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# Bombesin Stimulates Mucosal Growth in Jejunal and Ileal Thiry-Vella Fistulas

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## Objective

The authors determined whether the trophic effects of bombesin (BBS) on the small bowel mucosa are mediated by either nonluminal factors or endogenous luminal secretion.

## Summary Background Data

The gut hormone bombesin stimulates growth of small bowel mucosa. The mechanisms responsible for this trophic effect are not known.

## Methods

Rats underwent construction of a Thiry-Vella fistula (TVF) of either the jejunum or ileum. On postoperative day 10, the two groups were subdivided to receive either saline (control) or bombesin (10 μg/kg, subcutaneously, three times a day). After 14 days, rats were killed and the TVF was removed. The mucosa was scraped and weighed, and DNA and protein content was determined.

## Results

Bombesin significantly increased mucosal weight and DNA and protein content of both the jejunal and ileal TVF compared with the control rats.

## Conclusions

Bombesin-mediated stimulation of small bowel mucosal growth is mediated by factors that are independent of luminal contents and pancreaticobiliary secretion. Bombesin may prove to be an important enterotrophic factor for gut mucosal proliferation.

The complex regulation of gut mucosal growth involves multiple factors, including luminal nutrients,[1-4] pancreaticobiliary secretions,[5-8] and humoral factors such as gut hormones.[9-11] Bombesin (BBS), a tetradecapeptide originally isolated from the skin of the European frog *Bombina bombina,*[12] is equivalent to mammalian gastrin-releasing peptide.[13] Bombesin stimulates the growth of the pancreas, gastric mucosa, and colon.[14-19] In addition, we have shown that BBS prevents gut atrophy by feeding rats an elemental diet[20]; it also significantly enhances small bowel proliferation after methotrexate-mediated intestinal injury.[21,22] Taken together, these findings suggest that BBS may be involved in gut mucosal repair and regeneration. Further evidence to corroborate the importance of BBS as a trophic factor in the gut is provided by the demonstration of BBS-like immunoreactivity in the breast milk of some mammals[23,24] and the finding that exogenous BBS, adminis-

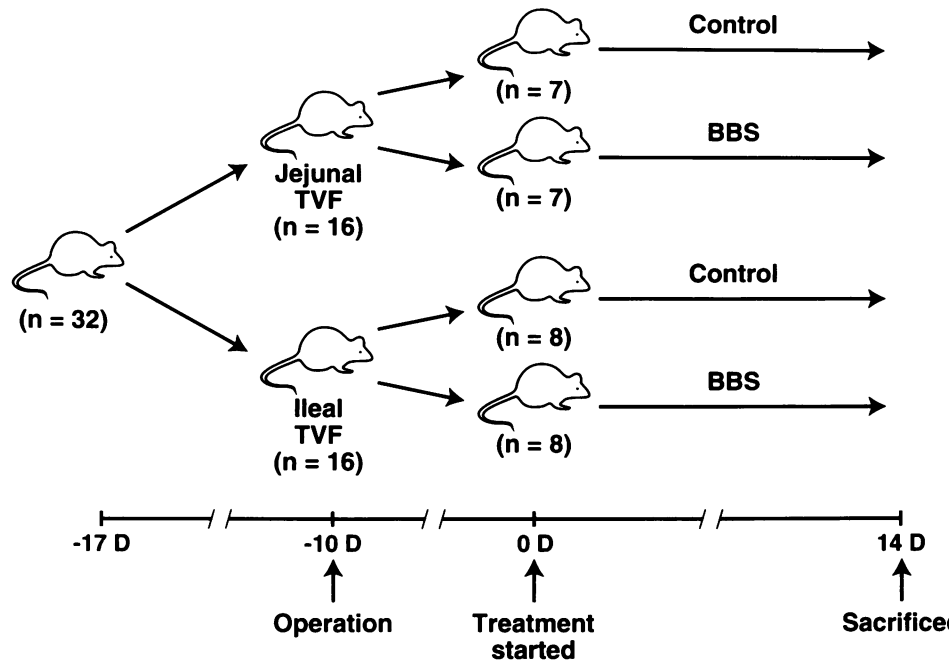


**Figure 1.** Schematic depiction of Experiment 1. (See Experimental Design for description).

tered either enterally or intravenously, promotes growth of neonatal small intestine.[16,25] The exact mechanisms for the trophic effect of BBS on gut mucosa are not completely known.

Bombesin can be considered a generalized "on-switch" in the gastrointestinal tract and may, therefore, stimulate the growth of gut mucosa by a variety of mechanisms. Bombesin could enhance gut growth as a result of its stimulation of luminal (pancreaticobiliary) secretions.[26–28] Nonluminal factors also may account for the trophic effect of BBS. For example, BBS stimulates release of most gut hormones, including neurotensin and enteroglucagon, which are known enterotrophic agents.[29–31] Furthermore, similar to the pancreas,[15,19] BBS may stimulate mucosal growth by a direct, receptor-mediated effect. To more precisely delineate the factors that regulate BBS-mediated gut growth requires examination of gut mucosa which is not in continuity with the luminal stream.

The purpose of our study was to determine whether the trophic mechanism of BBS on gut mucosa is mediated by nonluminal factors. To accomplish these goals, we constructed isolated loops of jejunum and ileum—Thiry-Vella fistulas (TVFs). Stimulation of mucosal growth in the TVF by BBS would suggest a mechanism that does not require the action of endogenous luminal secretions.

## METHODS

### Experimental Design

In the first experiment (Fig. 1), 32 male Fischer 344 rats (250–275 g; Harlan Sprague-Dawley, Indianapolis, IN) were randomly divided into two groups of 16 rats each. All groups were placed in individual wire-bottomed cages, acclimated for 1 week at a constant temperature (22 C) with 12-hour light/dark cycles, and fed standard chow (Ralston, Purina, St. Louis, MO) *ad libitum*. After an overnight fast, the rats underwent construction of either a jejunal or ileal TVF, as previously described[32] and as illustrated in Figure 2. Briefly, the small bowel was divided at 10 cm and 30 cm distal to the ligament of Treitz (jejunal TVF) or 10 and 30 cm proximal to the ileocecal junction (ileal TVF), taking care not to damage the neurovascular structures within the mesentery. The two ends of 20-cm segments of either jejunal or ileal TVF were exteriorized and sutured to the abdominal wall. Intestinal continuity was restored by end-to-end enteroenterostomy. On postoperative day 3, rats were allowed regular chow *ad libitum*. On postoper-

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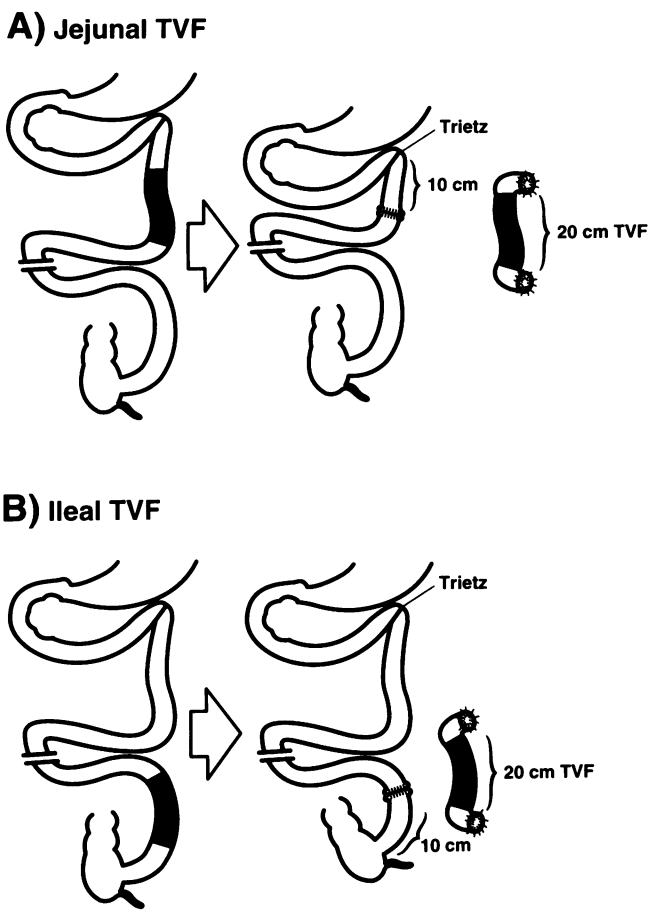


**Figure 2.** Schematic diagram of jejunal (A) and ileal (B) TVFs as described in detail in Methods. Bowel continuity was re-established with end-to-end anastomosis. Blackened areas denote gut segments removed for analysis.

ative day 10, rats were further subdivided to receive subcutaneous injections of either saline (control) or BBS (10 μg/kg; Bachem, Torrance, CA), administered every 8 hours. After 2 weeks of treatment, rats were weighed and killed, beginning at 8:00 A.M., and continuing sequentially with one rat from each group until all were killed (between 1:00 and 4:00 P.M.). The abdomen was opened, and the 20-cm TVF was removed, trimmed of mesentery, and suspended vertically with a 10-g weight to ensure constant lengths. The segments of small intestine then were opened longitudinally, blotted dry, and the mucosa was carefully scraped from the underlying seromuscular layer using a glass slide as a scraper. The mucosa and pancreas were weighed and immediately frozen at −20 C until assayed for DNA and protein content.

In the second experiment (Fig. 3), 32 male Fischer rats (250–275 g) were randomly divided into four groups of eight rats each, placed in individual wire-bottomed cages, and fed regular rat chow *ad libitum*. One group (chow control) was killed before any manipulation. A 20-cm segment of jejunum was removed, and mucosa was scraped and subsequently analyzed for DNA and protein content. After an overnight fast, the remaining three groups underwent construction of a 20-cm jejunal TVF as described in experiment 1. On postoperative day 10, one group was killed, and the jejunal TVF and pancreas were removed. The remaining two groups were started on subcutaneous injections of either saline (control) or BBS (10 μg/kg) every 8 hours. After 14 days of treatment, the rats were killed and the jejunal TVF and pancreas were removed, weighed, and analyzed for DNA and protein content.

## Peptide Preparation

A stock solution of BBS was prepared by first dissolving the amount needed for the study in 1 mL of sterile water containing 0.1% (wt/vol) bovine serum albumin (BSA; Calbiochem-Behring, La Jolla, CA), and then diluting the stock solution to the required concentration with saline containing 1% BSA. Equal portions of this solution, sufficient for single injections in all animals of a given group, were stored in plastic vials at −20 C.

Saline containing 1% BSA was likewise divided into equal aliquots and stored at −20 C. To prolong the rate of absorption after each injection, BBS in saline was mixed 1:4 (vol/vol) with 8% (wt/vol) hydrolyzed gelatin (Sigma Chemical Co., St. Louis, MO) before administration (final volume of 0.5 mL).

## DNA and Protein Measurement

Tissues were thawed and homogenized (Polytron, Kinematic GmbH, Kriens-Luzern, Switzerland). DNA content was measured by the Burton[33] modification of the diphenylamine procedure with calf thymus DNA used as the standard. Protein content was determined by the method of Lowry[34] with BSA used as the standard.

## Histology

Full-thickness segments from the jejunal and ileal TVFs were removed, fixed in 10% buffered formalin, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin and examined.

## Statistical Analysis

Mucosal weight and DNA and protein content were normalized per centimeter of intestinal length. Pancreatic weight and DNA and protein content were normalized by body weight. Values are expressed as the mean ± SEM and analyzed using a one-way analysis of variance. In the second experiment, the data were analyzed with

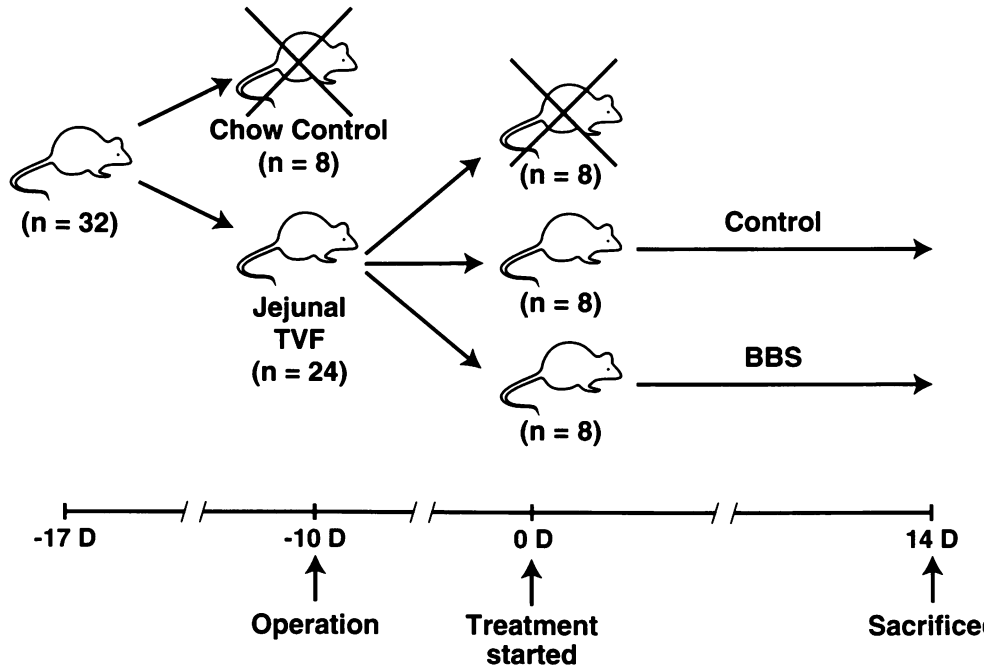


**Figure 3.** Schematic depiction of Experiment 2. (See Experimental Design for description). Crossed out rats denote groups that were killed before operation and BBS treatment.

Dunnett's procedure for multiple comparisons. In all instances, a p value $< 0.05$ was considered significant.

## RESULTS

### Experiment 1

Two rats in the jejunal TVF group died of anastomotic breakdown.

#### *Body Weight*

There were no differences in the initial and final body weights of control and BBS-treated rats in either the jejunal or ileal TVF groups (data not shown).

#### *Effect of BBS on Jejunal and Ileal TVFs*

The administration of BBS for 14 days stimulated significant mucosal growth of the jejunal TVF. Bombesin increased mucosal weight by 19%, DNA content by 42%, and protein content by 29% compared with control rats that received saline (Fig. 4A). In addition, BBS stimulated mucosal growth of the ileal TVF with mucosal weight increased by 61%, DNA content by 54%, and protein content by 58% compared with control rats (Fig. 4B).

Figure 5 shows representative photomicrographs from jejunal and ileal TVFs of rats treated with either saline or BBS. Consistent with our weight, DNA, and protein results, BBS significantly stimulated the mucosal growth in both jejunal and ileal TVFs.

#### *Effect of BBS on Pancreas*

The pancreas was removed and analyzed to confirm the bioactivity of BBS. As expected, BBS significantly stimulated pancreatic growth. Bombesin increased pancreatic weight by 59% and 56%, DNA content by 50% and 33%, and protein content by 105% and 99% in rats with jejunal and ileal TVFs, respectively (Table 1).

### Experiment 2

No deaths or complications occurred in this experiment.

#### *Body Weight*

In addition, there were no differences in either initial or final body weights of the rats (data not shown).

#### *Effect of TVF Construction and BBS on Jejunal Mucosal Growth*

After the jejunal segment was isolated from the luminal stream, significant atrophy was apparent by postoperative day 10, with decreases of mucosal weight by 44%, DNA content by 58%, and protein content by 49%. Although DNA content did not decline further after postoperative day 10, mucosal weight and protein content continued to decrease (Fig. 6). Bombesin administration stimulated gut mucosal growth in the jejunal TVF, with mucosal weight increased by 25%, DNA content by 28%, and protein content by 35%. Taken together, our findings demonstrate that isolation of the jejunal segment

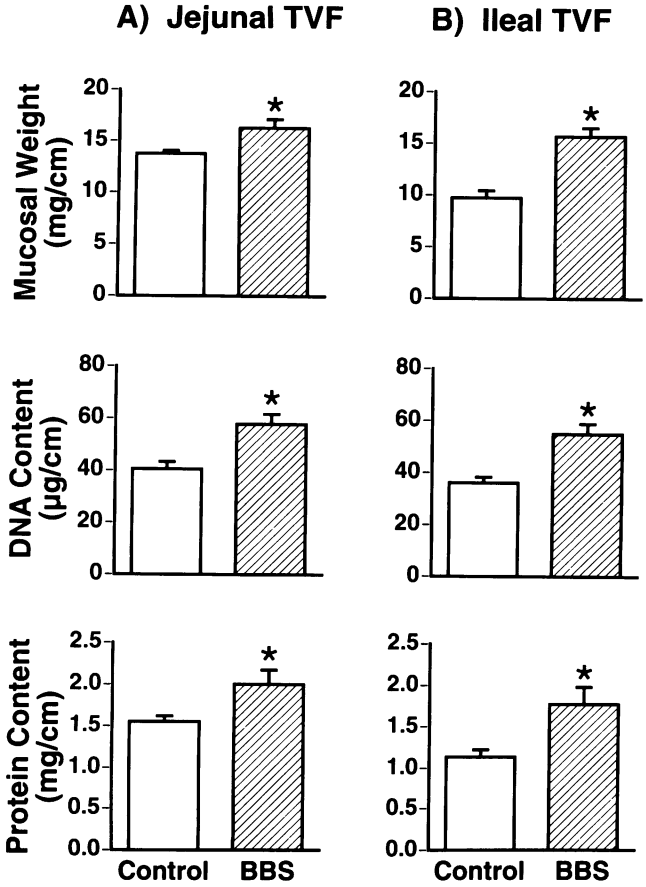


**Figure 4.** Mucosal weight, DNA, and protein content of jejunal TVF (A) and ileal TVF (B) after 14-day injections of saline (control, open bars) or BBS (10 µg/kg) (single-hatched bars). Mean ± SEM, * = $p < 0.05$ *vs.* control.

from the luminal stream leads to significant gut atrophy, and administration of BBS stimulates the hypoplastic gut mucosa. As expected, BBS significantly stimulated pancreatic growth, compared with rats treated with saline, thus confirming the bioactivity of the BBS dosage (data not shown).

## DISCUSSION

We have shown that BBS stimulates mucosal growth in isolated TVFs of both jejunum and ileum, suggesting that the trophic effect of BBS on small gut mucosa is mediated by factors that are independent of both luminal nutrients and pancreaticobiliary secretion.

The gut mucosa has a high rate of cell turnover, with proliferation tightly regulated by many factors that include luminal nutrients,[1-4] pancreaticobiliary secretions[5-8] and humoral factors such as certain gut hormones.[9-11] Construction of an isolated, exteriorized loop of small bowel (i.e., a TVF) facilitates analysis of gut mucosa that is not exposed to luminal contents but has an intact neurovascular supply. A trophic response of the mucosa in the TVF would suggest a mechanism that does not require exposure of the gut mucosa to the luminal environment. The findings in our present study demonstrate that the administration of BBS significantly stimulates gut mucosal growth in isolated loops of both jejunum and ileum. This trophic response is completely independent of luminal factors such as nutrients and pancreaticobiliary secretions, showing that the proliferative effect of BBS is due, at least in part, to a systemic mechanism that is not mediated by an enhanced luminal stream.

Because we have previously noted differential trophic effects of various gut hormones on segments of small bowel,[32] TVFs of both proximal jejunum and distal ileum were constructed and analyzed. In contrast to the gut hormone neurotensin, which has a trophic effect only on the proximal small bowel,[32] BBS stimulates growth of both jejunal and ileal mucosa, and to a similar degree. This generalized proliferative effect of BBS is characterized by both hyperplasia (weight and DNA content) and hypertrophy (protein content), and serves to further emphasize the importance of BBS as an important physiologic trophic factor involved in the regulation of growth of the entire small bowel.

Isolating loops of small bowel from the luminal stream

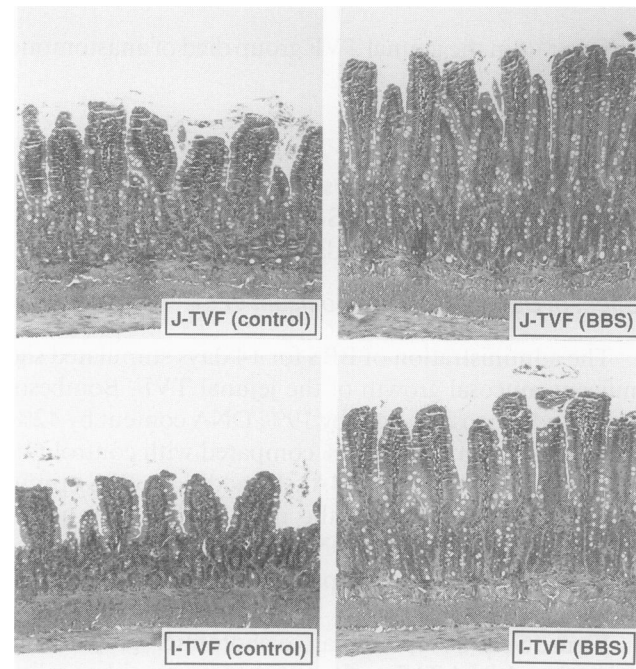


**Figure 5.** Histologic sections of jejunal and ileal TVFs after treatment with saline (control) or BBS. Administration of BBS produced mucosal hyperplasia in both TVFs compared with controls (original magnification for all sections × 100).

**Table 1. EFFECT OF BBS TREATMENT ON PANCREATIC WEIGHT, DNA, AND PROTEIN CONTENT**

| | Jejunal TVF | | Ileal TVF | |
|---|---|---|---|---|
| | Control (n = 7) | BBS (n = 7) | Control (n = 8) | BBS (n = 8) |
| Wt (mg/gBW) | 2.90 ± 0.15 | 4.61 ± 0.27* | 3.18 ± 0.09 | 4.96 ± 0.09* |
| DNA (μg/gBW) | 6.05 ± 0.45 | 9.09 ± 0.51* | 6.54 ± 0.27 | 8.71 ± 0.50* |
| Protein (mg/gBW) | 0.37 ± 0.03 | 0.75 ± 0.04* | 0.38 ± 0.03 | 0.75 ± 0.02* |

Mean ± SEM expressed relative to gram body weight (BW).
* $p < 0.05$ *vs.* control.

produces a significant mucosal atrophy.[35] To better determine the degree of atrophy and whether BBS acts to actually stimulate the atrophic gut mucosa or, conversely, whether its effect primarily is to prevent continued mucosal atrophy, we next examined jejunal mucosa in chow-fed control rats, jejunal TVFs 10 days after construction and jejunal TVFs after a 14-day administration of BBS. As noted by Hanson and colleagues,[35] significant gut atrophy is present 10 days after TVF construction. Bombesin stimulates the growth of this established atrophic mucosa, suggesting a possible role for BBS administration during periods of gut disuse or atrophy.

Our study provides clear evidence for a trophic effect of BBS on gut mucosa that does not require gut in continuity with the luminal stream; however, it does not address the specific mechanisms involved in this nonluminal growth effect. Possible mechanisms include either a direct, receptor-mediated action of BBS on gut mucosa or an indirect effect secondary to release of other trophic peptides (e.g., neurotensin).[29,31] Studies from our laboratory[15] and from others[19] have shown that BBS stimulates pancreatic growth by both a direct action of BBS and an indirect effect due to release of endogenous cholecystokinin. However, in contrast to the pancreas, which possesses BBS-binding sites on acinar cells, receptors for BBS have been localized only to the submucosal and muscular layer of the gut, not in the mucosa.[36–38] Therefore, these findings suggest that BBS-mediated release of other trophic peptides is a more likely possibility rather than a direct effect of BBS on the gut mucosa itself. Neurotensin is a gut hormone that is released by BBS[29–31] and exerts a potent trophic effect on small bowel mucosa,[30,32] and, therefore, must be considered a potential candidate for the proliferative effect of BBS. In contrast to BBS, neurotensin predominantly stimulates mucosal growth of proximal gut, not the ileum.[32,39] This differential effect of neurotensin does not necessarily exclude it as a possible factor involved in the trophic action of BBS, but it suggests that neurotensin is not the only factor involved.

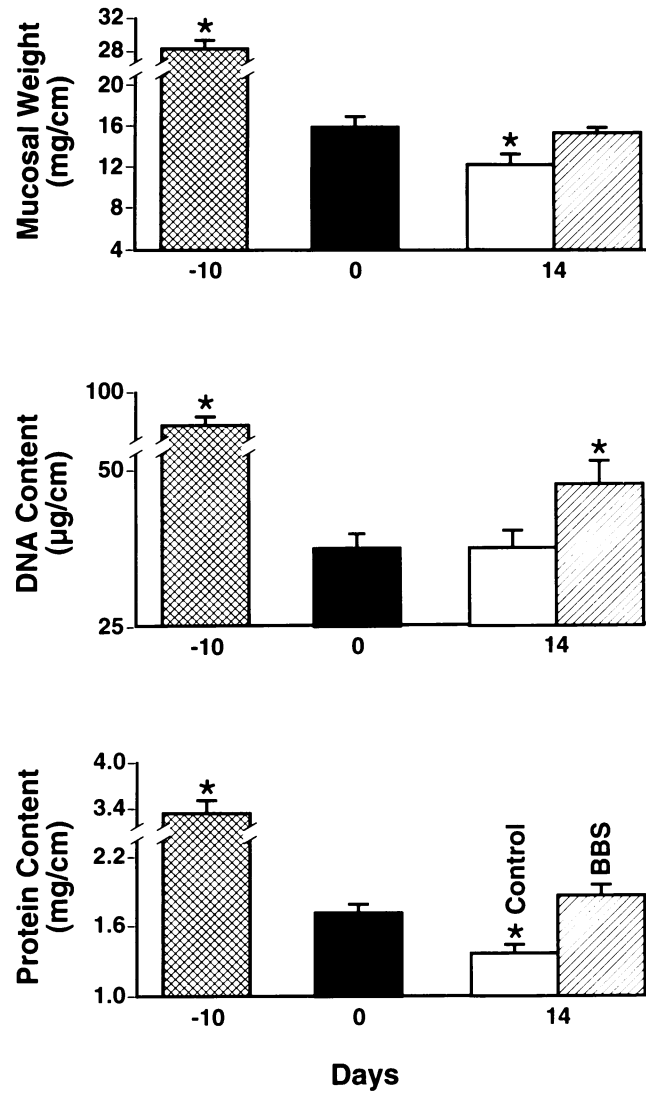


**Figure 6.** Mucosal weight, DNA, and protein content of jejunal mucosa before operation (chow control; cross-hatched bar), the jejunal TVF 10 days after operation (day 0; closed bar) and jejunal TVF after 14 days treatment with BBS (single-hatched bar) or saline (open bar). Mean ± SEM, n = 8; * = $p < 0.05$ *vs.* day 0.

In addition, BBS stimulates intestinal motility,[26,40] which also may contribute to the proliferative effects noted in the gut. Given its multiple functions in the gastrointestinal tract, BBS may play a major role in the tight regulation of gut mucosal proliferation.

We have shown that administration of BBS stimulates proliferation of both jejunal and ileal mucosa in isolated TVFs. These findings indicate that BBS can significantly enhance gut growth despite the absence of the normal components present in the luminal stream. Our findings further corroborate the importance of BBS as a physiologically important enterotrophic factor for both the proximal and distal small bowel mucosa and suggest that BBS may have clinical benefits during periods of gut disuse or atrophy.

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## Discussion

DR. R. SCOTT JONES (Charlottesville, Virginia): Dr. McDonald, Dr. Copeland, thank you for allowing me the privilege of the floor. This was an excellent, well-conceived study and well presented as usual. The conclusions appear fairly watertight, and I don't really have any comment to make about that.

But I would like to ask the authors some questions related to the general topic of atrophy and regeneration of the small bowel following exclusion or resection. And I think that the authors have concluded that perhaps bombesin as a pharmacological substance may have some value in managing patients who have atrophy or loss of some of their small intestine.

I would like to ask a physiologically oriented question. We know that there are physiological or pathophysiological mechanisms that exist to promote growth and integrity of the intestinal mucosa during pathological circumstances. I would like to ask the authors views on whether and how they believe that endogenous peptides play a role, apart from the skin of the *Bombina bombina.*

In other words, could you give us a brief analysis of the mechanisms you believe are responsible in humans who undergo resection of the small bowel?

Do bombesin-like peptides play a role there? The thing that's been interesting over the years is that patients with extensive small bowel resection, undergo hypergastrinemia. And this seems to go down as regeneration of the small bowel mucosa occurs. Perhaps you measured serum gastrin; I know you have the capability of measuring most every kind of peptide. Did bombesin release gastrin? So that gets to another question. Your experimental design proved, I think, conclusively that enteric content was not responsible for the response that you saw. But is it possible that another hormone release could have participated in this? There are other trophic hormones that bombesin may release which stimulate gut mucosa as well as pancreas.

It possible that that could have been the mechanism of the response that you saw?

I think this was an excellent experiment, and it's a pleasure to comment upon it. Thank you very much.

DR. WILEY W. SOUBA, JR. (Boston, Massachusetts): Thank you Mr. President and Mr. Secretary. I too rise to congratulate Dr. Thompson and his colleagues on yet another fine study. I appreciated the opportunity to read this manuscript, and I would recommend it to you when it comes out in print.

The investigators have shown that subcutaneous administration of bombesin stimulates intestinal growth in Thiry-Vella loops, indicating a trophic mechanism that is independent of nutrients and pancreaticobiliary secretions. The fistulas were excluded from the flow of luminal contents, but their neurovascular supply was intact.

While the local effects of bombesin could be direct, it is also possible, as pointed out by Dr. Jones, that its effects are mediated via some secondary or more distal hormone that is part of a complicated cascade. The authors address this possibility very nicely in their discussion and point to the likely role of neurotensin in this pathway.

I have three questions for the authors.

First, using techniques such as thymidine incorporation, have you been able to identify the specific enterocytes that line the villous on which bombesin exerts its principal effect? I would think these effects would be most pronounced in the crypt cells, but I wonder if they are also observed in the more mature enterocytes.

Second, in order to investigate the possibility of a direct effect of bombesin on enterocyte growth, studies in cultured intestinal cells may be useful. Although bombesin receptors have apparently not been identified in the gut mucosa, it is possible that bombesin may have a trophic effect on cells, perhaps by sharing another receptor. Could you comment on this, please?

And, finally, have you looked at these responses in vagotomized rats or in rats with small bowel autotransplantation? This would be one way of getting a handle on the role of neural innervation in this bombesin-mediated trophic response.

Again, I congratulate you on your work, and I appreciate the privilege of the floor.

DR. JOHN B. HANKS (Charlottesville, Virginia): Thank you very much. This very elegant study represents yet another important contribution by the University of Texas at Galveston, the group headed by Drs. Townsend and Thompson, and analyzes the physiology and possible clinical applications of gut peptide secretion.

This presentation shows very nicely that bombesin stimulates gut mucosal proliferation, as Dr. Souba and Dr. Jones have pointed out. The data strongly suggest that the trophic effect is independent of luminal contents, which would include pancreatic and biliary secretion.

The idea that's raised, that bombesin is an important trophic hormone in both proximal and distal small bowel, has great importance and possible significant clinical application. I have just a couple questions that are discussed directly or indirectly in the manuscript, but I think the membership would appreciate hearing it in detail.

My first question deals with the dosage of bombesin. How was this dosage picked, and could the authors give a range of bombesin levels that the target tissues might be seeing? Do they feel that these levels are physiologic or supraphysiologic?

Second, as Dr. Souba and Dr. Jones have pointed out, there's multiple trophic factors in the gut. Do they think that