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The effect of polyethylene glycol 1540 and Witepsol H12 suppositories on the store of rectal mucus in the rat

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Summary

The volume of rectal epithelial mucus was quantified histologically following a single suppository treatment. It was: unchanged by suppositories of Witepsol H12; reduced significantly by suppositories of PEG 1540 1, 6 and 24 h post-treatment but increased after 48 h.

A number of histological studies have shown in rats that some suppository bases induce significant changes in the structure of the rectal mucosa (Tupper et al., 1982; Reid et al., 1987). The changes are most clearly observed at the site of suppository insertion where they range from minor alterations in epithelial dimensions with some mucus secretion, to the complete desquamation of the surface epithelium and the mass secretion of stored mucus (Reid et al., 1987). Epithelial restitution is a relatively rapid event in the large intestine (Buck, 1986; Thomas et al., 1988; Waller et al., 1988), but the time scale for a complete regeneration of the epithelial mucus is unclear. The surface mucus gel is an important element in the absorptive barrier (Nimmefall and Rosenthaler, 1980) and therefore we have quantified the restoration of the epithelial mucus in rats following the administration of a rectal suppository.

Male Wistar rats were fasted overnight with free access to water. They were anaesthetised with sodium pentobarbitone (i.p. 75 mg/kg) and received a suppository (6.5 × 4.0 mm) of either Witepsol H12 (Dynamit Nobel, Slough, U.K.) or Polyethylene glycol (PEG) 1540 (Hythe Chemicals, Hythe, U.K.). The suppository was inserted just proximal to the anorectal junction. Groups of rats ($n = 6$) were killed 1, 6, 24 and 48 h after a single treatment with an overdose of the same anaesthetic. The anorectal region was removed, opened longitudinally, pinned to card and fixed in Bouin Hollande fluid. Tissue was process for histology using convention steps and sectioned at 6 μm. Five sections were collected at 60 μm intervals and stained with periodic acid basic fuchsin (Horobin and Kevill-Davies, 1971) to demonstrate intracellular stores of mucus. Point counting with an eyepiece graticule (10 × 10 mm square) was used to assess the volume fraction

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TABLE 1

Mean (± S.D.) values for % volume fraction of intracellular mucus in the rectal epithelium of each of the 6 rats in the control and experimental groups (WT12 = Witepsol H12; PEG = polyethylene glycol), also probability values (control / experimental group using the Mann-Whitney U-test) and means for all groups

Control	WT12 1 h	WT12 6 h	PEG 1 h	PEG 6 h	PEG 24 h	PEG 48 h
15.5 ± 2.3	17.9 ± 3.7	16.5 ± 5.6	7.7 ± 3.2	14.4 ± 2.7	15.9 ± 2.4	20.0 ± 5.2
16.6 ± 2.1	16.1 ± 4.8	17.1 ± 1.0	6.2 ± 1.7	9.6 ± 2.7	11.9 ± 1.9	19.0 ± 2.3
20.3 ± 2.7	15.7 ± 4.6	13.7 ± 2.7	8.7 ± 3.4	9.1 ± 1.2	11.5 ± 1.3	18.4 ± 1.1
13.0 ± 0.5	18.9 ± 1.1	11.2 ± 2.1	11.7 ± 2.1	9.1 ± 1.2	10.7 ± 0.6	15.7 ± 3.1
14.2 ± 2.8	13.1 ± 2.6	11.4 ± 3.5	6.5 ± 3.3	11.9 ± 5.3	13.6 ± 1.1	18.1 ± 2.3
15.5 ± 5.0	11.7 ± 0.5	11.1 ± 1.0	11.7 ± 3.7	7.8 ± 1.6	13.2 ± 2.2	22.9 ± 0.8
	Probability values					
	0.47	0.12	0.001	0.004	0.032	0.032
Mean values for the groups						
15.8%	15.6%	13.5%	7.8%	10.1%	12.8%	19.0%

(V_v) of mucus in the epithelium (V_v = points over mucus/total points over all epithelial cells: see Elias and Hyde, 1983). In each slide 3 sites were sampled: (1) the anorectal junction; (2) 1.5 mm from the junction; and (3) 3.0 mm from the junction. The V_v was expressed as a percentage ($V_v \times 100$) and values from the 3 sample sites in each rat were subjected to the Friedman test before being

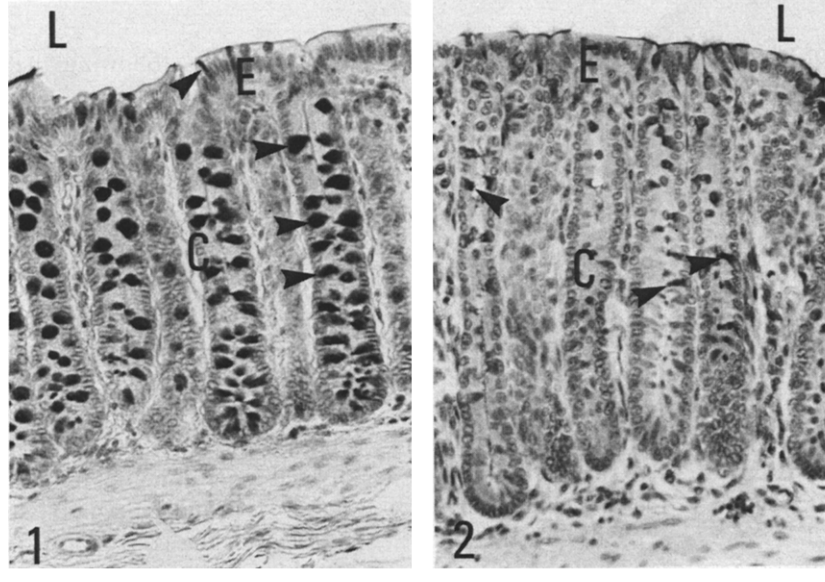

Fig. 1. Photomicrograph of the untreated rat rectal epithelium (E = surface epithelium; C = crypt epithelium) stained to illustrate the distribution of intracellular mucus (arrows) (L = lumen). ×240.

Fig. 2. Photomicrograph showing the appearance of the rectal epithelium (E = surface epithelium; C = crypt epithelium) 6 h following treatment with a suppository of PEG. Note there are fewer sites staining for intracellular mucus (arrows) (L = lumen). ×240.

expressed as a mean for each rat (Table 1). These means were used to compare control and treatment groups using the Mann–Whitney *U*-test. The means for some groups cover a wide range probably representing in part the variability of the tissue but also, particularly in the case of Witepsol, an uneven spreading of the base over folds in the rectal lining (Thomas et al., 1988).

In the untreated rectum the intracellular mucus accounted for approximately 15.8% (Table 1; Fig. 1) of the epithelial volume. From cell counts in vertical sections of crypts Chang and Leblond (1971) obtained a similar figure (16%) for the number of goblet cells in the crypts of the descending colon of the mouse. In this study, for the following reasons, point counting of intracellular mucus rather than cell counting was preferred: the method took into account the change in the total epithelial compartment following surface cell loss; it did not require perfect longitudinal profiles through crypts; it did not require a distinction to be made between goblet cells and mucus-secreting deep crypt cells. The latter cell types make up approximately 33% of crypt cells in the ascending colon of the rat, but gradually this figure declines to 5% in the descending colon (Altman, 1983).

Treatment with Witepsol H12 suppositories induced no significant change in the volume fraction of intracellular mucus 1 h (15.6%) and 6 h (13.5%) following treatment. This finding is in keeping with the conclusion that it was a non-irritant base (Reid et al., 1987). A number of studies have shown that different PEG bases induced surface desquamation (Tupper et al., 1982; Reid et al., 1987), and this was confirmed here. One hour following insertion the surface epithelium was completely desquamated and the V_v of intracellular mucus significantly reduced (7.8%). The surface epithelium was intact by 6 h and of normal dimensions by 24 h (Thomas et al., 1988); however, the volume of the intracellular mucus remained significantly less than control at 6 h (10.1%) (Fig. 2) and 24 h (12.8%) post-treatment. Jenning and Florey (1956) and Buck (1986) observed typical goblet cells in the intestinal epithelium 24 h following local irritation. 48 h following treatment the V_v of intracellular mucus (19.0%) exceeded the control value (15.8%) and from these studies, it is unclear whether this difference represents a rebound effect or an adaptive response of the mucosa to irritation.

The layer of surface mucus is a significant component of the barrier to intestinal absorption (Braybooks et al., 1974; Nimmefall and Rosenthaler, 1980; Kearney and Marriott, 1982). Changes in the thickness of the surface mucus gel and the quantity of mucus in the bulk phase will change the rates of drug dissolution (Kearney and Marriott, 1986) and drug diffusion from the bulk phase to the cell surface. The present morphometric studies suggest that, at the site of suppository insertion, there may be a paradox when considering drug absorption from a suppository formulation which induces mucus hypersecretion. Initially during the period of suppository liquifaction or melting, the quantity of mucus in the surface barrier and bulk phase would be increased with a consequential fall in drug uptake. Subsequently for a variable period, determined by the extent of mucus depletion, the dimensions of the surface mucus layer and the quantity of luminal mucus may be reduced and the absorptive process promoted.

References

Altmann, G.G., Morphological observations on mucus-secreting nongoblet cells in the deep crypts of the rat ascending colon. *Am. J. Anat.*, 167 (1983) 95–117.

Braybrooks, M.P., Barry, B.W. and Abbs, E.T., The effect of mucin on the bioavailability of tetracycline from the gastro-intestinal tract; in vivo, in vitro correlations. *J. Pharm. Pharmacol.*, 27 (1975) 508–515.

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

Chang, W.W.L. and Leblond, C.P., Renewal of the epithelium in the descending colon of the mouse. I. Presence of three cell populations: vacuolated-columnar, mucous and argentaffin. *Am. J. Anat.*, 131 (1971) 73–100.

Elias, H. and Hyde, D.M., *A guide to practical stereology*. Karger, Basel 1983, 305 pp.

Horobin, R.W. and Kevill-Davies, I.M., Basic fuchsin in acid alcohol: a simplified alternative to schiff reagent. *Stain Technol.*, 46 (1971) 53–58.

Jennings, M.S. and Florey, H.W., Autoradiographic observations on the mucous cells of the stomach and intestine. *Q. J. Exp. Physiol.*, 41 (1956) 131–152.

Kearney, P. and Marriott, C., The effect of an endogenous mucus layer on tetracycline absorption. *J. Pharm. Pharmacol.*, 34 (1982) 71P.

Kearney, P. and Marriott, C., The effects of mucus glycoproteins on the bioavailability of tetracycline. I. Dissolution rate. *Int. J. Pharm.*, 28 (1986) 33–40.

Nimmerfall, F. and Rosenthaler, J., Significance of the goblet cell mucin layer, the outermost luminal barrier to passage through the gut wall. *Biochem. Biophys. Res. Commun.*, 94 (1980) 960–966.

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.

Thomas, N.W., Lack, L.J., Woodhouse, B.A., Palin, K.J. and Gould, P.L., Formulation of Fenbufen suppositories. III. Histology of the rectal mucosa of rats following repeat dosing of Fenbufen in Witepsol H12 and PEG vehicles. *Int. J. Pharm.*, 44 (1988) 261–263.

Tupper, C.H., Copping, N., Thomas, N.W. and Wilson, C.G., Spreading of suppository bases assessed with histological and scintigraphic techniques. In Wilson, C.G., Hardy, J.G., Frier, M. and Davis, S.S. (Eds.), *Radionuclide Imaging in Drug Research*, Croon Helm, London. 1982, pp. 322–324.

Waller, D.A., Thomas, N.W. and Self, T.J., Epithelial restitution in the large intestine of the rat following insult with bile salts. *Virchows Arch. [A]*, 414 (1988) 77–81.