa,[19–21] and NT levels have been shown to be closely linked to the proliferative status of the small bowel mucosa.[22,24,32,33] We and others have shown that exclusion of nutritional stimuli to the small bowel mucosa during administration of an elemental diet or after heterotopic small bowel transplantation causes mucosal atrophy and a marked decrease in NT protein content[32] and N cell number[33] in the ileum. In contrast, small bowel resection induces hyperplasia in the remaining gut mucosa. We did not measure tissue levels of NT in the present study, but we have previously found that NT mRNA levels are increased in the rat ileum as early as 3 hours after proximal small bowel resection,[24] and Olsen and colleagues[22] have shown that the concentration of NT increases 3- to 15-fold in the residual gut after small bowel resection.

We have demonstrated here, for the first time, that NT augments the adaptive hyperplasia that occurs in gut mucosa after resection. Administration of NT for 7 days significantly increased weight, DNA, RNA, and protein contents after either proximal or distal enterectomy. Maximal gut hyperplasia occurs in the residual gut within 7 to 8 days after resection[4,5]; therefore, NT can further stimulate the proliferative response achieved by small bowel resection alone. Other studies have examined the effect of small bowel resection combined with administration of epidermal growth factor and prostaglandin E_2, agents reported to stimulate small bowel mucosa; however, these agents did not enhance mucosal regeneration of the distal gut when combined with intestinal resection.[34,35]

The mechanisms responsible for the augmentation of gut growth with NT in our study are not known. We have previously demonstrated that NT can stimulate mucosal growth by a direct systemic effect and also indirectly by the stimulation of pancreaticobiliary secretions[20]; therefore, a combination of factors may be involved.

In addition to stimulation of gut mucosal growth, resection of the distal (but not proximal) small bowel produces pancreatic hyperplasia.[11,12] Administration of the cholecystokinin antagonist CR-1409 suppresses this increase in pancreatic growth measurements, suggesting that increased cholecystokinin levels, which occur due to loss of the normal feedback control exerted by bile salts, are responsible for this phenomenon.[12] We found increases of pancreatic weight, DNA, and RNA content after distal small bowel resection in our current study. Neurotensin, trophic for normal pancreas, stimulated pancreatic growth in all sham-treated rats and rats treated with proximal small bowel resection. Neurotensin did not augment pancreatic growth after distal small bowel resection, however. These findings suggest that pancreatic growth stimulation was maximal and that administration of a perhaps weaker pancreatic trophic factor (NT) was unable to further augment this response.

Intestinal adaptation, after small bowel resection, also occurs in humans[28]; mucosal surface area may increase fourfold in the remaining gut. This adaptive response is oftentimes sufficient to compensate for limited resections of the intestine, but massive small bowel resection (*i.e.*, greater than 70%) is associated with increased morbidity and mortality rates because of a marked loss of intestinal absorptive surface.[36] Efforts to increase intestinal surface area have included innovative surgical techniques, such as longitudinal small bowel division and lengthening, construction of valves, and reversed segments of small bowel or colon, and small bowel transplantation.[37,38] These attempts have been, for the most part, unsuccessful. Identification of an agent that could augment the adaptive response of the residual gut after resection would be clinically important and may have potential therapeutic applications.

In conclusion, we have shown that both extensive small bowel resection and NT can independently increase gut mucosal growth. Administration of NT can further augment this adaptive hyperplasia of the remaining gut mucosa. Neurotensin appears to be an important enterotrophic factor and may be a useful agent during the early period of gut adaptation after resection of the small bowel to enhance mucosal regeneration.

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Discussion

DR. LESTER WILLIAMS (Nashville, Tennessee): Dr. Ochsner, Dr. Jones, Members and Guests, I have had the pleasure of reading the manuscript, which contains not only the material you heard but considerable additional information. And as one would expect as we hear presentations from Dr. Thompson's laboratory, we have seen a magnificent presentation of the data, and I could find almost nothing to quibble with, with respect to the data. So I have three questions, one of which is not fair because he did not have a chance to present the pancreas data.

The first question is whether the assumption is that neurotensin's mechanism is predominantly by a direct, systemic effect. If that is true, is it possible that the difference between the distal resection and proximal resection data could have been negated by increasing the dose of the exogenous neurotensin? Or do you have reason to believe that the mechanism is somehow or other different? If in fact it is simply a matter of the amount of neurotensin that is still in the animal because the distal bowel is there, then you would expect to be able to overcome these differences by increasing the exogenous dose.

The second question relates to the consequence of these changes in cellular phenomena. We do not have evidence that these cellular changes made a difference. Is there any functional significance to the cellular change? In fact, the all of resected animals lost weight, 5% to 6%. There was not a difference between the amount of weight lost in the animals that received neurotensin and those that received saline, and yet, the effect in terms of cellular phenomena were better with the neurotensin. Why did we not see functional significance in terms of the effect with treatment of these animals?

It is also true that in the manuscript you will see some information on the pancreas. And the fascinating phenomenon is that the pancreas goes differently than what would be predicted. The paper, I think, perhaps contains other evidence of a mechanism that we would have to explain. I certainly enjoyed the opportunity to see the manuscript and discuss the paper. Thank you.

DR. HIRAM POLK (Louisville, Kentucky): Mr. President, Dr. Jones, It is an interesting commentary to think back over 20 years ago and realize that the observations of Drs. Ellison and Zollinger would turn into this kind of sophisticated analysis of what now is the most important endocrine organ in the body, that is, the alimentary tract. It is an interesting turnaround from a thoughtful, clinical observation to something that is now fairly fundamental basic science. This paper is pretty typical of the work that Dr. Thompson, Dr. Townsend, and Dr. Evers have done in recent years. I do think that the questions and implications, however, are great.

There are some issues about the timing and duration of treatment. In other words, how late could you begin this treatment with potential value? And then how long should it be continued? Does it have a continued proliferative effect or does it plateau? I think the point that Dr. Williams made about functional significance is important, and it would be nice to see this sort of thing applied to a critical small bowel resection, the kind that would be incompatible with growth and normal life and to see if you could moderate that in a functional way, just as Drs. Dudrick and Rhoads did with parenteral hyperalimentation so long ago. Could