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Experimental desquamation of intestinal epithelium for in vivo studies of regeneration

J. Lamprecht, R. Figurski, Z. Lewicki and A. Pawłowski[1]

Department of Histology and Embryology, Institute of Biostructure, Medical School, Chałubińskiego 5, PL-02-004 Warsaw, and Department of Cell Pathophysiology, Medical Center of Postgraduate Education, Marymoncka 99, PL-01-813 Warsaw (Poland), 5 January 1979

Summary. Complete removal of villous as well as upper-crypt epithelium was achieved in vivo by vibration of an intestinal segment using tetraphenylboron sodium (TPB) as a disassociation agent.

Intestinal epithelium, being a rapidly proliferating and differentiating cell population, is a popular model for studies on regeneration. The common disadvantage of various experimental models in studies of intestinal epithelium regeneration[2-8] is either the damage of the underlying parts of the intestinal wall (ischaemia, thermic, chemical, or mechanical injury) or their influence on the whole body (radiation injury). Thus, most of these methods, when applied to the small intestine of animals, seem to be insufficiently accurate in evaluating the cellular events occurring in the course of the intestinal epithelium regeneration process.

The purpose of the present work was to adapt the in vitro Harrison and Webster vibration method[9] of cell isolation from everted segments of small intestine for studies on the regeneration of small intestinal epithelium. We expected that this method would have a less harmful effect on the lamina propria and other parts of the intestinal wall, thus allowing more precise and impartial observation of some phenomena occurring in the course of the regeneration process.

Materials and methods. 24 male Wistar strain rats with a weight range of 180–250 g were used. All animals were starved for 24 h and were given free access to water before the final experiment. The animals were anesthetised by i.p. injection of 3.6% chloral hydrate. The distal ileum was exposed after an abdominal wall incision in the left side of the body. The 2 ligatures were tied across the exposed intestine; the 1st one at 5 cm and the 2nd at 15 cm from the ileocaecal junction. The 10-cm-long segment of the ileum between the 2 ligatures formed a sac into which 1.5 - 2.0 ml of isolation medium was injected to the point of moderate dilatation of the intestinal wall. The sac with intact mesenteric vessels was placed in a vibrating holder (figure 1). The source of vibration was the Predom-Zelmer vibrating apparatus (100 Hz, amplitude 0.5 mm). The segments were vibrated for 15 min in all the experimental groups. The ileal sacs of 5 rats (group I) were injected with 0.15 M NaCl, 9 rats (group II) were injected with 5 mM EDTA + 0.14 M NaCl + 0.05 M sucrose, and 6 rats (group III) with 3 mM tetraphenylboron sodium (TPB) + 0.14 M NaCl + 0.05 M sucrose. Samples of vibrated segments of ileum and the control material taken 10 cm proximally were excised for histological examination immediately after vibration. Specimens were fixed in a mixture of 2.5% glutaraldehyde and 2.5% formaldehyde and embedded in epon. Semithin sections (1 µm) were stained with toluidine blue.

Results. The morphological appearance of segments vibrated with a medium consisting only of saline (group I) was insignificantly changed as compared with the controls. The mucosa had maintained its integrity and only the villi were somewhat shorter but of the same finger-like appearance. Epithelium of the lower parts of the crypts showed enhanced basophilia as compared to the controls; moderate

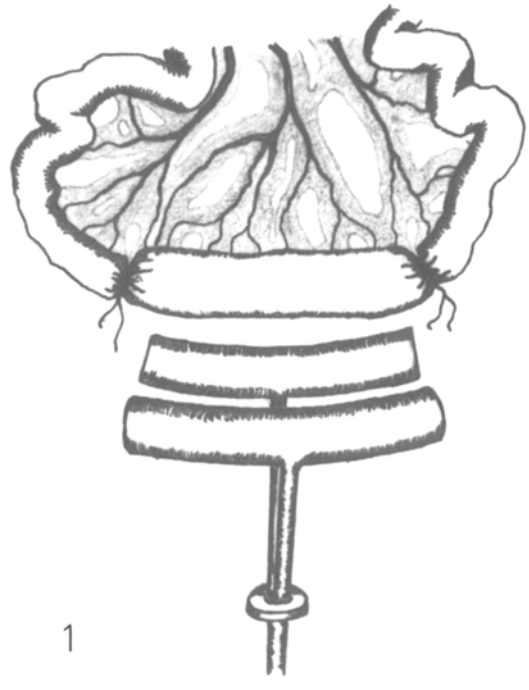

Fig. 1. Schematic drawing showing the vibrating holder and the intestinal loop with 2 ligatures.

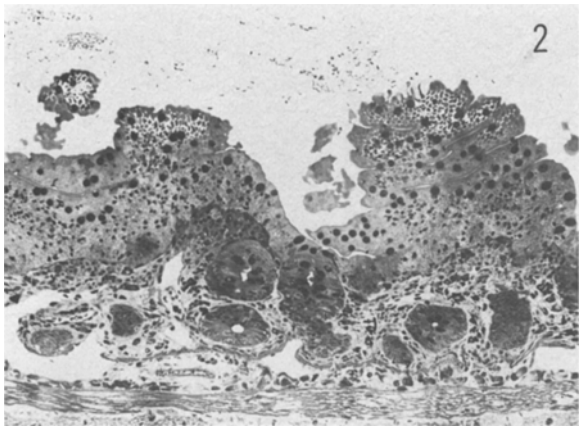

Fig. 2. Histological appearance of mucosa of rat ileum after vibration with EDTA.

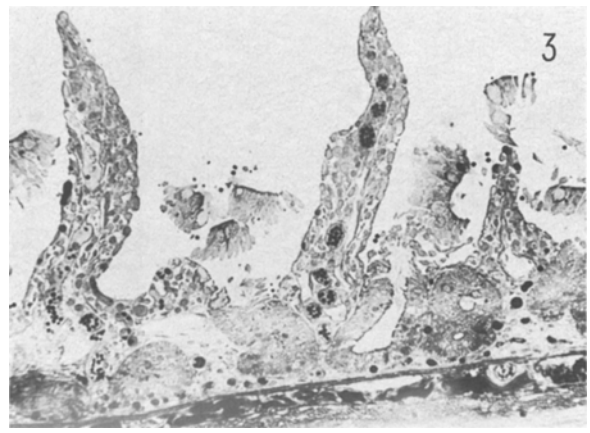

Fig. 3. Histological appearance of mucosa of rat ileum after vibration with TPB.

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].

1 Acknowledgments. The authors thank Hicol Industrieterrein De Bosschen for supplying TPB. Research was supported by MR-II 1.3.14.p.2 grant.

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Adrenergic reinnervation of the denervated rat urinary bladder[1]

P. Alm and M. Elmér

Institute of Pathology, University of Lund, Sölvegatan 25, and Institute of Physiology, University of Lund, Sölvegatan 19, S-223 62 Lund (Sweden), 29 January 1979

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