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Sprouting of bladder nerves into cystoplastic cecal segment in the rat

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Abstract Incorporation of bowel into the bladder (enterocystoplasty) has been widely used to increase bladder capacity. It has been reported by others that the response of smooth muscle from the cystoplastic segment of the intestine shifts from that of the intestine (relaxation to α-agonists and ATP) to that of the bladder (contraction to α-agonists and ATP). This suggests a functional integration of the intestinal muscle into the bladder; the mechanisms are unknown. The aims of the present study were (1) to elucidate if there are signs of bladder nerves sprouting across the anastomosis into the intestinal segment, and (2) to study what happens with the intrinsic innervation of the intestinal segment. As a model, we used cecocystoplasty in rats. The bladder was opened and a patch of cecum with intact vascular supply was anastomosed to the bladder. After two to 11 months the rats were sacrificed and the bladders mounted as wholemounts and stained for acetylcholinesterase-containing nerves, or embedded in paraffin for histology. A pronounced degeneration of the myenteric plexus was found in the cecal segments. In some areas, this had proceeded to the extent that the ganglia were isolated ovoid lumps of cells with no apparent connection to other ganglia. Areas lacking ganglia and nerve trunks but still with muscle could be found in all specimens. Abundant axon bundles were demonstrated sprouting from the cut bladder nerves close to the anastomosis. The bundles spread out in a fan-like pattern or were organized as fewer thicker nerves. There were many nerve bundles entering the cecal segment where they branched and the diameter decreased till they no longer became visible. Some nerves reached surviving lumps of myenteric ganglion cells. The results show that the bladder nerves sprout into the anastomosed cecal segment. It is reasonable to assume that these nerves are responsible for the changes in receptor pharmacological properties of the cecal smooth muscle towards that of bladder muscle.

Key words Urinary bladder · Enterocystoplasty · Cecocystoplasty · Innervation · Nerve growth · Rat

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Introduction

Incorporating bowel tissue into the bladder has been used widely in recent years for different types of enterocystoplasty with the purpose of increasing bladder capacity and/or decreasing bladder pressure. Although these procedures do have problems and may give rise to serious complications, thus requiring careful selection of the patients, they constitute important options for the treatment of patients with difficult clinical neurogenic bladder disorders. The major patient groups are those with detrusor hyperreflexia or instability [17, 18].

Research into the effects of chronic exposure of the intestine to urine has focused mainly on the mucosa. A number of studies have shown mucosal atrophy to be the major change both in experimental animals [6] and patients [1]. In the rat, hyperplastic changes are also common [15, 19] both in the urothelium and the intestinal mucosa, and tumors were not uncommon [15]. Interestingly, the atrophic changes seen were more pronounced [6] in intestinal mucosa deprived of contact with luminal chyme alone than in mucosa exposed to urine.

Little is known about the functional changes of the intestinal muscle following enterocystoplasty. Batra et al. [3] have shown that the response to α-agonists and ATP could differentiate rabbit intestinal muscle from detrusor muscle. Such substances relax intestinal but contract detrusor muscle. Also, electrical field stimulation produces an immediate contractile response in the rabbit detrusor and not the delayed response found in

intestinal muscle [12]. Intestinal muscle dissected from ileocystoplasties after 4 weeks to 3 months showed a contractile response to field stimulation [12], α-receptor activation and ATP stimulation [4, 5] that was intermediate to that for normal bladder and normal intestine.

There are several tentative explanations for this changed physiological pattern of the intestinal muscle. These include the effects of urine exposure, a changed pattern of distension and relaxation, and the effects of bladder nerves. Batra et al. [5] showed that exposure to urine alone has little effect and that detubularization was necessary for the changes to develop. Detubularization changes the mechanical environment for the smooth muscle cells and increases the anastomosis length between the bladder and the intestine.

To our knowledge nothing is known about the possible interaction between the bladder nerves and the intrinsic innervation of the intestinal muscle used for the enterocystoplasty. The aims of the present study were (1) to elucidate if there are signs of bladder nerves sprouting across the anastomosis into the intestinal segment, and (2) to study what happens with the intrinsic innervation in the intestinal segment. In preliminary experiments, we produced both ileocystoplasties and cecocystoplasties in rats. Due to the presence of villi, the ileocystoplasties were too thick to analyze using wholemounts. As the experimental model for the present study we therefore chose to use cecocystoplasties.

Materials and methods

A total number of 19 Sprague-Dawley female rats (weighing 220–250 g) were used. The animal ethics committee of Lund University approved the experiments.

The animals were anesthetized with Ketamine (Ketalar, Parke-Davis, Barcelona, Spain) 100 mg/kg body weight) and xylasine (Rompun, Bayer AG, Leverkusen, Germany) 15 mg/kg body weight). The abdominal cavity was opened using a lower midline incision and the urinary bladder and cecum were identified. The uppermost part of the bladder dome was removed. A segment of the cecum was then isolated with its vascular supply intact, opened by a longitudinal antimesenteric incision and trimmed to a 1×1 cm quadratic patch (the patch occupied about 20% of the surface of the whole specimen when measured on the wholemounts, see below). The patch was then incorporated with continuous 5–0 dexon sutures to the opened bladder. The cecum was sutured with 5–0 continuous dexon and the abdominal cavity closed in 2 layers of interrupted 3–0 dexon sutures.

After 1 to 11 months the animals were killed by CO_2 asphyxia, and the bladder together with the urethra and the cystoplasty was dissected. The ureters were ligated, and the urethra was cannulated. In 16 animals the bladder was then distended with 0.7–1 ml saline, and transferred to a 4% phosphate buffered formaldehyde solution. After 15 min the dorsal side of the bladder with its cystoplasty was opened longitudinally using a scalpel and the bladder was again transferred to the fixative. After fixation for 2 h the bladders were transferred to Krebs' solution containing hyaluronidase (0.33 mg/100 ml) and ISO-OMPA (10^{-4} M) for 4–12 h. The bladders were then stained for acetylcholinesterase according to a variation of the method of Karnovsky and Roots [13] and proposed by Baker et al. [2]. After the incubation (usually overnight) the bladders were opened further by cuts at right angles to the dorsal longitudinal incision; this enabled the bladders to be flattened out with minimal folds. After dehydration, the bladders were mounted in DPX on slides, and examined under a light microscope (for technical details see [21]). In some bladders only the anastomotic areas were mounted. This was done to diminish further the tendency of the specimens to wrinkle.

Control segments of the cecum were dissected at the same time as the bladders, and were fixed, stained and mounted in the same manner.

In a separate series, three cecocystoplastic bladders were distended with 0.7 ml saline and fixed in 4% phosphate buffered formaldehyde solution. Segments containing the anastomotic region were dissected and embedded in paraffin. Sections were then cut and stained for connective tissue and muscle using the Van Gieson method, or were stained with hematoxylin-eosin.

Results

The luminal surface of the cecocystoplasty was covered by a thin mucus layer, irrespective of the time that had elapsed after the initial operation. The anastomosis between the bladder and the intestinal segment was easily visible to the naked eye. In Van Gieson stained sections, we could observe an abrupt transition from the multicellular urothelial to the single cell-layered intestinal mucosa. Often hyperplastic zones of urothelial mucosa could be identified along this border zone. Occasionally areas with the appearance of transitional epithelium could be found in the cecal segment at some distance from the anastomosis. The crypts in the cecal segment were well preserved at all points in time studied. Usually some glands could be observed buried under the urothelium in the border zone, showing that a shift had occurred between the transitions for the different layers in the wall. The smooth muscle in the bladder was composed of bundles. In the intestinal segment the muscle constituted a more homogenous and often thicker layer than in the bladder. There were no areas devoid of muscle. The anastomotic region usually contained little muscle, but instead was rich in fibrillar collagen. A typical specimen is shown in Fig. 1. There was no inflammatory response in the anastomotic region.

The smooth muscle displayed a faint nonspecific acetylcholinesterase staining in the wholemounts. It was evident that the smooth muscle in the cecal segments was well preserved and with a similar thickness in the whole segment. The acetylcholinesterase reaction stained the bladder nerves heavily. The architecture of the intramural bladder nerves was similar to that described previously [21]; two ventral nerves, one from each side reached the cecal segment along the ventral longitudinal detrusor muscle. A few thinner dorsolateral nerves also reached the anastomotic region.

The myenteric plexus was heavily stained in the control cecum. The mesh formed by the ganglia and their connections was very regular, almost geometrical (Fig. 2). The long axis of the ganglia and its perikarya traveled in the same direction as the axis for circular muscle; conversely the axis of the interconnections had

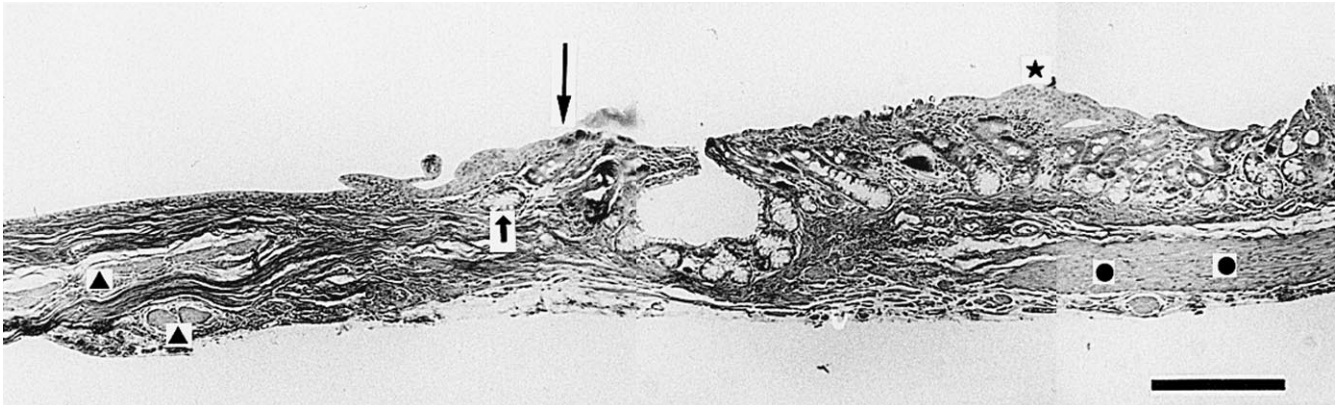


Fig. 1 Cecocystoplasty after 9 months. Anastomosis between bladder (*left*) and cecal segment (*right*). *Large arrow* indicates the point of transition from urothelium to intestinal mucosa. Note that intestinal glands (*small arrow*) can be found to the left of this transition zone, covered with urothelium. The *star* shows an area of urothelium in the cecal segment. There is no contact between the detrusor muscle (*filled triangles*) and intestinal muscle (*filled circles*). Van Gieson staining. Bar indicates 0.25 mm

the same direction as the outer longitudinal muscle. The perikarya were heavily packed in the ganglia.

In the cecocystoplasties, a varied pattern was observed. Some cecal segments had areas of myenteric plexus with a well preserved original pattern (Fig. 2). Other segments still had a dense mesh of nerves, but with a loss of the normal axes of the ganglia and interconnections (Fig. 2). In such specimens many ganglion cells had lost their elongated form, and were now almost spherical in shape, with no visible axons. This change had in other areas proceeded to the extent that the ganglia were isolated ovoid lumps of cells with no apparent connection to other ganglia. In such areas, nerves could be observed running with directions and lengths not normally found in the cecum (Fig. 2). Areas lacking ganglia and nerve trunks, but still with well-developed muscle, could be found in all specimens.

Generally, the best preserved areas of the myenteric plexus were found in the mid-portion of the cecal patch. Figure 3 shows that there was no correlation between the time after the initial surgery and the size of the area with a preserved pattern of innervation in the ten bladders that were distended enough to be evaluated in this way. In some cecal segments there was no normal pattern at all. The mean relative area with preserved original innervation was measured on micrographs using a planimeter. It amounted to 5 ± 5% ($n = 5$) at 6–8 months and 22 ± 7% ($n = 4$) at 11 months (mean value ± SE).

Contrary to what we found for the myenteric nerve plexus, we could easily see sprouting axon bundles from the cut bladder nerves in the vicinity of the anastomosis. The bundles were organized in two ways; either they spread out from the cut nerves in a fan-like pattern (Fig. 4, *left panel*), or they were organized in fewer, well defined thicker nerves (Fig. 4, *middle and right panel*). We could trace abundant nerve bundles originating from severed bladder nerves and entering the cecal segment (Fig. 4, *middle and right panel*). In such segments the nerves would then branch and their diameter would eventually decrease to the extent that they were no longer possible to stain (Fig. 4, *middle and right panel*). Some nerves reached surviving lumps of ganglion cells (Fig. 5, *left panel*).

The nerves crossing the border between the bladder and the cecal segment had all three characteristics (1) they can be traced from larger bladder nerves, and branch in the direction of and also in the cecal segment (2) they can be followed into areas of the cecal segment that are devoid of ganglion cells, and (3) their course in the cecal segment bears no resemblance to that of normal myenteric nerves.

We could not find any intramural ganglion cells in the rat bladder wall, except in a few cases where there were small ganglia close to the anastomosis with the cecal segment (Fig. 5, *right panel*). These ganglia seemed to have contact with nerves in both the bladder and the cecal segment.

Discussion

In recent years, incorporation of bowel into the bladder has become a common procedure to augment the bladder [17, 18]. Such surgery results in an interesting situation with close contact between two smooth muscle organs with different characteristics of smooth muscle and innervation pattern. In the rat, the bladder does not contain any intramural ganglion cells [9] but is innervated by nerves originating in the two pelvic ganglia. The intestine, on the other hand, has a high number of intramural ganglia arranged in a very regular pattern (see e.g. [8]).

There are pharmacological differences between detrusor and intestinal smooth muscle. Detrusor muscle contracts when exposed to alpha agonists and ATP, whereas intestinal muscle relaxes [3]. It has been reported that the characteristics of smooth muscle from the cystoplastic segment of the intestine shift from that of the intestine to that of the bladder [4, 5, 12] suggesting a functional integration of the intestinal muscle into the bladder.

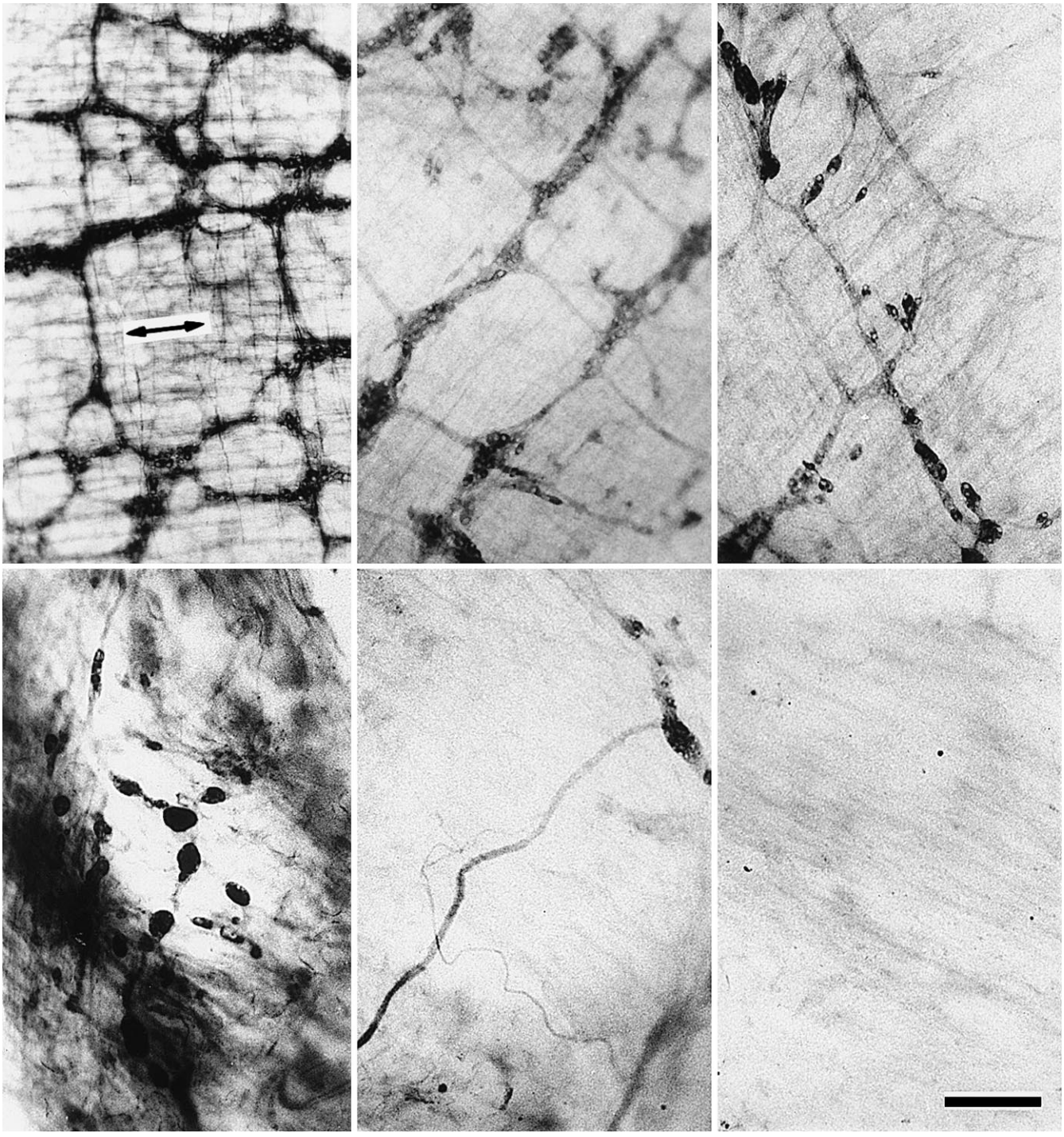


Fig. 2 Cecal and cecocystoplasty whole mounts. Acetylcholinesterase staining. *Upper left* Normal cecal segment. Typical appearance of myenteric plexus. Double arrow indicates the direction of the circular muscle. *Upper middle* Cecal segment. Cecocystoplasty after 11 months. A fairly normal pattern of nerve and nerve cell distribution is noted in this area. *Upper right* Another area in the same specimen. There is a distortion of the nerve pattern, and there seems to be a decreased number of nerves. The ganglion cells seem to be lumped together and have, at higher magnification, a spherical shape with few stainable axons. *Lower left* Cecal segment, cecocystoplasty after 6 months. The ganglion cells are found in isolated lumps and there are few nerves connecting them. *Lower middle* Cecocystoplasty 6 months. Most nerve cells have disappeared but a few lumps can be observed. Nerve bundles can be observed with an appearance completely different from that in normal cecum. *Lower right* Another area in the same specimen. No nerves are visible. Bar in lower right corner indicates 0.25 mm

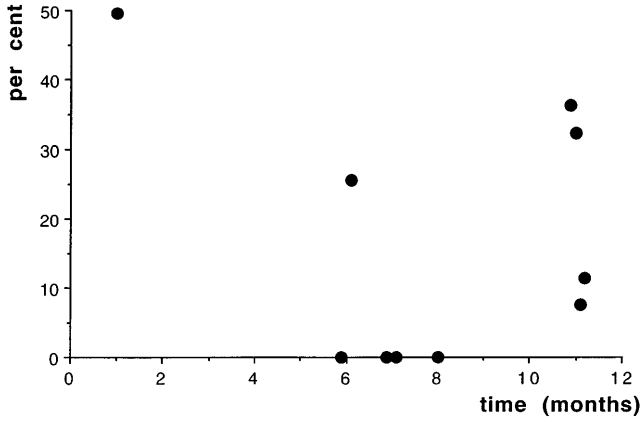


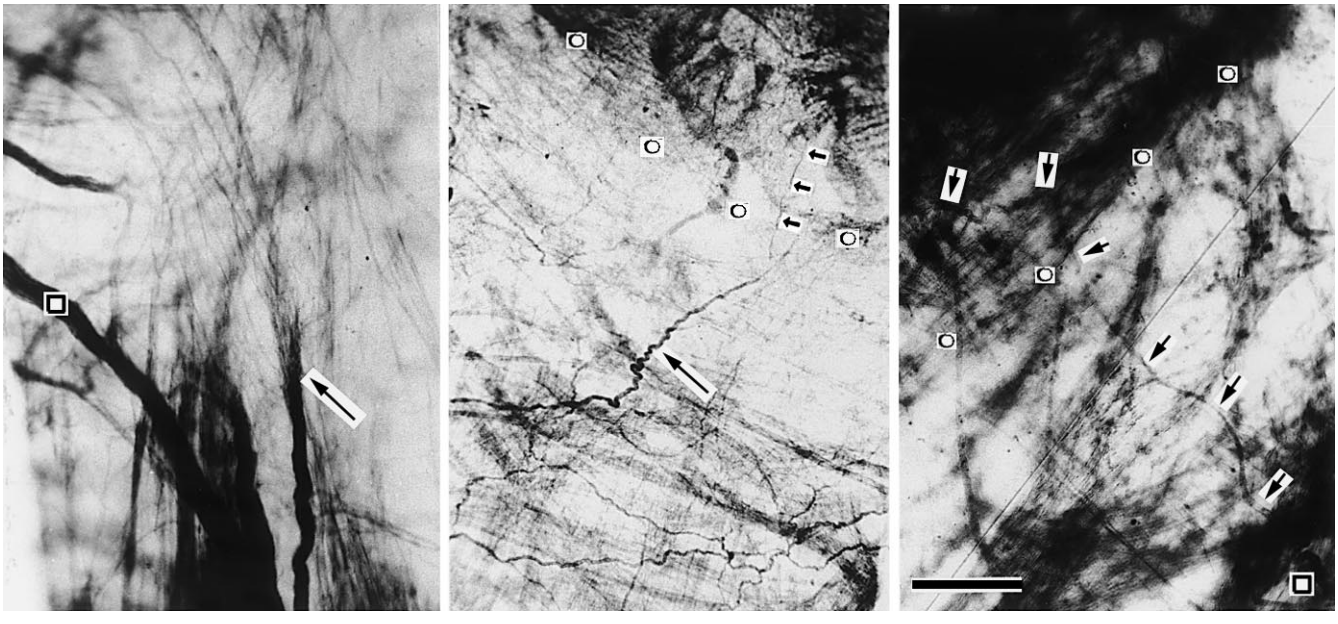
Fig. 3 Diagram showing the relative area of the cecal segment with preserved innervation (*upper middle* and *upper right* in Fig. 2) relative to the time after the cecocystoplasty operation. Most of the cecal segments lack a normal innervation but there is no apparent effect of time. The figure for each bladder represents the percentage of preserved innervation of the whole cecal segment. A total number of ten bladders could be evaluated in this manner

Fig. 4 Cecocystoplasty wholemounts. Acetylcholinesterase staining. *Left panel* Two major nerve trunks in the bladder wall. The cecal segment is just above the upper margin of the illustration. The left nerve trunk (square) looks completely normal. The right trunk has been severed during the cystoplasty operation 7 months previously. At the end of the nerve (*arrow*) sprouting axons are spreading out in a fan-like pattern towards the cecal segment. *Middle panel* Cecocystoplasty after 7 months. Part of the cecal segment is seen in the upper right, and is delineated by the circles. A branch (*small arrows*) from a large bladder nerve (*arrow*) can be followed into the cecal segment, which has lost its intramural ganglia. *Right panel* Cecocystoplasty after 6 months. A bladder nerve (*square*) gives off a thin nerve (*arrows*) which can be traced going into the cecal segment (*upper left*) delineated by circles. The cecal segment has lost its ganglion cells. Bar indicates 0.25 (*right and left panel*) or 1.0 mm (*middle panel*)

The stimuli causing the intestinal muscle cells to change their pharmacological properties are not known. Batra et al. [5] found that the muscle in tubular cystoplastic ileum retained its intestinal contractile characteristics, implying that the contact with urine is of little importance. Detubularization, on the other hand, changes the mechanical environment for the smooth muscle cells and also increases the contact area between the bladder and the intestine. It has not previously been reported if bladder nerves reach the smooth muscle in the intestinal segment.

The innervation of the urinary bladder is not static. There is a balance between the functional demands of the bladder and its innervation [10, 11]. Outlet obstruction leads to a rapid growth of the detrusor muscle, and also induces an increased total innervation of the bladder as evidenced by the increased total amount of choline acetyltransferase [16]. Partial denervation by removal of one pelvic ganglion leads to a rapid sprouting of the surviving nerves [21]. It is therefore not surprising that there are abundant nerves sprouting from the severed nerve trunks in the bladder wall. The sprouting axons either radiate out from the cut nerve trunk, or are organized into a limited number of thin nerves. We have been able to demonstrate that such nerves often cross the anastomosis and can be followed into the cecal segment. Normally the nerve trunks in the bladder wall are composed of a mixture of axons from motor and sensory neurons. It is reasonable to assume that this is the case for the sprouting nerves as well.

The results strongly suggest that the bladder nerves sprout into the cecal segment. The possibility that the nerves originate from the intestinal ganglia seems unlikely because there was a pronounced degeneration of the intestinal ganglia. Also, the new nerves in the cecal segment are distributed in a pattern that seems more related to that in the bladder than in the normal cecum.



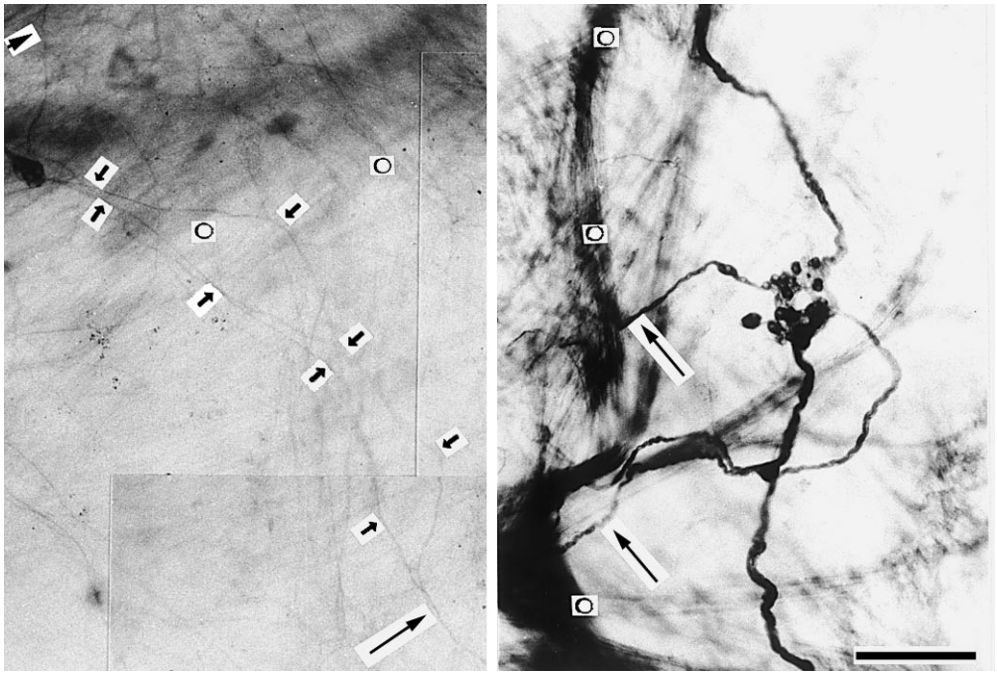


Fig. 5 *Left panel* Cecocystoplasty 11 months. A bladder nerve (*large arrow*), that can be traced back to a major bladder nerve trunk, is seen dividing into at least two thinner ones that can be traced (*small arrows*) into the cecal segment at the upper part of the plate (delineated by *circles*). Here they reach a lump of nerve cells, and also continue further (*arrowhead*) into the cecal segment. *Right panel* Small ganglion in the bladder wall close to cecal segment (at the *left* of the plate and delineated by the *circles*). The ganglion is in contact with bladder nerves and nerves going into the cecal segment (*arrows*). Bar indicates 0.25 mm

We have no clearcut explanation for the ganglionic degeneration found in the cecal segment. The vasculature was well preserved. Ganglionic degeneration could have been secondary to smooth muscle atrophy, but the cecal muscle layer was thick and well preserved. One tentative explanation could be that the intestinal patch is stretched during bladder filling to levels beyond that in the intact intestine; this could lead to overstretch of the nerves and nerve degeneration. Another interesting possibility is that the amount of smooth muscle in the cecal segment innervated by its intrinsic ganglion cells has decreased as a consequence of the ingrowth of bladder nerves, and that this has caused the decrease in intrinsic neurons.

Occasional small ganglia can be found in the bladder wall close to the anastomosis. We think that these neurons are of bladder origin. It has been shown that although intramural ganglion cells are not normally found in the rat bladder, they can appear after nerve lesions [20]. The structure of such ganglia is similar to those in the present study, and completely different from the rounded lumps of ganglion cells (Fig. 2) found in the cecal segment.

As the anastomotic area contains little muscle, it is unlikely that the smooth muscle of the detrusor would directly influence the muscle in the cecal segment, e.g. via myogenic spread, a possibility suggested by Gill et al. [12].

It is well known that motor nerves in skeletal muscle influence the contractile property of the muscle. Cross-innervation of a fast-twitch muscle with a nerve from a slow-twitch muscle changes its ultrastructure to that of a slow-twitch muscle [7] even if impulse activity is excluded. This suggests the existence of an activity-independent trophic factor that determines skeletal muscle fibre type. On the other hand, it has been suggested that the nerve activity pattern is important for the differentiation of muscle type [14]. It is reasonable to assume that the bladder nerves found in the cecal segment and the ganglionic atrophy are responsible for the changes in receptor pharmacological properties of the cecal smooth muscle towards that of bladder muscle, and that trophic factors or a changed contraction pattern are involved.

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