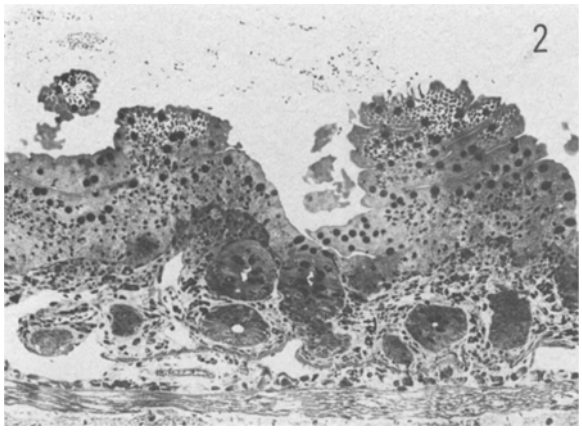

Fig. 2. Histological appearance of mucosa of rat ileum after vibration with EDTA.

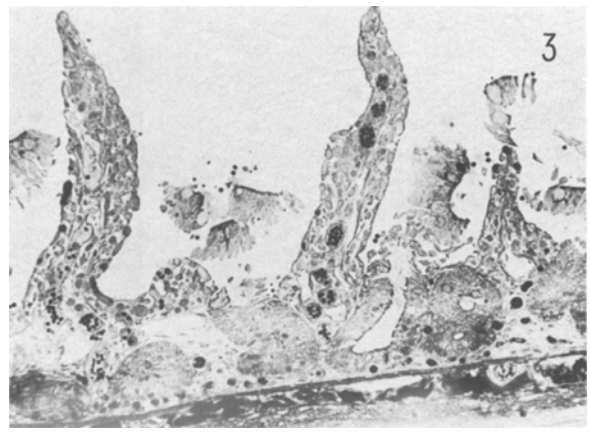

Fig. 3. Histological appearance of mucosa of rat ileum after vibration with TPB.

dilatation of blood vessels could also be observed. No detachment of villi and no desquamation of epithelium was noticed. Vibration with a mixture of EDTA + NaCl + sucrose (group II) resulted in varying effects. Sometimes, large areas of mucosa were deprived of villous epithelium; in other parts, villi were shortened and covered with epithelium (figure 2). Quite different effects were observed after vibration with a mixture containing TPB (group III). In all specimens we observed total desquamation of epithelium from villi up to the upper parts of crypts (figure 3). In the intestinal lumen, there could be observed a number of strands and free-lying groups of enterocytes or whole epithelial sheets in close proximity to villous stroma (figure 3). The crypt bases, lamina propria, and other underlying parts of the ileal wall maintained their integrity. The blood and lyphatic vessels of mucosa and submucosa were somehow dilated. There was no damage of villous stroma. The basement membrane seemed to be preserved and adherred closely to the stroma. After 24–48 h, the mucosa of vibrated and replaced segments displayed normal histological appearance.

Discussion. The adaptation of the Harrison and Webster method, using as a disassociation agent TPB instead of EDTA, is a simple and reproducible model for investigations of intestinal epithelium regeneration. On account of selective and complete desquamation of epithelium from villi and upper parts of crypts, this method seems to be particularly useful in investigating early events of epithelial repair. This model can also serve as a tool in evaluating both the specific influence of individual factors on the promotion of regeneration and the intercellular relation during the repair phenomena in healing of mucosal lesions. Lastly, this method can be used in studies concerning proliferation and cytodifferentiation of intestinal epithelium.

The results obtained indicate that potassium-complexing agents such as TPB markedly accelerate the desquamation of intestinal epithelium in vivo, whereas EDTA appears to have varying effects under these conditions. Similar conclusions were reached by Rappaport who performed disassociation of hepatic and brain cells[10]. However, there is a report on the smaller effectiveness of TPB in cell isolation in vitro[11].

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Adrenergic reinnervation of the denervated rat urinary bladder[1]

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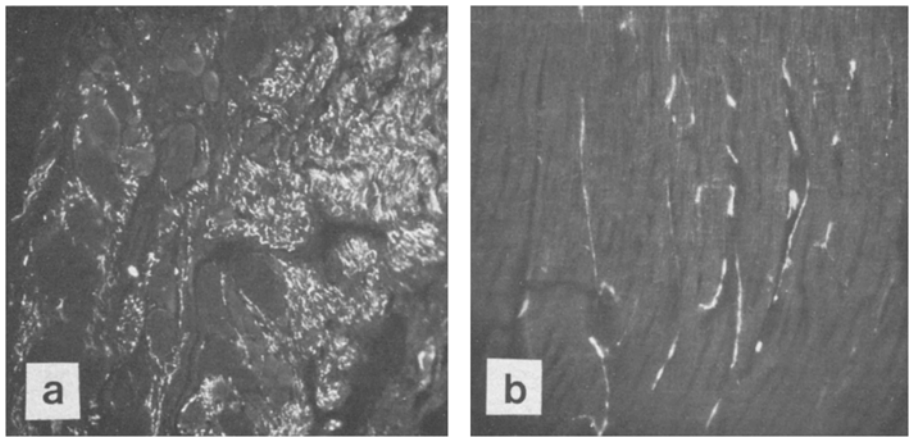
Summary. In rats undergoing unilateral extirpation of the pelvic ganglion, the adrenergic innervation disappeared on the ipsilateral side of the urinary bladder. It had reappeared after 6–9 weeks.

The detrusor muscle of the rat urinary bladder is supplied with adrenergic nerves, mainly affecting inhibitory β_2-receptors, although the presence of α-adrenoceptors has also been shown[2]. At 6–9 weeks after unilateral denervation, the stimulation of remaining intact nerves disclosed a predominant contractile α-receptor response, masking the relaxing β-receptor response. This might suggest a regrowth of adrenergic nerves, activating α-receptors in muscle fibres



Rat urinary bladder, denervated side, 9 weeks after total unilateral postganglionic denervation. Trigonum (a) and body/fundic (b) parts respectively show adrenergic innervation, not different from that in not-operated animals. *a* × 120 and *b* × 193.

normally not innervated by adrenergic nerves[3]. In the cat, 6–10 weeks after bladder denervation, the outgrowth of adrenergic nerves is seen concomittant with a change in the nerve-mediated response, from the normal inhibitory β-receptor response to an excitatory α-receptor response[4].

In view of this, the occurrence of a regrowth of adrenergic nerves in the denervated rat urinary bladder was studied for comparison with previous physiological findings[2,3].

Materials and methods. Male albino rats of the Wistar strain, weighing about 400 g, were used. Sympathectomy was performed by cutting the hypogastric nerves distal to the hypogastric ganglia below the bifurcation of the aorta. Total unilateral postganglionic denervation was achieved by extirpating the left pelvic ganglion; this procedure also results in a left-sided sympathetic denervation since the postganglionic fibres of the hypogastric nerves pass through the pelvic ganglion. In some experiments, total unilateral postganglionic denervation was combined with sympathectomy. The operations were performed aseptically under ether anesthesia.

The animals were killed at 10 days or 6–9 weeks after the denervation operations. The bladders were rapidly dissected out and divided by a longitudinal section from the top to the bottom into a left part and a right part. Each of these were then further divided by a transverse section into a neck-part and a part consisting of the body and fundic parts of the bladder, as described by Wakade and Kirpekar[5]. The tissue specimens were rapidly frozen in liquid propylene/propane cooled by liquid nitrogen, and then further processed according to the method of Falck and Hillarp for the histochemical demonstration of adrenergic nerves[6].

Results and discussion. The rat urinary bladder receives postganglionic adrenergic as well as postganglionic cholinergic nerve fibres from both the pelvic and hypogastric nerves[7]. After bilateral sympathectomy, the adrenergic nerve supply did not differ from that in normal bladders either at 10 days or at 6–9 weeks after the denervations. In contrast, 10 days after total unilateral postganglionic denervation, alone or in combination with sympathectomy, only some very few adrenergic nerve fibres were seen in the left half of the bladder, in the neck as well as in the body-fundic parts. In the corresponding right half the innervation pattern appeared almost unchanged. This strongly suggests that each half of the bladder is supplied with adrenergic nerves mainly via sacral nerve trunks and only to a very small extent via the hypogastric nerves. Furthermore, the very small number of nerves remaining on the denervated side, and the almost unchanged pattern on the contralateral side, after total unilateral postganglionic denervation, probably means that there is a very small overlapping in the adrenergic nerve supply between the halves of the bladder. In this respect, the adrenergic innervation differs from the cholinergic innervation, in which axons from either side distribute bilaterally in the detrusor muscle[8,9].

At 6–9 weeks after total unilateral postganglionic denervation, no difference in any regional part was seen between the denervated and non-denervated sides of the bladder (figure, a and b). The 2 halves of the bladder both demonstrated an adrenergic nerve supply as in normal, intact animals, verifying a regrowth of adrenergic nerves into the denervated side. At this time, the responses of the contractile α-receptors and the relaxing β-receptors to drugs were each as in non-denervated bladders. However, in nerve stimulation experiments, the β-receptor response was only seen after α-receptor blockade[2]. This probably means that after denervation the restored adrenergic innervation mainly innervates α-receptors, in contrast to the situation in non-denervated animals. The time reported herein for the regrowth of the adrenergic nerves is in line with findings from a similar study in cats[4].

The origin of the regenerating adrenergic nerves is unknown. The detrusor muscle of the rat, in contrast to that in the cat, contains no intramural ganglion cells[7,8,10]. Total unilateral postganglionic denervation implies the removal of the pelvic ganglion and its surrounding nerve trunks. This results in a large tissue deficiency, which then is filled with fibrotic scar tissue, making an ingrowth of adrenergic nerves to the bladder from the severed nerve trunks difficult. In view of this, it seems reasonable to assume that the reinnervation of the denervated side of the bladder is due to collateral sprouting of adrenergic nerve terminals from the contralateral, non-denervated half of the bladder, the sprouting process being induced by the peripheral target organ, here the denervated bladder part[11].

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