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A Useful Experimental Model of Short Bowel Syndrome

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ABSTRACT The short bowel syndrome is a well-known human clinical entity that produces serious metabolic disorders. This syndrome arises after a massive resection of more than 50% of the small intestine, when the intestine attempts to minimize the consequent irregularities by means of compensatory mechanisms. Many reports suggest that an exocrine and endocrine pancreatic dysfunction is associated with enterohormones and an abnormal altered nutrient flow. In this report, we present an experimental model of short bowel syndrome in rats. A massive intestine resection was performed in rats, followed by a histological study of the small intestine. We report the histological changes related to the compensatory changes that occurred in the remaining intestine. The residual intestine produces a hyperplasic response, and hypertrophy was seen in the portion proximal to the anastomosis. We believe this experimental model of short bowel syndrome could be a very useful tool for studying the enterohormonal changes related to an abnormal blood flow of nutrients.

KEYWORDS diabetes, experimental surgery, gut, short bowel syndrome, small intestine

The short gut syndrome is a well-known clinical entity. In current surgical practice, there is considerable interest in what is now designated short bowel syndrome (SBS). This syndrome can be described as the group of dysfunctions that arise after the resection of possibly more than three quarters of the small intestine [1]. Prevalence of SBS is increasing for two main reasons. First, the development of effective methods of postoperative nutrition now allows patients to recover after massive intestinal resections producing severe nutritional deterioration. Second, there have been important recent advances in so-called "bariatric surgery" in response to the large and increasing numbers of people, particularly in the Western world, who present a morbid obesity

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[2]. This surgery is also called "metabolic intestinal surgery" and involves using surgical "by-pass" procedures to render long segments of the intestine nonfunctional, with the aim of facilitating significant weight loss by the patient [3].

The surgeon must be aware of the pathophysiological mechanisms associated with SBS, and the processes of intrinsic adaptation effected by the organism to counteract the loss of functional segments of absorptive intestine. Recent research on SBS has increased understanding of this syndrome and its consequences, enabling postoperative care to be improved [4]. All this, in turn, has improved the prognosis of patients requiring the extirpation or the bypassing of a substantial intestinal segment, for any etiological reason [5].

The SBS produces several physio-pathological alterations in the absorption of nutrients. Due to reduced absorption surface, the compensatory mechanism includes an increase in the rate of absorption [1]. The nutrients most affected are carbohydrates and fatty acids [5, 6]. Also those organs involved in postabsorption processes will modify their functions. This system is mainly controlled by an enterohormonal axis. Enterohormones play a role in the processes of digestion, absorption, and indirect metabolism. Of particular importance are the upper portion of jejunum (releasing cholecystokinin, secretin, and GIP) and the ileum (secreting enteroglucagon, neurotensin, etc.) [7].

A special aspect of this is the relationship that exists between the jejunum and the exocrine and endocrine functions. Some authors have reported the abnormal metabolism of fatty acids, and precipitation in the exocrine glands of the pancreas and liver [8].

The effect on the endocrine pancreatic function seems to be the most important. Several authors have reported abnormal glycemia and insulinemia levels after an intravenous glucose load in humans with SBS [9, 10]. This is an important datum, since the findings of abnormal functioning are very similar to those presented by diabetes mellitus patients. This accords with current theories about the factors that produce the abnormal function of insulin release in the beta cells: oscillations in the glycolitic pathways, and malonyl-coenzyme A (CoA) and long chain acyl-CoA esters [11–15].

On the basis of these facts, we decided to create an experimental model of SBS. The aims of the project are to investigate the physio-pathological basis of SBS and evaluate the consequences of some of the most important parameters of intestinal resection. Moreover, this model should be of use in studying the behavior of the enterohormonal axis. The profound alterations that appear in this syndrome could also be useful for considering the functioning of the pancreas and other intestinal organs.

Different factors can be studied in this model by modifying the different parameters. These variables include the length of the resected small intestine, the location of resected portion, the time after operation (survival time), and the type and quantity of absorbed nutrients. All of these are aspects that often arise in clinical practice.

MATERIAL AND METHODS

We used 30 male Wistar rats, of 180–200 g weight. The animals were divided into 3 groups of 10 rats each. Group I was taken as the control, and we did not perform any intestinal intervention on these rats, although this group was anesthetized and underwent a laparotomy (sham laparotomy) similar to the other two groups. Animals in group II underwent an intestinal resection from the angle of Treitz to the ileocecal valve, followed by an end-to-end anastomosis with silk 00000 monoplane suture in interrupted points. In group III, 50% of the small intestine was resected. The resection included the distal portion of jejunum and the proximal portion of ileum, leaving the same length from the proximal jejunum as the distal ileum. The terminal portions were subsequently anastomized end-to-end, with silk 00000 monoplane suture in interrupted points. All the interventions were performed while under general anesthesia by inhalation of ether in a closed glass chamber. The animals were given conventional preoperative treatment, including a standard fasting period. In the postoperative period, animals were given a dose of analgesic (paracetamol) with water to avoid pain. The rats recovered normal activity a half an

hour after the intervention, and they began to drink 5 h later. The mortality was less than 5%.

The animals were fed with a standard diet until the day of the sacrifice, carried out 21 days after the surgical intervention. This sacrifice was carried out by decapitation to avoid unnecessary pain. The abdominal cavity was opened immediately. We extracted a 5-cm fragment from the proximal jejunum situated proximally 1 cm from the angle of Treitz and a 5-cm fragment from the distal ileum located 2 cm distally from the ileocecal junction. The intestinal segments were fixed in Bouin solution for 4 h, dehydrated using graded alcohols and xylol, and embedded in paraffin medium by routine procedures. Serial paraffin-embedding sections of 5 μm were obtained and mounted on albumin-coated glass slides. They were counterstained with modified Harris hematoxylin–eosin for histological study.

The morphometric parameters studied were complete epithelial height (TH), the villous height (VH), the villous cellular height (CVH), the number of cells per unit of villous length (CVH/VH), the mucous index (mi), the cryptal height (CH), and the mitotic index (MI). All the data obtained were analyzed by matching group I with II, group I with III, and group II with III, and applying Student's *t*-test.

The experiment was approved by the Ethical Committee for Animal Experimentation of the University of Cádiz, which certified the welfare of rats during the surgical and medical treatment.

RESULTS

The samples studied had perfect succession of the epithelium from the bottom of the crypt to the same vertex of the corresponding ciliary tissue. The distance between them was sufficient to avoid the possibility of a double contracted sample.

We analyzed the samples by taking three different morphological parameters: (1) the total height of the intestinal mucous epithelium (TH); (2) the villous characteristics (VH, CVH, CVH/VH, mi); and (3) the structure and proliferation of the crypts (CH, MI). From this analysis, the following findings are reported.

In the jejunum, it was demonstrated that the single intestinal anastomosis caused the total height of the crypt–villous system to increase, with this increase being statistically significant ($p < .0005$). After the intestinal resection, we found a similar increase, also statistically significant, when we compared it with the control samples. However, we did not find a statistical difference when the total heights of groups II and III were compared. This indicated that the jejunum undergoes a compensatory hypertrophy after the resection and the anastomosis.

When these parameters were studied in the ileum, it was found that the ileum did not undergo a compensatory hypertrophy after resection and anastomosis, since the values were similar to those of the control group, and the difference was not statistically significant.

The villous height of the jejunum increased significantly after resection and anastomosis, when compared with the control group. This indicated that in both processes there was a similar compensatory hypertrophic response. However, when we analyzed the number of villous cells (CVH), we found that there was a significant increase in these cells only after the intestinal resection (Figure 1). From this, we deduce that there was a concomitant hyperplasic response only in group III. This response was not seen in group III. The hyperplasia was more clearly manifested when we studied the number of cells per unit of villous length. There was an increase of 20% in this parameter in animals from group II, compared to the controls. This seems to demonstrate that after the intestinal resection of the jejunum there were increases in the length of the cilium (hypertrophy) and in the cellular density (hyperplasia). This phenomenon did not occur after the anastomosis.

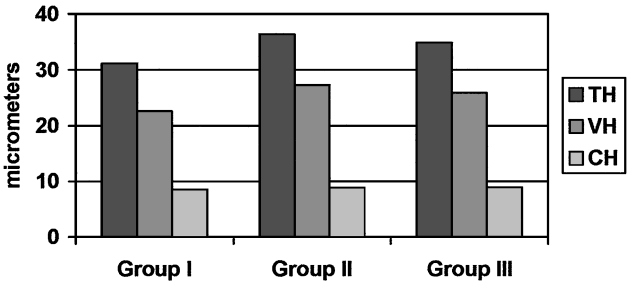

FIGURE 1 Parameters in the jejunum.

TABLE 1 Morphologic parameters in the jejunum

	Group I	Group II	Group III	Group II vs. group I	Group III vs. group I	Group III vs. group II
TH	31.12 ± 0.66	36.38 ± 0.63	34.82 ± 0.65	$p < .0005$	$p < .0005$	n.s.
VH	22.54 ± 0.57	27.25 ± 0.60	25.87 ± 0.55	$p < .0005$	$p < .0005$	n.s.
CVH	45.38 ± 1.29	49.88 ± 0.85	65.33 ± 0.81	n.s.	$p < .0005$	$p < .0005$
CVH/VH	2.10 ± 0.19	1.83 ± 0.14	2.52 ± 0.17	n.s.	$p < .01$	$p < .0005$
mi	14.16 ± 0.87	10.07 ± 0.54	7.87 ± 0.46	$p < .0005$	$p < .0005$	$p < .0005$
CH	8.58 ± 0.23	8.89 ± 0.20	8.94 ± 0.18	n.s.	n.s.	n.s.
MI	4.12 ± 0.61	4.78 ± 0.63	4.74 ± 0.63	n.s.	n.s.	n.s.

Note. TH, VH, and CH in μm; CVH, number of cells; mi and MI in number of cells per hundred; n.s., not significant.

The percentage of mucous cells in the villous of the jejunum decreased mainly after the resection, but also after the anastomosis (Table 1). This decrease was significant in all the groups. The decrease of the mucous index was a sign of regeneration in the intestinal epithelium, which reached its maximum level when it was accompanied by the processes of hyperplasia and hypertrophy.

The study of the parameter VH in the ileum demonstrated that a compensatory hypertrophic response was not present (Figure 2). However, we found an increase in the total cell count in the villous in samples from group III (Table 2). From this we deduce, that after ileum resection, there was a hyperplasic response, but a hypertrophic response was not present. In the ileum, we also found a similar decrease of the mucous index after the section (Table 2). This decrease was highly significant when we compared it to that of groups I and II.

The height of the crypts in the jejunum and ileum did not show significant differences among the three groups. The mitotic index was not altered in the jejunum (Table 1), which suggested that there were no proliferative changes after resection or after anastomosis. However, in the ileum we found a decrease in the mitotic index after resection and anastomosis (Table 2). Differences were not significant in the comparison between the samples with a lower mitotic count to the other groups.

DISCUSSION

The residual intestine compensates for the loss of digestive and absorptive function following massive small bowel resection, through an adaptative process. The pathogenesis of the adaptative process is complex, but probably involves luminal nutrients, gastrointestinal secretions, growth factors, and circulating hormones [4]. The changes that occur in the remaining intestine after the resection of the small intestine have been interpreted differently by various authors. Monari [16] found a lengthening and an increase in the number of villi per surface unit in the mucous membrane after resections in dogs. Flint [17] resected more than 70% of the intestine of dogs and found a duplication of the size of the cilium, which he interpreted as a quadruple increase of the area of absorptive surface. In 1939, West [18] demonstrated a hypertrophy in the residual intestine of a patient, who 2 years previously, had undergone a massive resection. Similar clinical findings were found by Althausen [19]. Bochkov [20] carried

TABLE 2 Morphologic parameters in the ileum

	Group I	Group II	Group III	Group II vs. group I	Group III vs. group I	Group III vs. group II
TH	36.18 ± 0.65	37.10 ± 0.66	34.92 ± 0.61	n.s.	n.s.	n.s.
VH	25.59 ± 0.63	27.82 ± 0.59	26.23 ± 0.54	n.s.	n.s.	n.s.
CVH	45.38 ± 0.97	46.85 ± 1.11	66.24 ± 0.9	n.s.	$p < .0005$	$p < .0005$
CVH/VH	1.77	1.68	2.52	n.s.	$p < .00006$	$p < .00003$
mi	13.85 ± 1.08	11.63 ± 0.51	7.51 ± 0.32	n.s.	$p < .0005$	$p < .0005$
CH	8.31 ± 0.2	9.28 ± 2.40	8.99 ± 0.21	n.s.	n.s.	n.s.
MI	6.89 ± 0.76	4.478 ± 0.44	5.76 ± 0.58	$p < .007$	$p < .007$	n.s.

Note. TH, VH and CH in μm; CVH, number of cells; mi and MI in number of cells per hundred; n.s., not significant.

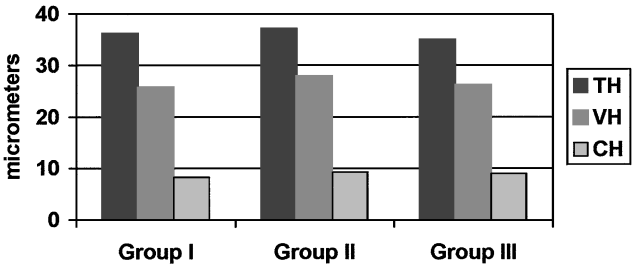

FIGURE 2 Parameters in the ileum.

out intestinal resections in rats and found a hypertrophic increase in the length of the villous. Loran and Althausen [21] demonstrated that, after intestinal resection, there was a 36% increase in the height of the jejunum villous and a 63% increase in the ileum. Nygaard [22] demonstrated a similar hypertrophic response in rats, and Tilson [23] found a 20% increase in villous height from rats that had undergone a 50% resection of the ileum.

However, other researchers report controversial findings. Porus [24] studied the compensatory response in the human intestine after massive resection and did not find an increase of the length of the villous (hypertrophy). However, he found a 22% increase in the number of cells per unit of villous length (hyperplasia). Kundtson [25] reported similar findings after massive resection of the small intestine in dogs. Hanson [26, 27] and Williamson [28, 29] found that the hyperplasic response is greatest at the ileum mucosa. This process persists for several months, according to Weser [30]. All these morphometric studies have been confirmed by measuring DNA and RNA values, as well as with tritium-labeled thymidine markers [31]. We appreciate that the mitotic index, as well as the morphology of the crypts, does not provide sufficiently reliable data to explain the increase, if it exists, of cellular proliferation after the sectioning.

Our results show evidence of both of the adaptative interpretations. On the one hand, we found a hyperplasic response in all the studied intestinal segments. We consider that this is the fundamental adaptative phenomenon. The hyperplasia found was greater in the ileum than in the jejunum, which correlates with similar findings reported previously [21, 23]. On the other hand, we have found a hypertrophic response in the jejunum. A possible explanation for this finding could be the interpretation offered by Lansky [32], who claims that it is a response mechanism to the partial functional obstruction that the intestine suffers at the level of the anastomosis.

An additional, possibly complementary, explanation is that this response may be a compensatory mechanism by which absorption is increased quantitatively at the jejunum and qualitatively at the ileum levels. In the first segment of the gut, which performs the bulk of the absorption process of fluids and nutrients, an increase in the villous height may be necessary to provide the required blood flow, but without an increase in the number of cells. However, at the ileum, where more selective absorption of nutrients is performed, an increased number of transporter cells may be needed; such an increase in the number of cells (hyperplasia), rather than in the volume of cells (hypertrophy), could be the most effective means of assuring sufficient energy for the transport processes.

The goal of this experimental model is to be able to modulate the flow of nutrients. Since the flow and absorption of nutrients seem to be important in the production of clinical syndromes, this experimental model would be useful, since these syndromes can be reproduced. This appears to be of special interest in the production of an experimental model of diabetes. In common with diabetes, short bowel syndrome presents an alteration in the management and/or flow of glucose and fatty acids. Thus, this easy-to-reproduce experimental syndrome could be an important model for the study of parameters related to pancreatic alterations.

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