GLP-2 augments the adaptive response to massive intestinal resection in rat

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GLP-2 augments the adaptive response to massive intestinal resection in rat. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G911–G921, 1998.—To determine whether treatment with a potent protease-resistant analog of human glucagon-like peptide 2 (GLP-2) might augment the adaptive response to massive intestinal resection, rats were divided into resected, which had 75% of the midjejunum removed, sham-resected, and nonsurgical groups. Within each group, animals were assigned to 21 days of treatment with the drug (0.1 μg/kg of the GLP-2 analog in phosphate-buffered saline) or vehicle alone subcutaneously twice daily. Food intake; weight gain; jejunal and ileal diameters, total and mucosal wet weights per centimeter, crypt depths, and villus heights; mucosal sucrase activity, milligrams of protein per centimeter, and micrograms of DNA per centimeter; and D-xylose absorption were measured. There was a significant increase in diameter, total and mucosal wet weights per centimeter, crypt-villus height, sucrase activity, milligrams of protein per centimeter and micrograms of DNA per centimeter in both the jejunum and ileum in response to resection and a significant additive response to the GLP-2 analog in the jejunum but not in the ileum. The ratio of milligrams of protein per centimeter to micrograms of DNA per centimeter of mucosa was not different among groups, consistent with hyperplasia. D-Xylose absorption was significantly reduced in response to resection; however, the GLP-2 analog enhanced the absorptive capacity in control animals and restored the absorptive capacity in resected animals. Thus the GLP-2 analog induces mucosal hyperplasia and enhances the rate and magnitude of the proximal intestinal adaptive response to massive resection.

intestinal adaptation; short gut; glucagon-like peptide 2; growth factor

Short Bowel Syndrome is a clinical condition characterized by rapid intestinal transit and malabsorption in individuals who have had a congenital or, more commonly, an acquired lesion leading to intestinal resection (32). Surgical outcome in a given individual depends on the extent and location of resection and how well the residual intestine adapts to the reintroduction of oral nutrition. All layers of the bowel wall have been shown to participate in a hyperplastic adaptive response to intestinal resection (34). In addition to a marked increment in the diameter and some increase in the length of the residual intestine (9, 17, 18, 23, 26, 34), there are structural, functional, and cytokinetic adaptations of the mucosa (34), with augmentation of mucosal surface area. In both animal and human studies, the adaptive increase in mucosal mass is associated with gradual functional compensation that is characterized by diminishing fecal losses and increased transit time associated with increased segmental absorption of water, electrolytes, and nutrients (32).

Identification of factors regulating the adaptive hyperplastic response to resection and an ability to increase the magnitude or rate of the adaptive response would improve the clinical care of patients with short bowel syndrome and reduce their utilization of health care resources. Traditional concepts of bowel growth recognize the principal determinants of intestinal epithelial proliferation as including quantitative and qualitative nutrient ingestion, locally produced growth factors that act through paracrine or autocrine mechanisms, and circulating growth factors that act in a humoral or endocrine manner, all of which are coordinated as part of an integrated biological response (10). Bloom and Polak (1) were among the first to investigate the hormonal pattern of intestinal adaptation to resection, injury, or inflammation and documented elevated levels of intestinal proglucagon-derived peptides (PGDPs). This, and a previous report (16) of a patient with a glucagonoma who presented with massive enlargement of the small bowel that abated after surgical removal of the tumor, prompted subsequent analysis of the relationship between PGDPs and intestinal epithelial proliferation. Small bowel growth was enhanced in nude mice carrying transplantable subcutaneous glucagonomas, and the PGDP with the most marked intestinotrophic activity was identified as glucagon-like peptide 2 (GLP-2) (12). GLP-2 is a 33-amino acid peptide-encoded carboxy terminal to GLP-1 in proglucagon. It is synthesized in and released from endocrine cells of the intestine (12). GLP-2 promotes small intestinal growth after either subcutaneous, intraperitoneal, or intramuscular administration and dose dependently increases small bowel mucosal mass (31). The increased small bowel and mucosal mass is detectable after only 4 days of administration (12) and will persist for prolonged periods with ongoing GLP-2 stimulation. However, the small bowel epithelium regresses to its normal state several days after cessation of treatment (30). GLP-2 stimulates crypt cell proliferation, suggesting that signal transduction after GLP-2-receptor activation is associated with a mitogenic signal and inhibits apoptosis in villus epithelium of the small bowel (30). Although the small intestinal growth and induction of intestinal epithelial proliferation by GLP-2 results in an increased capacity for nutrient digestion and absorption in vivo (3), the therapeutic potential of GLP-2 in...
the context of small bowel resection has not been studied in either animal models or humans.

The aim of this study was to determine, in a rat animal model, whether treatment with ALX-0600, a potent protease-resistant analog of human GLP-2 (13), might increase the rate or magnitude of the normal intestinal adaptive response to massive intestinal resection.

MATERIALS AND METHODS

Experimental procedures were approved by the University of Calgary (Calgary, Alberta) Animal Care Committee. Growing 200- to 225-g Sprague-Dawley rats purchased from the University of Calgary Life and Environmental Sciences Animal Resources Center were utilized in this study. The animals were housed in individual cages with wire mesh floors and standard bedding. Lighting conditions were controlled for a 12:12-h light-dark cycle.

Experimental design. The rats were divided into a resected group, which had a 75% surgical resection of the midjejunum, a sham-resected operated control group in which the intestine was sectioned and reanastomosed, and an unoperated control group. The 75% intestinal resection was chosen to maximize any adaptive response (18, 23, 34), and retention of equal portions of the proximal jejunum and distal ileum was based on the nutritional implications (15, 23, 29, 36) of removing the specialized absorptive capacity of the terminal ileum for vitamin B₉ and bile acids and the ileal brake. In the rat, retention of 25% of the small intestine, inclusive of a portion of distal ileum, is sufficient to allow resected animals to achieve the same growth rate as control animals (23, 26).

Because the enteric mucosal adaptive response to surgical resection occurs over a time interval of several weeks in the rat (9, 17, 23, 26), we planned to assess the morphological and functional response of the gut to resection and treatment with the GLP-2 analog at 6, 14, and 21 days. To demonstrate that within this 21-day time interval we had followed the course of the trophic response to treatment with GLP-2 analog out to the plateau phase of its trophic activity, an additional group of unoperated, GLP-2 analog-treated animals was killed and studied after 40 days of therapy. Equal numbers of animals in the resected, sham-resected, and unoperated groups were given twice daily subcutaneous injections of either the drug (0.1 µg·g⁻¹·dose⁻¹ of GLP-2 analog in phosphate-buffered saline) or, as a control for the effects of the twice daily injections, an equal volume of vehicle alone. This created a total of six treatment groups: resected drug treated (RD), resected vehicle treated (RV), sham operated drug treated, sham operated vehicle treated, unoperated drug treated, and unoperated vehicle treated in which food intake and growth, gross and microscopic small intestinal morphology, and functional evaluation of mucosal absorptive and permeability characteristics were evaluated longitudinally as detailed below.

Surgical preparation. Surgery was performed on animals anaesthetized with an inhaled mixture of halothane and oxygen. The animals were prepared with providone and draped, and through a midline abdominal incision, the length of the small intestine between the ligament of Treitz and the ileocecal valve was measured by running a length of umbilical braid with centimeter markings parallel to the course of the gut. This technique has been shown to have a mean error of 2.7% compared with measurements made after the intestine has been fixed in situ (before any handling) with a 4% formaldehyde solution, resected, and then freed of mesentery along its entire length so that it can be laid out linearly (23). Once the total length of the small intestine had been determined, a 75% resection of the mid-small bowel was made, leaving the proximal 12.5% of the jejunum and the distal 12.5% of the ileum. A primary end-to-end anastomosis was performed between the resection margins with a 7-0 silk interrupted stitch. Postanastomosis, patency of the lumen and integrity of the anastomosis were tested by injecting 1 ml of normal saline through the wall of the jejunum near the anastomosis with a 25-gauge needle. The sham-resected animals had transection and reanastomosis of the jejunum at a point 12.5% of the distance along the intestine from the ligament of Treitz toward the ileocecal junction. The musculature of the abdominal wall was closed with interrupted sutures of 3-0 dixon, and the skin incision was closed with interrupted sutures of 3-0 silk. The animals received a subcutaneous injection of 10 ml of Ringer lactate and a 5% dextrose solution on day 1 postoperatively, a liquid feed (Sustacal, Mead Johnson) on the second through the fifth days postoperatively, and rat chow thereafter. Resected animals were allowed rat chow ad libitum. To control food intake in the various groups so that intake and growth were not greater in the animals with minor compared with major surgical intervention, the unoperated and sham-operated treatment groups were given the measured mean daily food intake of the resected group for that postoperative day.

Clinical, morphological, and biochemical parameters. Groups of control, sham-resected, and resected animals treated with either vehicle or drug were killed on days 6 and 21 postoperatively (n ≥ 8 in each treatment group on each day). In each treatment group, the daily food intake and weight of individual animals were recorded. The animals were killed by anaesthesia with diethyl ether and then cervical dislocation. After death, a laparotomy was performed. Animals with a stricture at the anastomotic site, as evidenced by any distension of bowel proximal compared with distal to the anastomosis, were excluded from study (≤5% of the resected and sham-resected rats) and are not included in the ≥8 animals in each treatment group on each day. Total length of the small intestine was measured again by running a length of umbilical braid with centimeter markings parallel to the course of the gut. Once the length was determined, the intestine between the ligament of Treitz and the ileocecal valve was removed and cleared of feces by gently flushing with normal saline. The gut was then placed in oxygenated Krebs solution. Components of the Krebs solution were (in mM) 120.3 NaCl, 5.9 CaCl₂, 2.5 KCl, 1.2 MgCl₂, 15.4 NaHCO₃, 1.2 NaH₂PO₄, and 11.5 glucose.

Locations 12.5 cm distal to the ligament of Treitz in the proximal gut and 12.5 cm proximal to the ileocecal valve were determined in all animals and in the resected group represented the site of the original anastomosis (if no increase in gut length had occurred). The first 5-cm segments proximal to this in the jejunum and distal to this in the ileum were discarded because mucosal adaptation to resection is much augmented in the intestine immediately adjacent (within 5 cm) to the anastomotic site (23), and all structural and biochemical measurements were performed on jejunal tissue obtained from the next 6 cm in a proximal direction in the jejunum and a distal direction in the ileum. From these 6-cm length segments, a 1-cm-wide ring of tissue was cut for measurement of diameter and preparation for histological assessment of crypt height and villus length while the remaining 5-cm segment was saved for measurement of total segmental and mucosal wet weights per 5 cm and mucosal sucrase activity, total protein, and DNA content.
Because measurement of the luminal diameter and wall thickness will vary with the degree of stretch, we assessed the diameter under physiologically determined, standardized conditions. The 1-cm-wide rings of jejunum and ileum were suspended transversely between the base of a 20-ml tissue bath and an isometric force transducer (0.50 g; Harvard Apparatus model 50-7905, Kent, UK) and bathed in Krebs solution. Temperature was maintained at 37°C, and the bath was bubbled continuously with 95% O₂-5% CO₂. Mechanical activity detected by the isometric force transducer was amplified by a transducer amplifier (Harvard Apparatus model 50-7970), input to a bioelectric amplifier (Hewlett-Packard model 8811A), and recorded on an eight-channel chart recorder (Hewlett-Packard model 7858A). Segments were allowed to stabilize in the tissue bath for 20 min and then stretched until muscle tension began to increase with any further increase in muscle length (the initial length). At this point, the internal diameter of the tissue ring was measured. The Krebs solution was then drained from the tissue bath and replaced with Formalin for fixation for a period of 12 h before histological studies were performed.

The 5-cm segments of proximal jejunum and distal ileum were opened longitudinally, gently patted dry, and weighed. Then the mucosa and as much of the submucosa as possible were scraped from the muscularis propria with a glass slide and weighed. Preliminary histological evaluation of gut segments from which the mucosa had been scraped was performed to assess the effectiveness of the technique for removal of mucosa. Duplicate samples from eight animals were assessed. Scraped segments showed no residual mucosa; only a thin layer of submucosal tissue remained adherent to the muscularis propria in every sample. The mucosa and submucosa were then homogenized and assayed for sucrase activity (7), total protein (21), and DNA content (24).

Formalin-fixed tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Crypt depth (base of crypt to base of villus), villus height (base of villus to villus tip), and total length of the crypt-villus axis (crypt base to villus tip) were measured with an ocular micrometer and a standard image-splitting micrometer with a Leitz (Wetzlar, Germany) microscope at a ×250 power. The muscularis propria in every sample. The muscularis propria were then homogenized and assayed for sucrase activity (7), total protein (21), and DNA content (24).

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Functional evaluation: mucosal absorptive and permeability characteristics. An index of mucosal absorptive and permeability characteristics was obtained from measurement of the urinary clearance of an orally administered load of D-xylose (14). D-Xylose assimilation is independent of pancreatic exocrine secretion, bile salts, or mucosal brush border enzyme activity, and the amount absorbed and excreted in the urine is proportional to the extent of the intact mucosal absorptive surface and the duration of contact (transit time).

Tests of intestinal permeability are designed to measure intestinal barrier function, which is a sensitive indicator of intestinal injury or disease (22). Damage to the mucosal barrier may be assessed noninvasively by the use of nonhydrolyzable sugar probes that permeate through the transcellular or paracellular (tight junction) routes, are not metabolized once absorbed, and are then excreted in the urine. Differentiation of damage sites (gastric vs. small intestinal vs. colonic) can be made provided sugar probes that are selectively destroyed at different levels along the bowel are utilized: class 1 probes (sucrose) are destroyed after leaving the stomach, class 2 probes (lactulose and mannitol) are destroyed after leaving the small intestine, and class 3 probes (sucralose) are not destroyed (22). In this study, mucosal absorptive capacity and permeability were examined in vivo on days 6, 14, and 21 and just before death in the animals scheduled for morphological and biochemical evaluation on those days. After a 2-h fast, nonhydrolyzable oligosaccharide probes were delivered by gavage directly into the stomach. Each animal received a 2-ml bolus of a solution containing 250 mg of sucrose, 40 mg of mannitol, 60 mg of lactulose, 30 mg of sucralose, and 25 mg of d-xylose. The rats were denied access to water for 3 h after gavage, at which point they were allowed free access to water for the remainder of the collection period. Urine was collected separate from fecal material in metabolic cages for 18 h, the volume was measured, and the composition was analyzed by high-performance liquid chromatography ( Dionex HPLC, Dionex, Oakville, Ontario) at room temperature with 520 mmol/L of NaOH as the isotropic mobile phase. Peak identification was accomplished with the use of authentic standards and detected with pulsed amperometric electrochemical detection on a gold electrode. Samples were diluted as necessary after the addition of cellobiose as an internal standard.

Statistical analysis. Results are expressed as means ± SE, where n is equal to the number of rats in each treatment group at each time interval. Food intake and weight gain are plotted against time (days 0–21) in each group and expressed as an area under the curve. Significance of the difference between means was determined at P < 0.05 with one-way ANOVA for three or more means. Tukey’s procedure was utilized for the post hoc comparison of pairs of means where multiple comparisons were possible.

Preliminary evaluation of the data showed that, for any of the parameters assessed, there were no significant differences between the means of unoperated and sham-operated rats in the vehicle- or drug-treated groups. To simplify presentation, the unoperated and sham-operated data were combined for further analysis as the control vehicle-treated (CV) and control drug-treated (CD) groups.

RESULTS

There was no difference in mean daily food intake among CV (29.3 ± 0.4 g/day), CD (30.5 ± 0.3 g/day), RV (30.7 ± 0.7 g/day), and RD (31.9 ± 0.9 g/day) groups. Figure 1 shows the increase in body weight of the CV, CD, RV, and RD groups. The mean body weights of these four treatment groups were not significantly different on day 0. In each group, body weight increased significantly from days 0 to 21. The slight decrease in body weight noted in each group on days 6 and 14 reflects the fact that the animals were fasted for 18 h preceding the in vivo testing of the functional parameters of absorption (urinary clearance of orally administered d-xylose) and permeability (urinary sucrose clearance, lactulose-to-mannitol ratio, and sucralose clearance) at 0800 on days 0, 6, 14, and 21. There were no significant differences in the area under the curve of mean body weight with time for these four treatment groups.

On day 21, there was no significant difference in gut length (the distance between the ligament of Treitz and the ileocecal valve) in the CV (103.7 ± 2.6 cm; n = 21 rats) compared with the CD (109.3 ± 3.3 cm; n = 18 rats) group or in the RV (32.5 ± 1.0 cm; n = 8 rats) compared with the RD (31.2 ± 0.8 cm; n = 11 rats) group; i.e., there was no effect of drug treatment on small intestinal length in control or resected animals. The small increment in length of the residual intestine with time (day 0 postanastomosis to day 21) was not significantly different in either the RV group (day 0
immediately postanastomosis: 28.9 ± 0.6 cm; n = 7 rats vs. day 21: 32.5 ± 1.0 cm; n = 8 rats) or the RD group (day 0 immediately postanastomosis: 28.3 ± 0.6 cm; n = 11 rats vs. day 21: 31.2 ± 0.8 cm; n = 11 rats); i.e., there was no significant adaptive increase in small intestinal length in response to resection alone or resection plus treatment with the GLP-2 analog.

In contrast to the lack of a significant normal adaptive or drug-induced increase in intestinal length, there were significant effects of massive resection and drug treatment on the proximal jejunal and distal ileal diameters (Fig. 2). In both the proximal jejunum and distal ileum of the CV group, there was a small and insignificant increase in luminal diameter (between days 6 and 21), consistent with normal growth. Massive intestinal resection was followed by a trend (not significant) toward an increase in luminal diameter in the proximal jejunum and a significant increase in diameter of the distal ileum (RV group). In the proximal jejunum, there was a significant increase in luminal diameter in the RD compared with the CV group. In the distal ileum, there was no effect of drug treatment on luminal diameter in either the control or resected groups.

The effect of massive intestinal resection and drug treatment on total segmental wet weight and on mucosal weight per 5-cm length are shown for both the proximal jejunum and distal ileum in Figs. 3 and 4, respectively. In both the proximal jejunum and distal ileum of the CV group, there was an increase in segmental wet weight and mucosal weight (between days 6 and 21), consistent with normal growth. Massive intestinal resection was associated with a significant increase in total segmental wet weight and mucosal weight in both the proximal jejunum and terminal ileum (RV vs. CV group, P < 0.05). Drug treatment was associated with a significant increase in total segmental and mucosal weight in the jejunum and ileum of control animals on both days 6 and 21 (CD vs. CV group, P < 0.05) and a significant additional increment in total segmental and mucosal weights in the proximal jejunum but not in the terminal ileum of resected animals on both days 6 and 21 (RD vs. RV group, P < 0.05). In the separate group of control animals treated with the GLP-2 analog for a total of 40 days, there was no significant difference between mucosal wet weight per 5 cm on day 40 compared with that on day 21, suggesting that by day 21 a plateau phase of the trophic mucosal response to therapy had been achieved.

To determine whether the increase in mucosal mass (measured as weight/unit length) observed in the proximal jejunum and distal ileum in response to massive intestinal resection or drug treatment was contributed
to by an increase in thickness of the mucosa as well as an increase in gut circumference, we next measured the length of the crypt-villus unit in micrometers. In both the proximal jejunum and distal ileum, there was a significant increase in length of the crypt-villus unit in response to drug treatment (CD vs. CV group, \( P < 0.05 \)) and a significant additional increment in sucrase activity in the proximal jejunum but not in the distal ileum of resected animals (RD vs. RV group) on day 21.

To determine whether the adaptive increase in mucosal mass, crypt-villus height, and sucrase activity in response to resection and/or drug treatment was due to hyperplasia (an increased number) and/or hypertrophy (an increased size) of the enterocytes, we measured total protein per centimeter and DNA per centimeter of length of the proximal jejunal and distal ileal mucosae 6 and 21, consistent with normal growth. Massive intestinal resection was associated with a significant increase in sucrase activity in the proximal jejunum and in the terminal ileum on days 6 and 21 (RV vs. CV group, \( P < 0.05 \)). Drug treatment was associated with a significant increase in sucrase activity in the proximal jejunum but not in the distal ileum of control animals on days 6 and 21 (CD vs. CV group, \( P < 0.05 \)), with a significant additional increment in sucrase activity in the proximal jejunum but not in the distal ileum of resected animals (RD vs. RV group) on day 21.

To determine whether the adaptive increase in mucosal weight, crypt-villus height, and sucrase activity in response to resection and/or drug treatment was due to hyperplasia (an increased number) and/or hypertrophy (an increased size) of the enterocytes, we measured total protein per centimeter and DNA per centimeter of length of the proximal jejunal and distal ileal mucosae.

\[ \text{Fig. 3. Total segmental wet weight/5 cm in proximal jejunum (A) and distal ileum (B) on days 6 and 21 for CV, CD, RV, and RD groups. Values are means } \pm \text{ SE. Significantly different (} P < 0.05 \text{) from: } * \text{ CV group on that day; } ** \text{ RV group on that day.} \]

\[ \text{Fig. 4. Mucosal weight/5 cm in proximal jejunum (A) and distal ileum (B) on days 6 and 21 for CV, CD, RV, and RD groups. Values are means } \pm \text{ SE. Significantly different (} P < 0.05 \text{) from: } * \text{ CV group on that day; } ** \text{ RV group on that day.} \]
In the distal ileum but not in the proximal jejunum of the CV group, there was an increase in total protein per centimeter and DNA per centimeter between days 6 and 21, consistent with normal growth. Massive intestinal resection was associated with a significant increase in total protein per centimeter and DNA per centimeter in the proximal jejunum and terminal ileum on days 6 and 21 (RV vs. CV group, \( P < 0.05 \)). Drug treatment was associated with a significant increase in total protein and DNA per centimeter in the proximal jejunum and terminal ileum on days 6 and 21 (CD vs. CV group, \( P < 0.05 \)). Drug treatment was not associated with a significant additional increment in total protein and DNA per centimeter in either the proximal jejunum or distal ileum of resected animals (RD vs. RV group) on day 21.

The ratio of milligrams of protein per centimeter to micrograms of DNA per centimeter of length of proximal jejunal or distal ileal mucosa was not significantly different among treatment groups on day 6 or 21, a finding consistent with mucosal hyperplasia. If sucrase activity is expressed per milligram of protein or per microgram of DNA instead of per centimeter, no differences were observed among the treatment groups in response to resection or drug treatment. Thus the increased sucrase activity per centimeter is due to mucosal hyperplasia, not to increased activity per cell and, by expressing the activity per unit of protein or DNA, the absolute magnitude of any increase in sucrase activity occurring in response to resection or treatment is normalized for the degree of mucosal hyperplasia.

(Figs. 7 and 8, respectively). In the distal ileum but not in the proximal jejunum of the CV group, there was an increase in total protein per centimeter and DNA per centimeter between days 6 and 21, consistent with normal growth. Massive intestinal resection was associated with a significant increase in total protein per centimeter and DNA per centimeter in the proximal jejunum and terminal ileum on days 6 and 21 (RV vs. CV group, \( P < 0.05 \)). Drug treatment was associated with a significant increase in total protein and DNA per centimeter in the proximal jejunum and terminal ileum on days 6 and 21 (CD vs. CV group, \( P < 0.05 \)). Drug treatment was not associated with a significant additional increment in total protein and DNA per centimeter in either the proximal jejunum or distal ileum of resected animals (RD vs. RV group) on day 21.

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Fig. 5. Villus height and crypt depth in proximal jejunum (A) and distal ileum (B) on day 21 in CV, CD, RV, and RD groups. Values are means ± SE. * Significantly different length of crypt-villus unit, \( P < 0.05 \) per no. of comparisons (Bonferroni correction factor).

Fig. 6. Mucosal sucrase activity in proximal jejunum (A) and distal ileum (B) on days 6 and 21 in CV, CD, RV, and RD groups. Values are means ± SE. Significantly different (\( P < 0.05 \)) from: * CV group on that day; ** RV group on that day.
To obtain an in vivo assessment of intestinal absorptive capacity, we measured the urinary clearance of an orally administered load of D-xylose on days 0, 6, 14, and 21 (Fig. 9). The fractional excretion of xylose did not change significantly with time (P > 0.05 by ANOVA) in the CV group. Compared with the CV group, the area under the curve of the fractional excretion of xylose with time was significantly decreased by massive intestinal resection (RV vs. CV group, P < 0.01 by two-tailed Student’s t-test) and significantly increased by drug treatment (CD vs. CV group, P < 0.01 by two-tailed Student’s t-test). The fractional excretion of xylose was significantly reduced in the RD compared with the CV group on day 6 (P < 0.001 by two-tailed Student’s t-test) but by day 21 was significantly increased (RD vs. CV group on day 21, P < 0.005 by two-tailed Student’s t-test). Thus, by day 6, drug treatment was unable to compensate for the reduced absorption as a result of massive intestinal resection, but by day 21, drug treatment had restored absorption in the resected group to levels above that seen in the CV group.

Urinary sucrose clearance, lactulose-to-mannitol ratio, and sucralose clearance were evaluated in vivo as a functional measure of gastric, small intestinal, and total intestinal permeability in animals in each treatment group on days 0, 6, 14, and 21. The data are shown in Table 1. There were no significant differences between the RV and CV groups (effect of resection alone), CD and CV groups (effect of drug alone), or RD, RV, and CV groups (effect of drug + resection vs. resection alone vs. no treatment, no additive effect).

**DISCUSSION**

In summary, in this rat model of the effect of a GLP-2 analog on massive midjejunoileal resection, there was a significant increase in diameter, total and mucosal wet...
weights per centimeter, crypt-villus height, sucrase activity, milligrams of protein per centimeter, and micrograms of DNA per centimeter in the proximal jejunum and distal ileum in response to resection and a significant additive increment in these parameters in response to the GLP-2 analog in the proximal jejunum but not in the terminal ileum. The ratio of milligrams of protein per centimeter to micrograms of DNA per centimeter of mucosa was not different among groups, consistent with a hyperplastic response. The GLP-2 analog significantly enhanced mucosal absorptive capacity (xylose clearance) in control animals. Although absorptive capacity was significantly reduced in response to resection, treatment with the GLP-2 analog restored absorptive capacity in resected animals to levels significantly above that of the CV group. No changes in permeability were detected in response to resection or treatment with GLP-2. Thus the GLP-2 analog induced mucosal hyperplasia and significantly enhanced both the rate and the magnitude of the proximal intestinal adaptive response to massive resection.

The validity and accuracy of the techniques we employed for the measurement of gut length and diameter have been previously established. The initial intestinal length measured in our resected and sham-resected treatment groups on day 0 before any intervention were not significantly different from each other. At the conclusion of the study on day 21, there was no effect of drug treatment on small intestinal length in either the control or resected treatment groups. Although there was a small increment in residual intestinal length with time in the resected vehicle- or drug-treated animals (112 and 110% of initial resected length, respectively), this change did not reach significance. There is inconsistency in the literature over whether there is an adaptive increase in intestinal length after resection. Nygaard (23) reported that the residual ileum increased its length by 139% of predicted (P < 0.001) after 75% proximal resection but found that the residual jejunum increased its length by 108% of predicted (P < 0.05) after 75% distal resection in rats. However, other authors have reported that intestinal resection caused no significant increases in the length of the residual small intestine in rats (20, 26) or in dogs with resections of up to 80% (15). Studies in humans (2, 8, 34) suggested that the potential for adaptive increases in intestinal length after resection may be greater in preterm versus full-term newborn infants, but several authors (33, 35) have suggested that an increase in intestinal length is not part of the adaptive response in adult humans. If there is a tendency to increased length of residual intestine after resection, its magnitude and physiological relevance in other than preterm infants would certainly appear to

Table 1. Mucosal permeability

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<tr>
<td>All animals</td>
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<td>0.408 ± 0.02</td>
<td>14</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>RD</td>
<td>17</td>
<td>0.42 ± 0.04</td>
<td>16</td>
<td>0.375 ± 0.02</td>
<td>16</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>13</td>
<td>0.83 ± 0.18</td>
<td>13</td>
<td>0.279 ± 0.01</td>
<td>13</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>CD</td>
<td>14</td>
<td>0.90 ± 0.15</td>
<td>12</td>
<td>0.248 ± 0.01</td>
<td>14</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>RV</td>
<td>8</td>
<td>0.77 ± 0.14</td>
<td>8</td>
<td>0.307 ± 0.06</td>
<td>8</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>RD</td>
<td>7</td>
<td>0.72 ± 0.19</td>
<td>4</td>
<td>0.207 ± 0.03</td>
<td>7</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>16</td>
<td>0.95 ± 0.21</td>
<td>16</td>
<td>0.275 ± 0.01</td>
<td>7</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>CD</td>
<td>19</td>
<td>0.81 ± 0.11</td>
<td>19</td>
<td>0.253 ± 0.02</td>
<td>8</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>RV</td>
<td>7</td>
<td>0.52 ± 0.07</td>
<td>7</td>
<td>0.246 ± 0.02</td>
<td>4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>RD</td>
<td>9</td>
<td>0.76 ± 0.12</td>
<td>9</td>
<td>0.291 ± 0.03</td>
<td>5</td>
<td>5.8 ± 1.0</td>
</tr>
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</table>

Values are means ± SE in percent urinary excretion of oral dose administered for sucrose, of ratio of lactulose to mannitol (Lact/Mann), and in percent excretion for sucralose; n, no. of rats/group. CV, control vehicle treated; CD, control drug treated; RV, resected vehicle treated; RD, resected drug treated.
be of less importance than the adaptive changes in intestinal diameter or circumference.

Our data and those of others (9, 23, 26, 36) show that there is a significant increase in luminal diameter or external circumference of the residual small bowel after extensive resection. It is the increment in this parameter that, in association with mucosal hyperplasia, permits dramatic increases in mucosal surface area and contributes to enhanced segmental absorption (34).

An increased tissue weight per centimeter of intestinal length is a consistent feature of the adaptive response to massive intestinal resection (17, 18, 23, 37). Nygaard (23) measured dry weight, Hanson and colleagues (17, 18) measured wet weight and documented that the percent water content was identical in control and resected tissues, and Wittman et al. (37) measured wet weight and documented increased protein content in resected versus control tissues. Thus, in each of these reports, the increased weight represented increases in tissue mass, not just in water content. Our data showing an increase in both total segmental and mucosal tissue mass in the proximal jejunum and terminal ileum of the RV group compared with control animals are consistent with the literature. Treatment with the GLP-2 analog was associated with a significant increase in mucosal mass in the proximal jejunum and terminal ileum of control animals and with a significant additional increment in mucosal mass in the proximal jejunum but not in the terminal ileum of resected animals. It is interesting that although the residual ileum is much more efficient and effective at intestinal adaption than the jejunum (23), the structural trophic responses that augmented the adaptive response to resection in animals treated with the GLP-2 analog were observed in the jejunum and not in the ileum. This suggests that under normal circumstances the hyperplastic response to resection has multiple mediators (GLP-2 does not act alone), that there are site-specific mediators of the hyperplastic response to resection, or that in the ileum (but not in the jejunum) the endogenous adaptive trophic response to 75% resection is nearly maximal and cannot be further stimulated.

The increase in mucosal mass per centimeter and in villus-crypt height observed in the jejunum and ileum of vehicle-treated rats after resection, in the jejunum and ileum of drug-treated control animals, and in the jejunum (but not in the ileum) of drug-treated resected animals is very clearly due to mucosal hyperplasia. This is consistent with previous studies (8, 17, 18, 23, 34) demonstrating that the mechanism of the increase in mucosal mass and the increase in crypt-villus height after resection is a mucosal hyperplastic response.

We identified functional as well as structural adaptation to resection and to treatment with the GLP-2 analog. Resection induced a significant increase in mucosal sucrase activity in both the jejunum and ileum, and treatment with the GLP-2 analog was associated with a significant additive increment in sucrase activity in the jejunum but not in the ileum of resected animals. However, there were no differences in sucrase activity in response to resection or to the GLP-2 analog if results are expressed as units of activity per milligram of protein or per microgram of DNA. The ratio of milligrams of protein per centimeter to micrograms of DNA per centimeter of jejunal or ileal mucosa was similar among treatment groups. This is consistent with mucosal hyperplasia and with previously published data showing that enhanced segmental absorption of water and glucose after intestinal resection correlates with the adaptive increase in mucosal weight and villus height but is achieved by a larger number of epithelial cells (hyperplasia) rather than an increase in their individual absorptive capacity (35).

One of the most important findings of this study was the demonstration that intestinal absorptive capacity (measured by the fractional urinary excretion of xylose) was significantly increased in control animals in response to treatment with the GLP-2 analog, significantly decreased by massive intestinal resection, and, in resected animals treated with the GLP-2 analog, was restored to levels above those seen in the CV group. Restoration of absorptive capacity likely reflects the trophic mucosal effects of the GLP-2 analog and the hyperplastic mucosal response to its administration. However, a potential for the GLP-2 analog to mediate an increase in the number of carrier proteins or an increase in the affinity of the transport protein for xylose cannot be excluded. In fact, recent in vitro studies of enterocytes after intestinal resection have demonstrated a transcriptional increase of transport proteins within hours (19) and an increased capacity of individual cells for transport and enzyme activity (27). In in vivo studies, vascular infusion of GLP-2 increased the D-glucose maximal transport rate in perfused segments of rat jejunum (6).

Recurrent catheter sepsis is a common clinical problem in patients with short gut syndrome, particularly small infants, and enteric pathogens are frequently implicated (32). This may occur as a result of colonization of skin and environment due to the large volume enteric losses in these patients, and the recovery of enteric organisms from the bloodstream does not necessarily imply bacterial translocation as a result of concomitant bacterial overgrowth, associated mucosal injury, and increased mucosal permeability (32). However, this study undertook to measure alterations in gastric, intestinal, and colonic permeability as a consequence of massive intestinal resection. The data accumulated does not support an increase in gastrointestinal permeability in resected animals compared with control animals.

A review of mediators regulating the adaptive response to massive intestinal resection is beyond the scope of this paper. The process is clearly multifactorial, and the literature supports three categories of factors: luminal nutrients (soluble fiber, fatty acids, triglycerides, glutamine, polyamines, and lectins), pancreaticobiliary secretions, and hormonal and/or systemic factors [growth hormone, epidermal growth factor, transforming growth factor-α, enteroglucagon,
insulin-like growth factors I and II, keratinocyte growth factor, GLP-2, gastrin, peptide YY, neurtensin, bombesin, and cytokines including interleukin (IL)-3, IL-5, and IL-11 (8, 10, 35). Previous studies (3, 12, 30, 31) have shown that the prolonged administration of GLP-2 to mice or rats rapidly stimulates a persistent small bowel trophic response characterized by crypt cell proliferation and an increase in mucosal cell mass, brush border enzyme activity, and absorptive capacity. However, this is the first demonstration that treatment with a GLP-2 analog promotes a trophic effect in addition to the normal adaptive intestinal response to massive intestinal resection.

What are the clinical implications of the fact that treatment with the GLP-2 analog is associated with a trophic proximal small intestinal mucosal response, which is additional to that of the intrinsic adaptive response to massive intestinal resection and actually restores a functional measure of mucosal absorptive capacity to normal? Obviously, patients who have had major intestinal resections complicated by malabsorption, with dependence on exogenous nutrient supplementation, would benefit (10). In addition, patients with a functionally short intestine, that is, reduced mucosal surface area as a result of inflammation (e.g., Crohn's disease) or injury (radiation or chemotherapy), might also benefit from treatment (10). In this respect, the data demonstrating that the trophic response and increased absorptive capacity is maintained during prolonged courses of treatment (3, 30); is intestine specific compared with other factors with potent intestinal trophic properties such as growth hormone, epidermal growth factor, insulin-like growth factor I, or IL-11 (10); is a more potent agent for increasing small and large bowel mass than epidermal growth factor, insulin-like growth factor, and human growth hormone (11); and is safe in long-term animal trials (30) are very encouraging. As well, recent attempts to employ parental growth hormone, glutamine supplementation, and a high carbohydrate diet to enhance adaptation and avoid long-term home parental nutrition in humans have had disappointing results (25). In a randomized, double-blind, placebo-controlled crossover study in eight patients with well-established short bowel syndrome, Scapolio et al. (25) found only modest improvements in electrolyte absorption and delayed gastric emptying but no improvements in small bowel morphology, stool losses, or macronutrient absorption in response to active treatment with growth hormone, glutamine, and a high-carbohydrate, low-fat diet. The doses of growth hormone and glutamine were similar to those used in less rigorously designed and previously published trials (4, 5) that had suggested a potential benefit of these agents (20). The lack of benefit of current therapeutic modalities and the incremental trophic effect that we have shown for treatment with the GLP-2 analog compared with the adaptive response to resection alone suggests that a well-designed therapeutic trial of the GLP-2 analog in human short bowel syndrome is warranted. Success in such a human trial would potentially reduce both the morbidity and mortality of individuals with short bowel syndrome and the costs associated with the prolonged hospitalization and home parenteral/enteral nutrition of these patients.

We are grateful to Roberta Funk for typing the manuscript and to Daimen Tan, Kim Tran, and Matthew Lauzon for expert technical assistance.

This work was supported by an operating grant from Allelix Biopharmaceuticals, Mississauga, Ontario, Canada L4V 1V7. Address for reprint requests: R. B. Scott, Dept. of Pediatrics, Faculty of Medicine, Health Science Centre, The Univ. of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 1N4. Received 25 February 1998; accepted in final form 7 july 1998.

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