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A light and scanning electron microscope study of the albino rat ileum after partial obstruction

Tarek Mahdy*, Gamal Mohamed, Adel Elhawary

Mansoura Faculty of Medicine, Egypt

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Summary

Purpose: Induction of an obstruction could be resorted to as a definitive line of management in some cases of short bowel syndrome (SBS). The goal of this study has been to elucidate histological and morphometric alterations in the albino rat ileum after surgically induced partial obstruction.

Methods and materials: Thirty adult male albino rats (240–250 g) were used in this investigation. They were divided into two equal groups: control and experimental. Small pieces of the ileum of the control and experimental animals were processed for histological and scanning electron microscope study.

Results: The ileum of the experimental animals proximal to the site of obstruction showed an apparent enlargement in the Peyer’s patches and an increase in the thickness of both the mucosa and muscle layers. The villi showed significant elongation and thickening. Both widening and deepening of the crypts were detected. There was an apparent increase in the goblet cell number and lymphocytic infiltration in both the corium and submucosa. In scanning electron microscopic examination, the microvilli showed scattered areas of shortening and irregular orientation. The surface was more frequently interrupted by goblet cell orifices.

Conclusions: Partial ileal obstruction resulted in hypertrophy of the ileal wall with considerable structural alterations oral to the obstruction site. Thus, the procedure apparently increased the absorptive surface area together with reduction in the speed of intestinal transit. These effects could support taking this technique into consideration as one of the suggested lines of treatment of some cases of SBS to eliminate the patient’s need for parenteral nutrition and all of its associated complications.

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*Corresponding author.
E-mail address: tmahdy@yahoo.com (T. Mahdy).

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Introduction

The small intestine is the site of terminal food digestion. It is also constituted for absorption of nutrients. This function is enhanced by several structural devices responsible for a 600-fold increase in the total intestinal surface area compared to the simple flat intestinal surface, i.e. without these structural devices. These are the plicae circulares, the myriad villi and the microvilli on these structural devices. These are the plicae circulares, the myriad villi and the microvilli on the epithelial cells (Fawcett and Jersh, 2002; Junqueira and Carneiro, 2005). Another important function of the small intestine is the transport of its luminal content to the colon. Any condition that interferes with this is classified as obstruction. The latter may be complete or partial; its causes may be vascular, neural or mechanical. The latter may be attributed to factors within the lumen, in the wall or outside it, as a result of compression (Woolf, 1998; Rubin and Farber, 1999; Ross and Pawlina, 2006).

Although any portion of the gut may be obstructed due to its narrow lumen, the small intestine is most commonly affected (Williams et al., 1993; Kumar et al., 2003). In the human, mechanical intestinal obstruction is a common surgical problem which is most often caused by post-operative adhesions or by hernia (Sabiston and Lyerly, 1997; Suri et al., 1999). The causes of intestinal obstruction also differ in children, where necrotizing enteritis and ascariasis are the common causes in tropical countries (Elhinnawy, 2004). Congenital bands, urachal remnants, annular pancreas and multiple segmental muscular defects are also among the causes of intestinal obstruction in children. (Itagaki et al., 2005; Hulvat et al., 2006; Kella and Rathi, 2006). In animals, such as horses, the ileum is the most common site for small bowel obstruction, while in cattle, obstruction may be duodenal, jejunal or ileal. There are several reasons for intestinal obstruction in small animals such as linear foreign bodies, focal intestinal neoplasia, feline infections, peritonitis and mega-colon (Macphail, 2002; Nuss et al., 2006).

Induced partial intestinal obstruction has been thought to be a last resort as a definitive line of treatment in short bowel syndrome (SBS) resulting from extensive bowel resections, in order to avoid the necessity of lifelong use of parenteral nutrition (Tannuri, 2003; Keller et al., 2004; Chang et al., 2006). The primary goal of such a surgical intervention in SBS is to optimize the intestinal function (Di Baise et al., 2004; Park et al., 2004). Thus, partial intestinal obstruction could be regarded as a surgical disorder or a suggested remedy for another disorder. Hence, the idea of the current investigation arose with the intent of presenting a vivid picture of the histological and morphometric alterations in the albino rat ileum after induced partial obstruction.

Materials and methods

Thirty adult male albino rats (240–250 g) were used in the current study. They were categorized in two equal groups: control and experimental. The animals were handled in accordance with guidelines of animal welfare prior to the experiment. The animals of the experimental group were subjected to ketamine hydrochloride anaesthesia (45 mg/kg). Under aseptic techniques, the abdomen was opened via a midline incision and a loop of ileum approximately 2–4 cm from ileocecal junction was exposed and placed on sterile gauze soaked in warm sterile saline solution. Through an incision in the mesentery between two blood vessels close to the gut, a plastic ring was tied around the intestine. The ring diameter exceeds that of ileum by 1–2 mm leaving the ring free to turn initially (Plate A, Figure 1). The intestine was then returned back into the abdominal cavity and the abdominal incision was closed. The animals were operated in the morning and solid food was not allowed until the following morning. Postoperatively, the animals were monitored daily with regard to body weight and abdominal distension. The control rats were subjected to sham operation where the plastic ring was placed around the ileum as in the experimental animals but it was removed before closing the abdominal cavity (Williams et al., 1993; Bertoni and Gabella, 2001).

Two weeks after the operation, under the same form of anaesthesia, skin of the control and experimental rats was incised along the ventral midline and the chest was exposed. Through the wall of the left ventricle, a cannula was inserted, the right auricle was cut to allow drainage and vascular perfusion was begun. About 100 ml of 100 mM phosphate-buffered saline (PBS) (pH 7.3) were injected via syringe and under manual pressure, through the cannula into the ascending aorta. The abdominal cavity was then opened with a midline incision and the small intestine excised and immersed in PBS. A 10 cm long segment of maximally hypertrophic ileum of the experimental rats (Plate A, Figure 2) and a similar segment of the control ileum were cleared of their contents by injection of PBS into the lumen then draining it. Each segment was cut into two equal loops, which were isolated with cotton thread ligature at both
ends and distended by injecting fluid into the lumen through a small needle piercing the wall at a small angle (Bertoni and Gabella, 2001).

Table 1. Comparison of data from the control (sham-operated) and experimental animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>Mean</td>
<td>245.6</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Circumference of the wall (mm)</td>
<td>Mean</td>
<td>15.2</td>
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<tr>
<td></td>
<td>SEM</td>
<td>0.2</td>
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<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Surface index</td>
<td>Mean</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Length of ileal villi (μm)</td>
<td>Mean</td>
<td>479.1</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Width of the base of ileal villi (μm)</td>
<td>Mean</td>
<td>108.1</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Density of the ileal villi (mm²)</td>
<td>Mean</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Height of the columnar absorptive cells (μm)</td>
<td>Mean</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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<tr>
<td>Height of the microvilli (μm)</td>
<td>Mean</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.2</td>
</tr>
<tr>
<td>Density of the microvilli (mm²)</td>
<td>Mean</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diameter of the crypts (μm)</td>
<td>Mean</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Thickness of the muscle layer (μm)</td>
<td>Mean</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;0.05</td>
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</tbody>
</table>
Tissue preparation

Small pieces of the ileum of the control and experimental animals were fixed in 10% neutral formalin, dehydrated, cleared and embedded in paraffin wax. Paraffin sections (6 μm) were prepared and stained with H&E and periodic acid Schiff (PAS) (Bancroft and Stevens, 1982). Other rectangular specimens of ileum 1 mm in thickness and 3–8 mm in size were fixed in 2% glutaraldehyde in phosphate buffer at pH 7.3 and proceeded for scanning electron microscopic study. The specimens were examined and photographed with the JEOL JSM 5300 scanning electron microscope.

Statistical analysis

Measurement of the outer circumference of the wall of the ileum was achieved by applying a plastic ring around and in close contact with the wall at 10 points for each animal. Measurement of the villous length (from the tip to base), the width of ileal villi at the base, height of enterocytes (from the surface to the base passing through the nucleus), the diameter of the intestinal glands (crypt lumen) as well as the thickness of the muscle layer was performed using a Leitz micrometer eye piece, on 10 randomly selected sections for each animal. Density of the ileal villi/mm² and also the height of the microvilli and their density/μm² were estimated on scanning electron micrographs. The surface index is defined as surface length divided by the section (intestinal) length. This index was calculated from digital images using a personal computer. Surface length was the length of a line drawn over the surface of the intestine, outlining the villi, but not extending into the crypts. The intestinal length was the length along which the villi were measured (Collins et al., 1996) (Plate A, Figure 3). The measurements obtained from the control and experimental animals were expressed as mean ± SEM. They were subjected to the test for significance, in Mansoura University Computer Center, according to the formula of Daniel (1987).

Results

All utilized rats survived throughout the entire period of the present study. Variable degrees of abdominal distension were observed in the experimental animals. The mean body weight of the control rats and that of the experimental group at the end of the second week was 245.6 ± 0.9 and 258.4 ± 1.0 g, respectively. Thus a significant weight gain was achieved (P < 0.05).

Macroscopically, at the final laparotomy, there was an apparently uniform distension of about 15 cm long segment of ileum proximal to the obstruction location. This distension was gradually less evident in the more oral parts. Distal to the obstruction, the terminal segment of ileum more or less resembled that of the control (Plate A, Figures 1 and 2). The outer circumference of the ileal wall significantly increased (P < 0.05) from 15.2 ± 0.2 mm in the control animals to 43.1 ± 0.4 mm oral to the partial obstruction site in the experimental rats after the elapse of 2 weeks (Table 1).

Light microscopic examination of the ileum of the experimental group proximal to the obstruction site, 2 weeks after the operation, revealed an apparent shortening of the villi over Peyer’s patches (Plate B, Figures 4, Table 1). There was also an apparent thickening of the mucosa. Some villi ended in small knobs. Fusion between adjacent ileal villi might take place. The mean villous length and basal width were 710.0 ± 6.3 and 115.4 ± 0.2 μm, respectively. Goblet cell number apparently increased in the villous and crypt epithelium. The lamina propria housed a considerable cellular infiltration (Plate B, Figure 5, Table 1). The fused adjacent villi contained a marked amount of lymphocytic infiltration in their core and a very large number of goblet cells in their covering epithelium. Apparently long ileal villi with a concomitant deepening of the lamina propria (Plate B, Figure 6). A high power magnification of Figure 5 showing fusion of the adjacent villi which show marked lymphocytic infiltration (asterix) in their core and a very large number of goblet cells (arrows) in their covering epithelium (H&E, × 250).

Plate B. Figure 4: A photomicrograph of a paraffin section of the albino rat ileum proximal to the obstruction site 2 weeks after the operation showing a part of Peyer’s patch (P), a short villus (V) and a crypt (C) (H&E, × 100). Figure 5: A photomicrograph of a paraffin section of the albino rat ileum proximal to the obstruction site 2 weeks after the operation showing an apparent thickening of the mucosa. The apical parts of some villi end with small knobs (K). Fusion of adjacent villi may be seen (F). Goblet cell (G) number apparently increases in the villous and crypt epithelium with appearance of small subepithelial spaces (crossed arrows). The lamina propria lodges considerable cellular infiltration (asterix). The muscle layer shows separation of the individual smooth muscle fibres (head arrows) (H&E, × 100). Figure 6: A high power magnification of Figure 5 showing fusion of the adjacent villi which show marked lymphocytic infiltration (asterix) in their core and a very large number of goblet cells (arrows) in their covering epithelium (H&E, × 250).
crypts could be observed proximal to the obstruction site (Plate C, Figure 8, Table 1). Small spaces intervened between the individual smooth muscle fibres of the hypertrophied muscle layer which became 178.9 ± 3.2 μm thick (Plate C, Figure 9, Table 1). The obviously increased number of goblet cells and their intensely positive PAS reaction were noticed (Plate C, Figure 10).

The scanning electron microscopic examination of the ileal mucosal surface of this experimental group proximal to the obstruction site showed hypertrophy of the villi with focal

Plate D. Figure 11: A scanning electron micrograph of the mucosal surface of the terminal part of albino rat ileum proximal to the obstruction site 2 weeks after the operation showing hypertrophy of the villi (V) with focal detachment of their epithelial layer (arrow) (scale bar 100 μm). Figure 12: A scanning electron micrograph of the mucosal surface of the terminal part of albino rat ileum proximal to the obstruction site 2 weeks after the operation showing, in addition to the villous hypertrophy, noticeable detachments of parts of the epithelial layer (arrows) (scale bar 100 μm). Figure 13: A scanning electron micrograph of the mucosal surface of the terminal part of albino rat ileum proximal to the obstruction site 2 weeks after the operation showing a more pebbled mucosal surface (arrows) which contains the orifices of several goblet cells that may be seen extruding mucus (M) or not (crossed arrow) (scale bar 1 μm). Figure 14: A scanning electron micrograph of a fractured columnar absorptive epithelial cell of albino rat ileum proximal to the obstruction site 2 weeks after the operation offering a side view of the microvilli showing focal shortening and irregular orientation (arrows). Notice the luminal mucous debris (asterix) (scale bar 1 μm).
detachment of some apical epithelial cells (Plate D, Figures 11 and 12). The mean density of the ileal villi and microvilli were $6.9 \pm 0.2/\text{mm}^2$ and $98.8 \pm 0.7/\mu\text{m}^2$, respectively. The mucosal surface exhibited a more pebbled appearance. This surface was more frequently broken by small orifices of goblet cells which may or may not contain mucus (Plate D, Figure 13, Table 1). A fractured columnar absorptive epithelial cell offered a side view of the microvilli which showed focal shortening and irregular orientation at multiple sites. The mean height of these microvilli was $1.1 \pm 0.01 \mu\text{m}$ (Plate D, Figure 14, Table 1).

Discussion

Intestinal obstruction is a common surgical problem caused by several etiologic factors (Kumar et al., 2003). Then again, an induced partial intestinal obstruction could be resorted to as a supposed eventual line of treatment of SBS (Chang et al., 2006; Sukhotnik et al., 2006). The present study has been performed to evaluate the histological and morphometric alterations in the albino rat ileum after its surgically induced partial obstruction utilizing the light and scanning electron microscopes.

The mean body weight of the experimental rats, 2 weeks after the partial ileal obstruction in the current study, significantly increased as compared with that of the control animals. This could be due to distension of the intestine with the retained contents together with intestinal hypertrophy. Such a finding contradicted that obtained by Collins et al. (1996), who denied any increase in the body weight of the animals with partially obstructed intestine. This controversy could be attributed to differences in the experimental animals used and in the surgical technique applied.

At the final laparotomy, the experimental rats in the present work exhibited an obviously uniform distension of a segment of the ileum oral to the site of partial obstruction. The outer circumference of this distended segment increased several times in comparison to that of the control group. This distension could be due to both stagnation of intestinal contents and thickening of the wall. Similar observations have been reported by Collins et al. (1996), Shoenberg and Kluth (2002) and Archer et al. (2006).

The mean length and the basal width of the villi showed significant increases oral to the obstruction site in the experimental animals compared to their corresponding control values. Thus the mean villous length became six to seven times the mean villous basal width in the experimental rats, while in the control this mean villous length was four times the mean villous basal width. This finding was supported by the significantly increased surface index in the experimental rats. Scanning electron microscopic examination also confirmed the hypertrophy of the ileal villi. The whole mucosal surface became more pebbled and exhibited more numerous orifices of goblet cells. In turn, the density of the villi significantly decreased in the experimental group. These observations in addition to the significant widening and the apparent deepening of the crypts denoted the occurrence of mucosal hypertrophy. The latter was accompanied by a significant increase in the height of the columnar absorptive cells. On the contrary, mucosal hypertrophy without changes in the size of epithelial cells has been observed by Riecken (1988) in the ileum after its partial resection and by Bertoni and Gabella (2001) in the ileum following its partial obstruction.

The mechanisms involved in mucosal hypertrophy are obscure. Intraluminal nutrients are perhaps the most important trophic inducers to intestinal mucosa. This mucosa becomes atrophic in the absence of intraluminal nutrients (Wilmore et al., 1971; Morgan et al., 1987). Moreover, Gabella (1984) hypothesized that intestinal distension stretches sensitive elements in the mucosa. Both the altered innervation and the increased blood supply may exert additional trophic influences. In addition, Snider and Johnson (1989) offered a similar interpretation and postulated that some local growth factors are liberated within the gut wall. Uchiyama et al (2000) explained the changes in gut after a valve construction to be a compensatory mechanism to maintain the bowel contents and to increase the intestinal absorption. Scolapio (2001) proposed that the luminal nutrients, beside their direct trophic effect, could stimulate both pancreatic and intestinal peptide secretion, which also promotes growth and function of the intestine.

The mean height of the microvilli, in the present investigation, exhibited insignificant variation between control and experimental rats. Meanwhile, the density of the microvilli per $\mu\text{m}^2$ showed a significant increase proximal to the partial obstruction site in comparison with the controls. The obtained mean microvillous height, more or less, was similar to that obtained by Mayhew and Middleton (1985) and Bertoni and Gabella (2001), whereas the packing density was vividly different. Variations amongst these studies could be owed to differences in strain, age and weight of the experimental rats and also in the methodology employed. However, Penzes and Regius (1985)
denied the occurrence of large-scale changes in the microvilli dimension through the entire life of the animal except possibly during pregnancy.

The ileal muscle layer proximal to the partial obstruction site showed a significant increase in the mean thickness, recording a three-fold increment compared to the control value. Such a change could be attributed to muscle hypertrophy because of the extra effort exerted to push the luminal contents via the partial obstruction. The small spaces between the individual smooth muscle fibres of this layer could be owed to interstitial oedema. This finding is in accordance with that of Williams et al. (1993) who observed that a hypertrophied segment of the same unit length had an approximately 10-fold increase in the weight of the muscularis externa layer. Bertoni and Gabella (2001) also stated that distension of the obstructed ileum is a passive mechanical effect of the accumulated ingesta, while hypertrophy is an active process of growth of the intestinal wall, mainly the musculature. A similar observation was made by Bettini et al. (2006) in the obstructed intestine of cats. They attributed that finding to stimulating factors in the intestinal wall. Moreover, Meylan et al. (2003) reported that the myoelectric activity in the ileum of cows immediately oral to the obstruction site was characterized by abolition of the submucosal and myenteric plexus neurons. In the myocytes, but no alterations were seen in the intramural ganglia of the hypertrophic intestine of the guinea pig. J. Neurocytol. 13, 73–84.

In light of the present results, it could be concluded that partial ileal obstruction resulted in hypertrophy of the ileal wall with considerable structural alterations oral to the obstruction site. Such a procedure apparently increased the absorptive surface area in conjunction with slowing of intestinal transit. These effects could support taking this technique into consideration as a promising line of treatment in some cases of SBS to eliminate patient dependence on parenteral nutrition and its associated complications.

References


