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Hypertrophy of ileal smooth muscle after construction of ileal reservoir in the rat

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Summary. An experimental model of the continent ileostomy reservoir used in clinical practise has been adapted for rats in order to evaluate changes in the smooth muscle layers of the ileal wall. The reservoir was constructed in the distal part of ileum, with intestinal continuity in order to avoid emptying problems. The rats were sacrificed 3–7 months after construction of the reservoir. There was a significant dilatation of the reservoir with a 26-fold increase in the mean reservoir volume. Morphometric studies of the smooth muscle in the intestinal wall revealed an increased thickness of the circular and longitudinal layers after construction of the reservoir ($p<0.001$). The number of muscle cell nuclei per section in samples of the ileal reservoir was reduced ($p<0.01$), whereas the mean size of the individual nuclei was greater ($p<0.001$). These results indicate that the enormous dilatation of this ileal reservoir is related to a clear hypertrophy of the smooth muscle layer. Whether there is a concomitant hyperplasia remains to be studied.

Key words: Ileum – Small intestine – Smooth muscle – Ileostomy – Hypertrophy

Introduction

An obstruction of the gastrointestinal tract leads to dilatation of the intestine proximal to the obstacle. If the obstruction develops slowly, pre-stenotic dilatation is generated concomitant with muscular hypertrophy of the proximal intestinal wall (Gabella 1975). An increased intraluminal pressure is necessary for continued propulsion of intestinal contents through the stenotic area and often leads to typical colic pains and symptoms of obstruction.

A continent ileostomy is constructed by fashioning the intestine into a reservoir. In this reservoir the typical propulsive motor action of the intestine is eliminated (Kock 1969). Intraluminal pressure studies show that

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pressure waves are seldom generated during filling of the reservoir until its maximal volume has been reached (Berglund et al. 1984). In spite of the low intraluminal pressure, a considerable dilatation of the reservoir occurs during the first weeks after its construction leading to a 5–10 fold increase in volume. This dilatation takes place without clinical signs of obstruction and, indeed, it is remarkable that such a considerable enlargement can take place without discomfort for the patient. Whether or not this dilatation is related to changes in the smooth muscle layers of the intestine has not been previously studied. The aim of the present investigation was to study the fate of these smooth muscle layers after construction of an ileal reservoir. Previous experiments have shown that immense dilatation occurs when such a self-emptying reservoir is created in the distal ileum of the rat (Philipson et al. 1983).

Materials and methods

Surgical procedures. Twenty-one male Sprague-Dawley rats weighing 325–450 g were fasted overnight and anaesthetized with Na-pentobarbital (30 mg/kg b.w. intraperitoneally). A midline laparotomy was performed and two continuous, 7 cm long segments were selected in the distal ileum, the proximal segment being used for control studies. The circumference of the segment was measured and the serosal surface area calculated. Thereafter, the lumen at both ends and the mesenteric vessels were ligated allowing the segment to be excised. The total volume of the segment, including muscle and lumen, was determined by immersing it in a graduated cylinder and recording the amount of fluid displaced. The segment then was opened longitudinally, carefully cleaned, weighed and spread out on filter paper, making sure that the breadth (former circumference) and length were the same as before excision. After being pinned out on cork, it was fixed in 10% buffered formaldehyde.

The continuity of the intestine was restored by an end-to-end anastomosis using interrupted 5-0 plain catgut. Subsequently, the distal segment was opened longitudinally along the antimesenteric border and a reservoir was constructed. The technique used for this construction has been described earlier (Philipson 1975) but in brief, the segment was bent in a U form, the two arms of the U were united with continuous 5-0 plain catgut and then folded as shown in Fig. 1. The reservoir was closed and no outlet was created to the abdominal wall, the contents emptying into the caecum via the remaining short segment of distal ileum.

Ten of the rats were sacrificed after an observation-time of 3 months and 11 rats after 7 months. At sacrifice the rats were anaesthetized in the same manner and re-laparotomized. The inlet and outlet of the reservoir were ligated as soon as possible in order to obtain a fixed intraluminal volume without escape of faeces or gas. After separation of the mesentery, the reservoir was removed and its volume registered by the same fluid displacement technique. This also permitted the calculation of the serosal surface area under the assumption that the reservoirs were spheres. Thereafter, a short incision was made in the reservoir releasing the contents. After rinsing and cleaning, the reservoir was weighed and then the incision was closed by suture. The emptying of the reservoir also lead to a gradual contraction of the wall similar to a deflation of a balloon. Therefore 10% formaldehyde was injected into the reservoir until it reached its previous volume. The whole reservoir was then immersed into a beaker of 10% formaldehyde and, thus, fixed under approximately the same distension as in vivo. After this fixation, sections were taken for histological examination. The suture lines from the construction of the reservoir were still discernible and the original direction of the ileum could be traced. The specimens from both the control segment and the reservoir were taken transversely to the longitudinal direction of the gut and, after orientation, dehydration and sectioning at 1.5 µ, they were stained with McManus PAS reaction.

Morphometry. Morphometry was performed with a Leitz microscope where a grid composed of 32 parallel lines corresponding to the length of 1 mm could be projected on the intestinal section. Each specimen was studied with regard to the *thickness of muscle layers:* under the

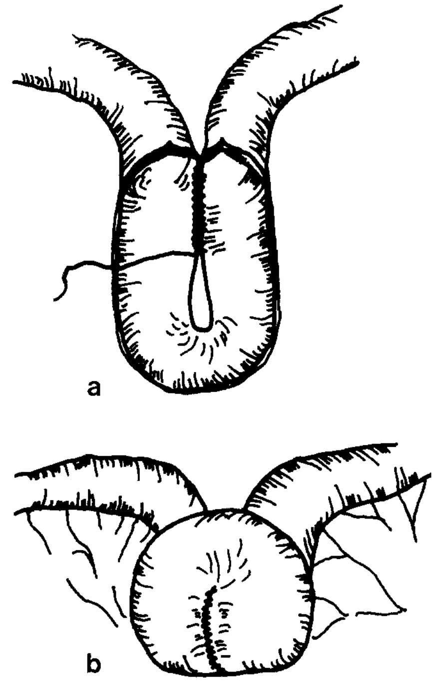

Fig. 1 a, b. Surgical procedure employed for the construction of ileal reservoirs in rats. After an antimesenteric longitudinal enterotomy the intestine is positioned as a U and the two arms of the U are seen together (**a**). The opened intestine is then folded and closed to form a reservoir (**b**)

Table 1. Macroscopic observations

(n=21)	At construction	At sacrifice
Body weight (g)	408 ±6.7	461 ±0.06
Weight of specimen (g)	0.56±0.03	4.75±0.51*
Volume of specimen (ml)	1.1 ±0.06	28.3 ±4.1*
Calculated serosal area (cm^2)	8.20±0.33	43.2 ±4.0*

* $p<0.001$

magnification of 40 ×, each of the three muscle layers – inner longitudinal (muscularis mucosae), middle circular and outer longitudinal – were measured in three individual sections. In addition, *the total number of muscle cell nuclei* in each of these sections was recorded. Under the magnification of 100 × *the area of muscle cell nuclei* was approximated from the length × the width of ten randomly chosen nuclei in each of three sections. These measurements of nucleus area were done only in the middle circular layer of the muscle wall – where, due to the direction of sectioning, the nuclei were displayed as ovals when compared to the small circular nuclei of the inner and outer muscle layers (see Fig. 2 and 3).

Statistical analyses. Results are expressed throughout as means (±Standard Errors). Statistical comparisons were performed with a paired "t"-test design.

Results

The difference in postoperative observation time did not lead to differences between the groups, and we have therefore presented the results in one group.

Fig. 2. Transverse sections of the smooth muscle layer in the control specimen of rat ileum. *Arrows* indicate circular layer

The rats appeared healthy throughout the experiment. Food intake and fecal output were normal, and there was a slight increase in bodyweight (Table 1). There was a significant dilatation of the reservoir which had an estimated volume of 9–90 ml at sacrifice. Compared with the control segment there was a 26-fold increase in the mean reservoir volume, a 9-fold increment in weight and a 5-fold enlargement of the calculated serosal area.

Fig. 3. Transverse section of the smooth muscle layer in ileal reservoir in the same rat, as in Fig. 2, seven months after construction. *Arrows* indicate the circular layer

The thickness of the muscular layers was increased in the whole muscular wall after construction of the ileal reservoir (Fig. 2 and 3). A comparison of the individual layers showed that the circular and the outer longitudinal layers were responsible for the increased thickness, the inner longitudinal not being altered (Table 2).

Table 2. Thickness of smooth muscle layer in control segment and ileal reservoir (μ)

Layers ($n=21$)	Control	Reservoir
Inner longitudinal	23.3 ± 0.6	25.9 ± 2.4
Middle circular	37.9 ± 1.8	115.0 ± 7.0*
Outer longitudinal	28.0 ± 1.1	72.7 ± 7.0*
Total	89.2 ± 3.0	213.6 ± 14.4*

* $p<0.001$

Table 3. Density of muscle cells nuclei and size of muscle cell nucleus in control segment and ileal reservoir

($n=21$)	Control segment	Reservoir
Number of muscle cell nuclei per section	126 ± 6	101 ± 3**
Area of cell nucleus (μm^2)	44.0 ± 2.5	66.9 ± 2.8*

* $p<0.001$ ** $p<0.01$

The number of muscle cell nuclei per section decreased in the reservoir compared with controls, while the *area of the muscle cell nuclei* in the reservoir was increased (Table 3).

Discussion

The present results clearly demonstrate that the dilatation of a surgically constructed ileal reservoir is related to a hypertrophy of the smooth muscle wall. This increased thickness of the wall, the enlarged muscle cell nuclei, and the apparent reduced number of nuclei per section are characteristics indicative of much larger muscle cells.

Earlier studies of ileal reservoirs constructed in rats (Philipson et al. 1983) demonstrated the phenomenal capability of the small intestine to dilate. The creation of this reservoir, which must be looked upon as a "stagnant loop", does not interfere with normal food intake or fecal passage, as judged from the gain in body weight and the continued health of the rats during the length of the experiment. The reason for the 10-fold variation in the reservoir volume between different animals is not known, but may possible be due to individual variations in the diameter of outlet, to angulation or kinking of the outlet in relation to the pouch, to fecal viscosity or to other factors influencing the flow of intestinal contents. For instance, it is not known whether the emptying of the reservoir is due to an overflow into the efferent ileal segment or if peristalsis in the segment leads to a suction of contents out of the pouch. In clinical practise it has been observed that, by prolonging the intervals between emptyings of continent ileostomy reservoirs, the volume of these reservoirs can be considerably increased above the average capacity.

Morphometric calculations of intestinal samples are always afflicted with the fact that intestinal tissue is very elastic and methodological errors may easily arise due to excessive stretching or contracting of the specimen. Deprivation of the blood supply invariably leads to contraction of the smooth muscle, for example, an unavoidable methodolocigal difficulty with samples studied with morphometry. In the present experiments care was taken to minimize these errors by fixing the reservoir after distension to a volume corresponding to the displacement volume of the reservoir immediately after excision. The treatment of the control segment followed the same principles.

The creation of an ileal reservoir in these experiments cannot be compared directly to the continent ileostomy constructed in patients. It must be born in mind that a patient empties the reservoir by catheterization at intervals. The intervals for emptying are slowly prolonged from 1-hour-periods early after construction until 7 or 8-hours-periods 8–10 weeks after the operation (Kock et al. 1977). The rat reservoirs never become catheterized and hence may be constantly filled with gas and feces, exposing them therefore to a different intraluminal pressure.

However, a number of points can be made. If the intestine utilized for the pouch had a normal propulsive motility pattern it would result in intermittent emptying of the reservoir. If this was the case, no dilatation would follow the surgical procedure. Consequently, the increased volume of the pouch can be taken as a proof of disturbed propulsion. A higher than normal intraluminal pressure in the pouch would result in reflux of the luminal contents and an obstacle to passage into the outlet. However, the smooth postoperative course with gain in body weight throughout the long experimental period is not in agreement with a state of chronic obstruction. We have assumed, therefore, that the intraluminal reservoir pressure was lower than normal just as has been shown in patients with continent ileostomies (Berglund et al. 1984).

Regardless of intraluminal pressure there was a significant increase in the weight of the pouches as a result of hypertrophy and/or hyperplasia. Earlier studies of mucosa from similar reservoirs gave evidence of mucosal hyperplasia (Philipson et al. 1983) but we can now also attribute some of the weight-gain to the muscular layer. Hypertrophy refers to enlargement of existing cells whereas hyperplasia refers to formation of new cells. However, after experimental stenosis of small intestine in guinea-pigs and rats Gabella found a marked increase in volume of the muscular coat. This was due not only to hypertrophy but also to hyperplasia, although the importance of the relationship between these two processes could not be deduced (Gabella 1975). Our data also clearly show that there was a significant hypertrophy of the muscle cells but, it was not assessed whether there was a coexisting hyperplasia as in the cited experiments.

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