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Formulation of Fenbufen suppositories. III. Histology of the rectal mucosa of rats following repeat dosing of Fenbufen in Witepsol H12 and PEG vehicles

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During the initial phase of suppository liquifaction a small region of the rectal lining is exposed to high concentrations of the vehicle and drug. Reid et al., (1987) observed that a variety of pure suppository bases interacted with the rectal lining and some induced significant surface desquamation. Similar desquamation was produced by suppositories of ethanolamine Fenbufen in Witepsol H12; however, 24 h following treatment, a complete epithelial barrier was restored (Young et al., 1987). The time scale of formulation/tissue interaction is an important consideration for all routes of delivery, particularly per rectum, where local acute effects can occur. In the present study two aspects of suppository/tissue interaction were investigated. Firstly, the time scale of re-epithelialization following a single dose, and secondly, the effect of chronic dosing on this process.

Suppositories (6.5 × 0.4 mm) of pure Witepsol H12 (Dynamit Nobel, Slough, U.K.), or pure polyethylene glycol (PEG) 1540 (Hythe Chemicals, Hythe, U.K.) or these bases containing 1.4 mg ethanolamine Fenbufen (Cyanamid of Great Britain, Gosport, U.K.) were administered to groups ($n = 6$) of male Wistar rats anaesthetised with sodium pentobarbitone (i.p. 75 mg/kg). The animals were allowed to recover and then killed with an overdose of sodium pentobarbitone either 1, 2 and 5 h after a single dose, and 5 h after either a second or third daily dose. The rectums were removed and prepared for histological examination as previously described (Reid et al., 1987).

The epithelial cell height in the terminal 1 cm of rectum was measured at 4 sites on each of 5 longitudinal sections from the rectal tissue of each animal; slides and measurement sites were selected randomly. Reduced cell height is an index of cell loss from the epithelium (Johnson et al., 1978) and also of epithelial restitution following complete desquamation (Reid et al., 1987).

Treatment with Witepsol H12 alone induced no disruption of the rectal surface, but all other suppositories (Witepsol H12 + drug, PEG alone, and PEG + drug) induced cell loss and a significant reduction in epithelial cell height 1 h after treatment (Table 1). Desquamation extended into the upper parts of some crypts, and goblet cells were emptied of stored mucus (Fig. 1b). There was no disruption of the subepithelial connective tissue

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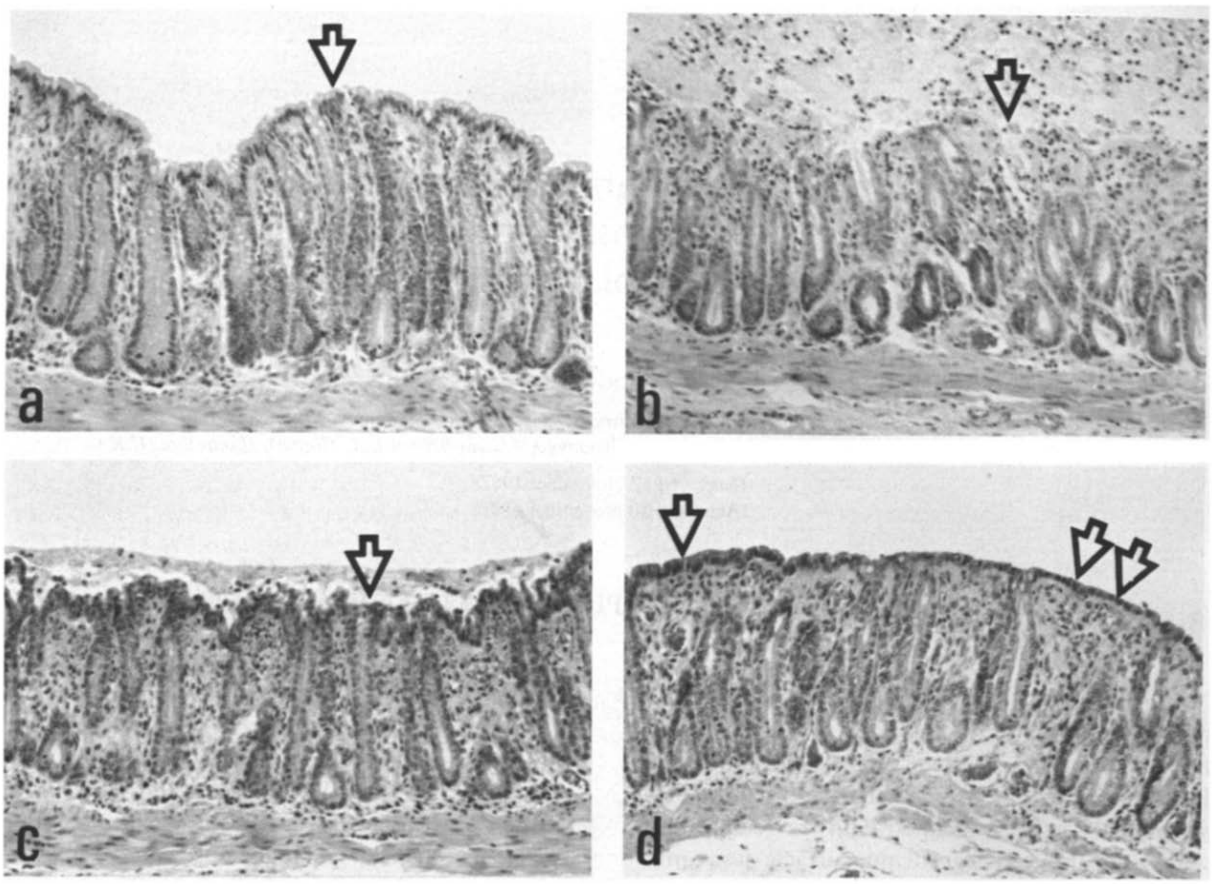

Fig. 1. Photomicrographs of rectal mucosa. a: control untreated tissue showing a normal columnar surface epithelium (arrow) and intact glands. b: mucosa following 1 h exposure to a PEG/Fenbufen suppository showing disruption of the surface epithelium (arrow), many detached cells in the gut lumen. c: mucosa 5 h following treatment with a PEG/Fenbufen suppository showing evidence of the resitution of an irregular but complete surface epithelium (arrow). d: mucosa 5 h following 3 daily doses with PEG/Fenbufen suppositories. Micrograph (d) shows a junction between two areas, one (single arrow) with a columnar epithelium indicating that the area was not desquamated by treatment, the other (double arrows) where the epithelium is cuboidal in type with a reduced cell height, indicating an area of recovery after surface desquamation. All micrographs at ×133.

although there was evidence of local hyperaemia. The irritancy of the PEG suppositories was not increased by the incorporation of the drug. Two hours after a single treatment there was a significant restitution of the surface epithelium, following all treatments and only a proportion of the animals in each group showed isolated foci of exposed basal lamina. The surface epithelium contained regions of cuboidal and squamous cells reflecting the regenerative process, while goblet cells had not replenished stored mucus. A similar picture was observed 5 h after a single dose (Fig. 1c).

Buck (1986) observed the first signs of cell migration in the rectum of mice 20 min after a physical insult, and a more or less complete epithelium was restored after 4 h, while Holyhead et al. (1983) observed in rats an intact epithelium at the sites of surface damage 6 h after treatment with Brij 35 suppositories. Here we observed evidence of surface restitution within a shorter time scale. In the stomach of the rat, and some other species, the restitution of a surface epithelium is an extremely rapid process (Ito et al., 1984); cell migration commences within minutes of an insult and by 1 h the lining of the whole stomach is

TABLE 1

Rectal epithelial cell height (μm) following suppository treatment with and without drug (mean ± S.D., n = 6)

Time	Witepsol	Wit./Drug	PEG	PEG/Drug
First dose				
1 h	23 ± 2.9	20 ± 2.7 *	14 ± 3.4 *	13 ± 2.9 *
2 h	26 ± 2.3	21 ± 7.0	20 ± 2.0 *	18 ± 2.3 *
5 h	27 ± 1.9	21 ± 2.0 *	19 ± 1.0 *	21 ± 1.8 *
Second dose				
5 h	25 ± 2.5	24 ± 4.0	20 ± 2.0 *	21 ± 2.0 *
Third dose				
5 h	22 ± 2.2	24 ± 3.0	18 ± 1.6 *	19 ± 1.6 *

* Significantly different from control: Student's *t*-test: $P < 0.05$.

almost completely restored. Ito et al. (1984) used the term 'gastric mucosal restitution' to distinguish this process from the slower process of ulcer healing.

Five hours after the second and third repeat doses the tissue showed no evidence of increased sensitivity to suppository treatment (Fig. 1d). After the PEG and PEG + drug treatment, the mean epithelial height was again significantly less than the untreated control but not less than 5 h after the first dose. A minority of animals in each group had foci of desquamation. This reflects the large individual variability in response that was observed throughout the study. In the case of the Witepsol H12 + drug-treated groups, the variability may also reflect poor spreading characteristics; 1 h after treatment a patchy distribution of suppository was observed over the surface of the rectum.

In conclusion, epithelial reconstitution represents the normal response of the bowel lining to minor surface damage. These studies have demonstrated, in rats, that the response to a suppository insult is rapid and importantly is not compromised by repeat dosing. However, the possibility of local interactions between a formulation and the intestinal barrier should not be disregarded because disruption of the intestinal barrier, even for a short time, may affect drug absorption and provide a route for pathogen entry.

References

Buck, R.C., Ultrastructural features of rectal epithelium of the mouse during the early phases of migration to repair a defect. *Virchows Arch.*, 51 (1986) 331–340.

Holyhead, E.M., Thomas, N.W. and Wilson, C.G., The regeneration of rectal epithelium in the rat following wounding with suppositories of polyoxyethylene (23) lauryl ether. *Br. J. Exp. Pathol.*, 64 (1983) 456–461.

Ito, S., Lacy, E.R., Rutten, M.J., Critchlow, J. and Silen, W., Rapid repair of injured gastric mucosa. *Scand. J. Gastroenterol.*, 19, Suppl. 101 (1984) 87–95.

Johnson, L., Lundquist, P.G., Nordstrom, H. and Nylander, G., Experimental studies on fluid pathophysiology in small intestinal obstruction in the rat. IV. Effects of intraluminal hyperosmolality and simultaneous intravenous infusions on mucosal micromorphology. *Scand. J. Gastroenterol.*, 13 (1978) 373–384.

Reid, A.S., Thomas, N.W., Palin, K.J. and Gould, P.L., Formulation of Fenbufen suppositories. I Quantitative histological assessment of the rectal mucosa of rats following treatment with suppository bases. *Int. J. Pharm.*, 40 (1987) 181–185.

Young, C., Palin, K.J., Reid, A.S., Thomas, N.W. and Gould, P.L. Formulation of Fenbufen suppositories. II Selection of a suppository base using dissolution studies and histological studies in rats. Int. J. Pharm., 40 (1987) 187–191.