Time-dependent intestinal adaptation and GLP-2 alterations after small bowel resection in rats

K. LJUNGMANN,1,2 B. HARTMANN,4 P. KISSMEYER-NIELSEN,1 A. FLYVBJERG,3 J. J. HOLST,4 AND S. LAURBERG1
1Surgical Research Unit, Department of Surgery L, 2Stereological Research Laboratory, and 3Department of Medicine M, Aarhus University Hospital, DK-8000 Aarhus C; and 4Department of Medical Physiology, the Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark

Received 18 January 2001; accepted in final form 8 May 2001

Ljungmann, K., B. Hartmann, P. Kissmeyer-Nielsen, A. Flyvbjerg, J. J. Holst, and S. Laurberg. Time-dependent intestinal adaptation and GLP-2 alterations after small bowel resection in rats. Am J Physiol Gastrointest Liver Physiol 281: G779–G785, 2001.—Existing data on morphological adaptation after small bowel resection are obtained by potentially biased methods. Using stereological techniques, we examined segments of bowel on days 0, 4, 7, 14, and 28 after 80% jejunoileal resection or sham operation in rats and correlated intestinal growth with plasma levels of glucagon-like peptide-2 (GLP-2). In the jejunum and ileum of the resected rats, the mucosal weight increased by 120 and 115% during the first week, and the weight of muscular layer increased by 134 and 83%, compared with sham-operated controls. The luminal surface area increased by 190% in the jejunum and by 155% in the ileum after 28 days. The GLP-2 level was increased by 130% during the entire study period in the resected rats. Small bowel resection caused a pronounced adaptive response with plasma levels of the intestino-trophic candidate GLP-2.

This study aimed at correlating the adaptive response to intestinal resection in the rat (29), and plasma levels of GLP-2 have been correlated with the intestinal hyperplasia seen in streptozotocin-induced diabetic rats (9, 32). The role of endogenous GLP-2 in the adaptive intestinal response after resection has not been established yet.

The purpose of this study was to provide new and firm estimates of the time-dependent adaptive changes in the intestinal wall and surface area of the small and large bowel after small bowel resection and thus establish whether all layers of the bowel wall participate, whether there are regional differences, and to what extent the proximal colon participates in the adaptive response. Finally, this study aimed at correlating the adaptive response with plasma levels of the intestino-trophic candidate GLP-2.

MATERIALS AND METHODS

Animals

One hundred female Wistar rats (Møllegården Breeding Centre, Ejby, Denmark) weighing 174–227 g were used. They were housed individually and acclimatized for 1 wk before surgery. Animal quarters were lit in alternate 12-h cycles. All procedures were carried out in accordance with the Danish legislation regarding the care and use of laboratory animals.

Design

Rats were randomized to either resection or sham operation and were observed for 0, 4, 7, 14, and 28 days.
Surgical Procedures

Nonfasted rats were given atropine sulfate (0.05 mg/kg) and ampicillin (Anhypen, 100 mg/kg) subcutaneously and were anesthetized by subcutaneous injection of fentanyl-fluanisone (Hynopnorm, 0.3 ml/kg) and midazolam (Dormicum, 3.75 mg/kg) before surgery. A subcutaneous injection of 10 ml of isotonic saline was given preoperatively to prevent dehydration. After a midline laparotomy, the small bowel was marked 5 cm distal to the ligament of Treitz and 10 cm proximal to the ileocecal valve, marking out ~80% of the small bowel. The rats were then randomized to either resection or sham operation. Before transection of the small bowel at the distal marking, a nonabsorbable suture (6–0 Prolene) was placed 2 cm distally and proximally to the anastomosis to achieve standardized jejunal and ileal segments of equal length and location, which could later be compared without considering changes in length caused by the experimental settings. In addition, a 4.5-cm proximal colon segment (2 cm distal to the cecum) was marked out (Fig. 1). The resected small bowel segment was rinsed, and the weight and length were determined. Sham-operated rats were transected and reanastomosed. The abdominal wall was closed in two layers. The rats received a single dose of buprenorphine (Temgesic, 0.01 mg/kg) subcutaneously for postoperative pain treatment.

Food Intake

To secure similar food intake in the two groups, the sham-operated group was given the measured mean daily food intake per gram body weight of the resected group for a given postoperative day. All the food offered was readily consumed by the sham-operated rats.

Experimental Procedures

Stereological analyses. The determinations of luminal surface areas (2) and volume fractions (10) were based on vertical sections of the tissue. The sampling procedure for vertical sections has recently been described in detail (22), and this procedure was followed (Fig. 1).

Briefly, the sections were counted by use of an Olympus BX50 microscope with a personal computer and a monitor connected to a video color camera mounted on top of the microscope. With the use of the Computer Assisted Stereological Toolbox-Grid software (Olympus), the stereological probes (points and cycloids) were superimposed onto the video images of the tissue sections viewed on the monitor (Figs. 2 and 3). Fields of vision were systematically randomly sampled in the sections by using a fixed step length (1,600 or 800 μm) accomplished by a motorized specimen stage controlled by the program. The estimates of the weights of the different intestinal layers are based on the assumption that the specific gravity is equal and ~1.0 in all layers of the bowel wall. All stereological counting procedures were performed in a blinded fashion to prevent observer bias.

The growth rates in the jejunal and ileal segments were compared to investigate possible regional differences in the small bowel adaptive response. This was done by testing differences in the means of the ratios of corresponding jejunal and ileal layers on days 0 and 28 among resected rats, as follows

\[ \frac{x_{28} - x_0}{x_0} = \frac{y_{28} - y_0}{y_0} \Rightarrow \frac{x_0}{y_0} = \frac{x_{28}}{y_{28}} \]

where \( x_n \) is the weight of the jejunal layer on day \( n \) and \( y_n \) is the weight of the ileal layer on day \( n \).

Blood samples and assays. All animals were killed within 3 h, from 0900 to 1200. Blood was sampled from the inferior vena cava on days 0, 4, 7, 14, and 28, a few minutes before sacrifice of the bowel specimens. Plasma enteroglucagon and GLP-2 concentrations were measured after extraction of plasma with 61% and 75% ethanol (vol/vol, final concentration). The enteroglucagon RIA was directed against a mid sequence of the glucagon molecule comprising residues nos.
6–15 (antibody code no. 4304) and therefore also measures glucagon-containing peptide moieties of intestinal origin (gli- centin and oxyntomodulin) (14, 15). For this assay, sensitiv-
ity was below 2 pmol/l, intra-assay coefficient of variation
was below 6% at 40 pmol/l, and recovery of standard (human
glucagon identical to rat glucagon), added to plasma before
extraction, was 100% when corrected for losses inherent in
the plasma extraction procedure. GLP-2 concentrations in
ethanol-extracted plasma were measured using an RIA em-
ploying antiserum code no. 92160 and standards of human
GLP-2 (proglucagon 126–158, a gift from Novo Nordisk,
Bagsvaerd, Denmark) and monooiodlated Tyr-12 GLP-2, spe-
cific activity >70 MBq/nmol (13). The antiserum is directed
against the N-terminus of GLP-2 (identical for rat and hu-
man GLP-2) and therefore measures only fully processed
GLP-2 of intestinal origin. Sensitivity was below 5 pmol/l,
and intra-assay coefficient of variation at 60 pmol/l was 6%.
The IGF-I RIA was based on a polyclonal rabbit antiserum
(Nichols Institute Diagnostics, San Juan Capistrano, CA),
monooiodated 125I-IGF-I (Novo Nordisk) and was calibrated
against rhIGF-I (Amgen Biologicals). The intra- and interas-
say coefficient of variation percents were 5 and 10%, respec-
tively.

Statistics

As expected and clearly depicted in the figures illustrating
the small bowel adaptive growth, the time-dependent growth
was different in the two groups, which was confirmed by a
two-way analysis of variance (P < 0.001). However, the same
test applied on the colonic morphological data could not
confirm a difference in the time-dependent growth. Data on
GLP-2, enteroglucagon, and morphological ratios were natu-
ral log-transformed for better approximation to the normal
distribution. The Student’s t-test was used to compare the
groups of interest, and 95% confidence limits (CL) for differ-
ences between means were calculated. Equal variances were
not assumed for the morphological data.

RESULTS

Body Weight

Changes in percentage of the preoperative weights
are shown in Fig. 4. Both study groups lost weight
toward day 4, whereafter they started to gain weight.
There was no difference in weight gain at postoperative
day 28.

Small Bowel Morphology

The most pronounced growth took place in the first
postoperative week, when the growth of the jejunal and
ileal mucosa was 120 (P < 0.05, CL = 0.12–0.32 g) and
The muscular layer demonstrated similar growth with CL5115% (0.096–0.21 g) and 133% (0.22 g), respectively. Correspondingly, the muscular layer displayed the same pattern as the mucosal growth, without reaching statistical significance.

**Enteroglucagon and Glucagon-like Peptide-2**

Enteroglucagon and GLP-2 measurements displayed an almost identical pattern of response to small bowel resection. Enteroglucagon levels were on average (data pooled from day 4 to day 28) increased by 106% (P < 0.0001, CL = 77–137%) in the resected group compared with the sham-operated group, and correspondingly GLP-2 levels were increased by 130% (P < 0.0001, CL = 89–179%) (Fig. 6).

**IGF-I**

On day 4, serum IGF-I levels were reduced by 49% (P < 0.001, CL = 178–294 µg/l) in resected rats and by 49% (P < 0.001, CL = 190–323 µg/l) in sham-operated rats compared with day 0 values (Fig. 6). Baseline values were not reached for either of the two groups within the study period.

**DISCUSSION**

The existing data on intestinal adaptation after small bowel resection are, because of the methodological problems and limitations of previous studies, potentially biased. Accordingly, the present study contains the first firm and unbiased measurement of the adaptive small bowel and colonic growth during the first 4 wk after extensive small bowel resection. There was a significant, early, and persistent adaptive growth response in the remaining small bowel, which involved the entire intestinal wall and markedly increased the luminal surface area, whereas the early growth response in the colon was of a temporary nature. In contrast to what is generally believed, we found that the adaptive growth was stronger in the jejunum than in the ileum. The continuously elevated GLP-2 level in the resected rats is consistent with the hypothesis that GLP-2 plays an important role in the adaptive response after small bowel resection.

Review articles on the subject of intestinal adaptation after intestinal resection have primarily focused on the adaptive response of the mucosa, which is probably justified from a clinical viewpoint (5, 27, 37),

### Table 1. Mean differences in percentage between resected and sham-operated rats for the mucosal, submucosal, and muscular layers

<table>
<thead>
<tr>
<th></th>
<th>Mucosa</th>
<th>Submucosa</th>
<th>Muscularis Propria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jejunum</td>
<td>Ileum</td>
<td>Colon</td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95% (0.17 g)</td>
<td>81% (0.27 g)</td>
<td>30% (0.07 g)</td>
</tr>
<tr>
<td></td>
<td>[0.11–0.23 g]</td>
<td>[0.23–0.32 g]</td>
<td>[0.02–0.1 g]</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>120% (0.22 g)</td>
<td>115% (0.40 g)</td>
<td>41% (0.1 g)</td>
</tr>
<tr>
<td></td>
<td>[0.12–0.32 g]</td>
<td>[0.32–0.49 g]</td>
<td>[0.04–0.2 g]</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td>178% (0.40 g)</td>
<td>91% (0.45 g)</td>
<td>26% (0.06 g)</td>
</tr>
<tr>
<td></td>
<td>[0.29–0.51 g]</td>
<td>[0.34–0.57 g]</td>
<td>[0.03–0.1 g]</td>
</tr>
<tr>
<td><strong>Day 28</strong></td>
<td>298% (0.48 g)</td>
<td>162% (0.69 g)</td>
<td>13% (0.04 g)</td>
</tr>
<tr>
<td></td>
<td>[0.55–0.61 g]</td>
<td>[0.52–0.82 g]</td>
<td>[–0.03–0.11 g]</td>
</tr>
</tbody>
</table>

Numbers in parentheses are mean differences in absolute values and numbers in square brackets are 95% confidence limits.
although previous studies suggest that all intestinal layers are involved in the adaptive response (6, 11, 30). The present data clearly support the theory that the adaptive response is a transmural process, in proximal as well as distal small bowel. The significant adaptive hyperplasia of the muscularis propria layer observed at present and previously (30) deserves more attention, because it might have functional importance in relation to small bowel contractility and motility (4).

The most marked and inevitable effect of small bowel resection is the loss of absorptive area, which has prominent clinical consequences and in the most severe cases can lead to short bowel syndrome. It is well established that the adaptive response in some part compensates for the bowel loss by increasing the surface area of the remaining intestine. However, so far these important increases in surface area have not been quantified, probably because of a lack of proper methods. Modern stereological techniques offer a good solution to these problems, as demonstrated in this study.

The typical end points used in previous small bowel morphology studies have been villus height and cell counts along the crypt-villus axis. Besides the fact that these variables have limited value for interpreting the biological significance of changes in bowel morphology (23), another problem lies in the various sampling procedures used. Specimens are usually sampled from the bowel at one or two specific sites, which have not been defined before surgery. When the specimens are being examined microscopically, certain selection criteria are applied, e.g., the ten tallest villi or a specified number of perfectly sectioned crypts. As a result, the specimens may be neither representative nor comparable, and the fields of vision are not chosen randomly and independently of content and observer.

To our knowledge, the methods used in the present study and a number of previous experimental studies (19, 20, 22) represent the only existing practical solution to these problems. The marking out of small and large bowel segments during surgery provides standardized segments for later comparison and allows a reliable quantification of alterations in mass and surface area. Furthermore, the sampling requirements for vertical section stereology (2) secure a systematic and independent sampling and accordingly an unbiased quantification. We find the present unbiased stereo-
logical techniques highly recommendable for future experimental studies in this field.

It is generally assumed that the ileum displays a more robust adaptive response to resection than the jejunum (5, 35, 37) and that this should be explained by the unaccustomed spill of richer than normal chyme into the ileum and by relative increments in the luminal bulk received by the ileum in resected animals. Several studies using a short bowel model resembling the present one have indicated a greater adaptive potential of the ileum (11, 12, 21, 36), whereas we have only managed to find one study in which the data indicate the opposite (34). That study and the present one share in common the fact that similar food intake was ensured in the resected and sham-operated groups, in contrast to the studies cited supporting a greater adaptive potential of the ileum. It has been demonstrated that sham-operated rats are capable of eating greater amounts of food compared with resected animals (24, 26), implying that the jejunum of the sham-operated animals in this situation receives a greater luminal bulk of nutrients compared with the jejunum of the resected animals. Bearing in mind that luminal nutrition probably is the prime stimulus to intestinal growth (8, 21), it seems most likely that these essential differences in feeding regimens explain the conflicting results between the present and other studies.

However, it must be emphasized that, with the present knowledge, the general belief of a greater adaptive capacity of the ileum possibly holds true in short bowel models in which either an extensive proximal or distal resection (including the terminal ileum) has been performed (1, 6). It is also well recognized that in humans jejunal resections are often better tolerated than ileal ones. A contributory factor to the lack of jejunal adaptation in patients with ileectomy may be the removal of GLP-2-producing tissue (17). In contrast, elevated GLP-2 (and GLP-1) concentrations have recently been demonstrated in ileum-resected short bowel patients with the colon in continuity (18), which may contribute to the intestinal adaptation usually observed in these patients.

The contribution of endogenous GLP-2 to the development of intestinal hyperplasia has been studied in rodent models of experimental diabetes (9, 32), in which increased levels of circulating and intestinal GLP-2 were observed. These are correlative observations that suggest a link between intestinal growth and secretion of endogenous GLP-2. The present results show a similar, significant correlation between intestinal hyperplasia and an endogenous GLP-2 response and furthermore add new insight to the time-dependent changes of GLP-2 during the adaptive process in the rat short bowel model.

The most active adaptive growth took place during the first postoperative week, at which point we observed a significant peak in the circulating levels of GLP-2. This GLP-2 peak was surely not caused by an elevated food intake (17), because the intake was at a minimum during this period. At this early postopera-

The technical assistance of Edith Kirkedahl is greatly appreciated.

This work was supported by the Danish Medical Research Council, the Institute of Experimental Clinical Research, University of Aarhus, Denmark, and the Aarhus University-Novo Nordisk Centre for Research in Growth and Regeneration (Danish Medical Research Council Grant no. 9600822).

REFERENCES

10. Hanson WR, Osborne JW, and Sharp JG.
13. Hanson WR, Osborne JW, and Sharp JG.