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Epithelial restitution in the large intestine of the rat following insult with bile salts

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Summary. In situ loops of large bowel of anaesthetised rats were used to observe epithelial restitution following surface desquamation using solutions of bile salts. The treatment induced complete surface desquamation but no disruption of the basal lamina. There was evidence of, cell migration at 30 min, and a complete surface epithelium two h post treatment. Neither a continuous contact between migrating cells and the basal lamina, nor a complete covering of secreted mucus, appeared necessary for epithelial restitution to occur.

Key words: Epithelium – Intestine – Desquamation – Restitution – Rat

Introduction

The surface epithelium of the gastrointestinal tract is maintained by the balance between cell loss, resulting from cell ageing and luminal insult, and cell proliferation and migration from within the the gastric or intestinal glands. Recent studies using a number of species have shown that the epithelium of the stomach is reformed quickly and almost completely following surface desquamation (Morris and Wallace 1981; Lacy and Ito 1984). Silen and Ito (1985) described this process as gastric restitution in preference to their earlier term of gastric reconstitution. They distinguished it from healing which takes place over a longer time scale following an injury involving the deeper layers of the gut (McMinn 1969). The process has been observed in large intestine, notably near the rectoanal junction, following surface destruction with either an irritant suppository (Holyhead et al. 1983) or physical insult (Buck 1986).

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In the present study, in situ loops containing a solution of bile salt were used to investigate epithelial restitution in the large intestine, at sites distant from the anus.

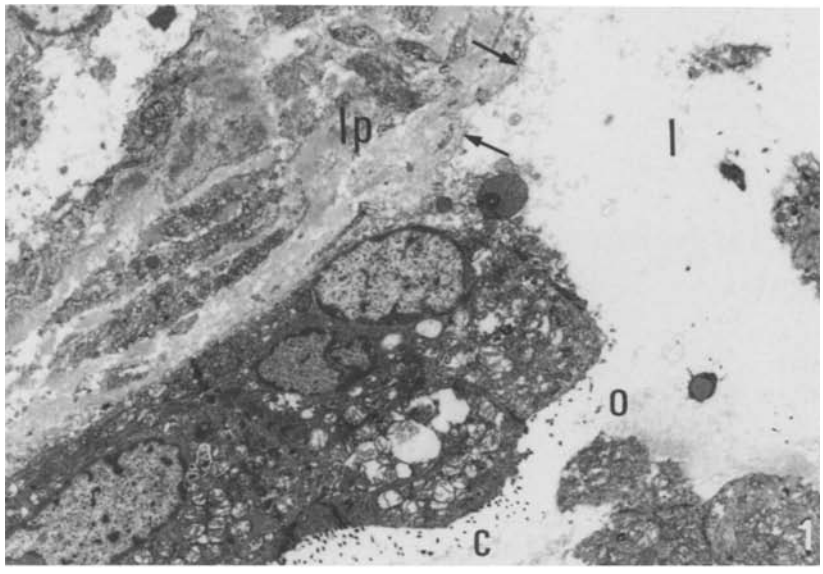
Material and methods

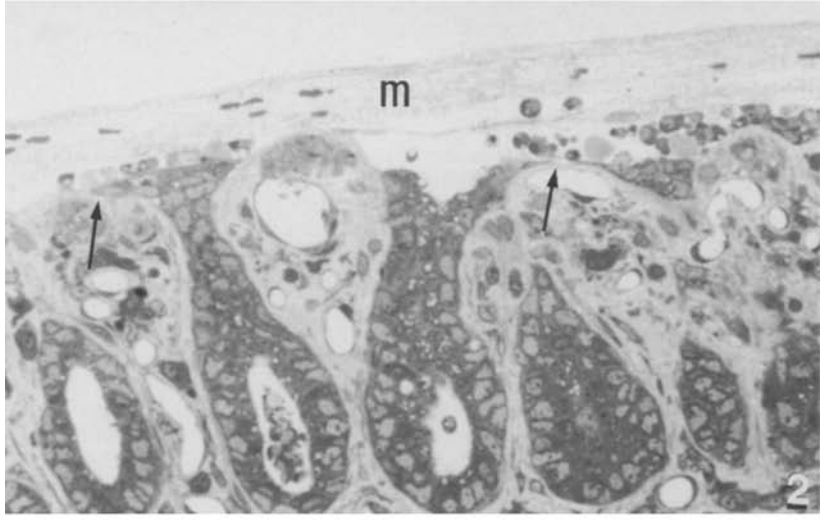
Groups of male Wistar rats ($n=6$; 180–200 g body weight) were fasted overnight with free access to water. They were anaesthetised with sodium pentobarbitone (i.p. 60 mg/kg body weight). The abdomen was incised and a cannula tied into the large intestine approximately 6 cm from the anus. The segment of bowel was flushed clean with Krebs buffer (pH 7.1) and the anus was clipped. A 1 ml solution of 25 mM sodium deoxycholate in Krebs buffer was introduced into the in situ loop via the indwelling cannula. During a 20 min period the loop was emptied and filled on three occasions with fresh bile salt solution. Thereafter the segment was emptied and flushed once with fresh buffer. One group of rats was killed immediately with an overdose of sodium pentobarbitone, others were killed in a similar way at intervals (30 min, 1 h and 2 h) after treatment. Loop tissue between 4 and 5 cm from the anus was removed and fixed in either Carnoy's fluid for wax embedding, or 2% glutaraldehyde in 0.1 M phosphate buffer with post fixation in OsO_4 for resin embedding.

Wax sections were prepared and stained with haematoxylin and eosin. Thick plastic sections were stained with toluidine blue. Ultrathin sections mounted on copper grids were stained with lead citrate and examined in a Philips 410 electron microscope.

Results

After 20 min exposure to the bile salt solution the entire surface epithelium was lost (Fig. 1). A layer comprising secreted mucus and desquamated cells was present within the gut lumen. The basal lamina was not disrupted but it was distorted, and cell debris was present on its luminal surface. Desquamation extended as far as the neck region of the crypts. Here cells had vacuolated cytoplasms and swollen mitochondria but they retained their normal relationship with adjacent cells and the basal





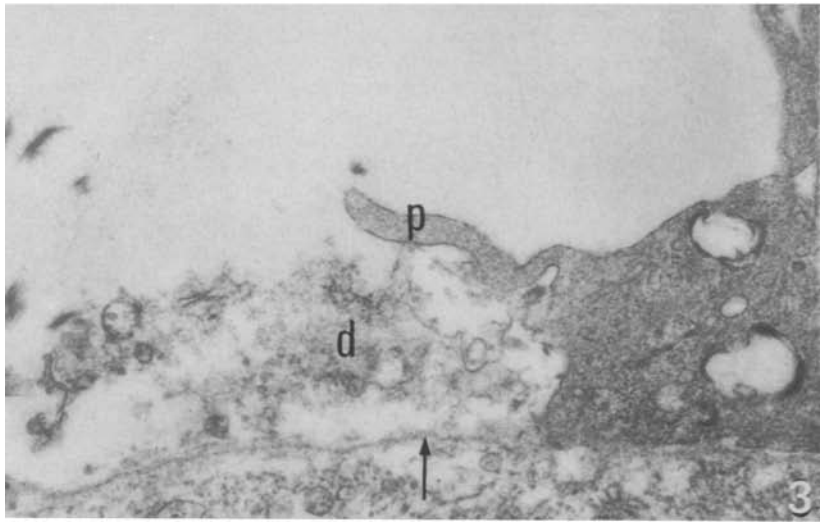
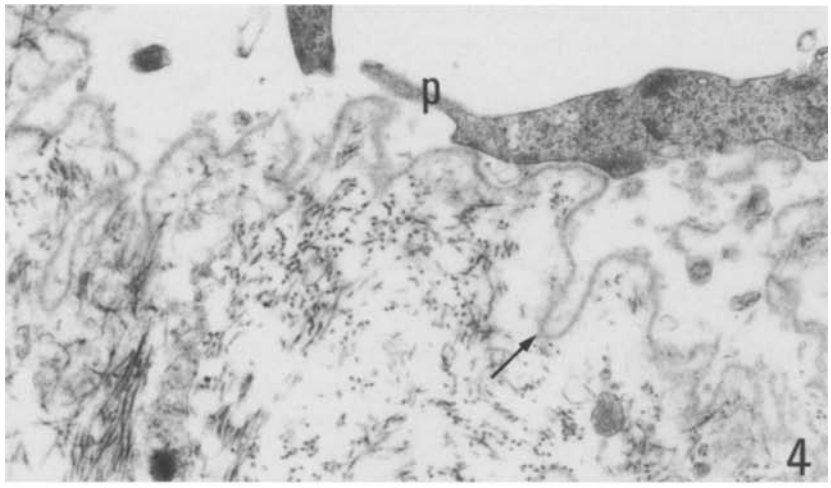
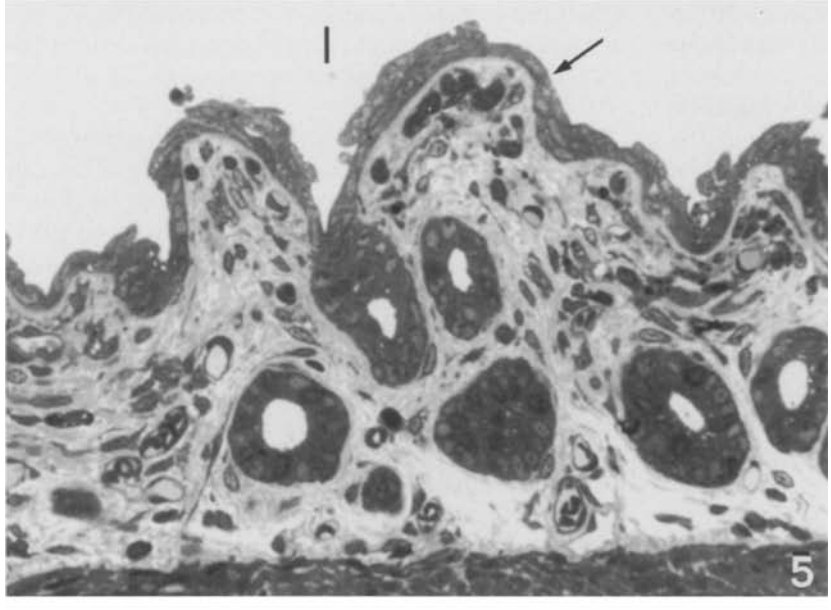


Fig. 1. Electron micrograph of tissue immediately following 20 min treatment with bile salt. The surface epithelium has been lost revealing the basal lamina (*arrows*). Cells at the ostia (o) of a crypt remain organised as an epithelium but have an electron dense vacuolated cytoplasm and swollen mitochondria. l, gut lumen; (*lp*) lamina propria; (*c*) crypt lumen. ×4000

Fig. 2. Photomicrograph of a thick plastic section of tissue 30 min following the experimental treatment. The luminal surface is covered with a mucous layer (*m*) and goblet cells had discharged their intracellular stores of mucin. Flattened cells extend from the ostia of the glands (*arrows*) and desquamated cells lie between the mucosa and the covering mucus. ×500

Fig. 3. Electron micrograph of the tissue 30 min following treatment showing a cytoplasmic process (*p*) extending over cell debris (*d*) associated with the luminal surface of basal lamina (*arrow*). ×24180





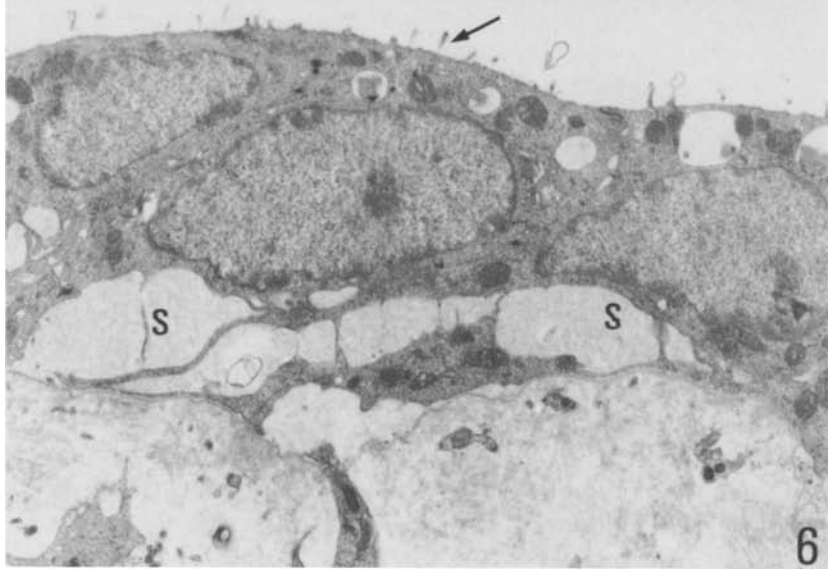


Fig. 4. Electron micrograph of the tissue 1 h following treatment showing the extension of cytoplasmic processes (*p*) across the plicated basal lamina (*arrow*). ×16500

Fig. 5. Photomicrograph of a thick plastic section of tissue 2 h following treatment showing an intact surface epithelium of squamous cells (*arrow*) and no inflammatory changes in the lamina propria (*l*) gut lumen. ×500

Fig. 6. Electron micrograph showing a surface epithelium of cuboidal cells 2 h following treatment. The intercellular spaces are distended (*s*) and microvilli (*arrow*) are sparse on the luminal surface. ×5830

lamina. Passing into the crypts, globet cells were depleted of mucin but there was no morphological evidence of cytoplasmic damage. Some connective tissue cells immediately beneath the luminal surface showed vacuolated cytoplasms, but the architecture of the lamina propria remained intact and there was no evidence of vascular disruption.

Thirty min following treatment squamous cells were present at the margins of the ostia to the glands (Fig. 2) and epithelial cell processes extended over the basal lamina and adherent cell debris (Fig. 3). These processes bridged irregularities in the basal lamina so that a continuous contact between surface cells and the basal lamina was not always observed. A layer of mucus, desquamated cells and fibrin covered the mucosal surface. Stores of intracellular mucin were not present within crypt cells at this time. One h following treatment the majority of interglandular regions were characterised by cell processes extending over irregularities in the basal lamina (Fig. 4); a minority of the interglandular regions were re-epithelialized. The luminal surface was not covered with a continuous layer of mucus. Two h following insult the greater part of the surface epithelium was reconstituted by squamous and cuboidal cells (Fig. 5). Some epithelial cells contained mucous vacuoles. Typically the intercellular spaces in the reformed epithelium were distended and cell processes extended between adjacent cells, and between cells and the basal lamina (Fig. 6). At some sites beneath the reconstituted epithelium the basal lamina was plicated although the basal plasma membrane was smooth. Microvilli were sparse and a layer of secreted mucus was an inconsistent feature of the mucosal surface. The organisation of the lamina propria during this period appeared normal.

Discussion

A number of studies have shown that dilute solutions of bile salts interact with the colonic epithelium to produce epithelial desquamation (Gaginella et al. 1977; Rafter et al. 1986). However, in one study (Georg et al. 1982) desquamation was not observed, unless the tissues were washed vigorously with buffer after exposure to bile salts and prior to fixation for histology. To ensure the complete desquamation of the surface epithelium in these studies we used a high concentration of bile salt, and also changed the solution during the period of insult. Importantly, neither the basal lamina, nor the connective tissue framework of the lamina propria were disrupted. Tissue recovery was therefore defined in terms of epithelial restitution rather than healing a process typical of lesions involving the deeper layers of the mucosa (McMinn 1969).

Cell migration from the undamaged epithelium at the ostia of the crypts initiated recovery. It resembled the process described in the stomach (Morris and Wallace 1981; Lacy and Ito 1984; Silen and Ito 1985), and the rectum (Holyhead et al. 1983; Buck 1986). Plication of the basal lamina reflects the loss of epithelial tension following desquamation, and its presence 2 h post treatment may reflect the slower rate of cell migration here compared with the stomach. The way in which cell processes bridged the irregularity in the basal lamina indicated that a continuous contact between the migrating cells and the basal lamina was not necessary for restitution to proceed. However, a continuous basal lamina is a prerequisite for the restoration of a normal structural relationship between epithelium and connective tissue (Vrako 1974; Black et al. 1985).

While epithelial restitution in the gastrointestinal tract is a rapid phenomenon, the rate of restitution observed in these studies appears to be slower than that observed in the stomach (Ito et al. 1984; Ito and Lacy 1985). This difference may represent an inherent difference between the two epithelia, for McMinn (1969) commented on the less extensive epithelial migration in the large bowel of man and animal species, or it may reflect the responses to different insulting agents. Experimental desquamation in the bowel is usually accompanied by a mass discharge of mucin. In the stomach the surface epithelium may be detached as a continuous sheet of cells (Lacy 1985; Sellers et al. 1987) and together with the secreted mucin give rise to a thickened mucoid (Wallace and Whittle 1986) or gelatinous layer (Lacy 1985; Sellers et al. 1987). This layer limits a plasma rich microenvironment (Ito and Lacy 1985) adjacent to the basal lamina which facilitates cell migration, and its removal significantly delays epithelial restitution (Lacy 1985; Wallace and Whittle 1986). En mass detachment of the epithelium was not observed here, and the mucous layer, which lacked the dimension (Sellers et al. 1987) and tethering elements (Morris et al. 1984) found in the stomach, was not present at the later sampling times. In addition normal levels of goblet cell mucin are not regained in the colon for at least 24 h (Florey and Webb 1931; Butterworth et al. 1988).

In conclusion bile salts induced surface desquamation in the large bowel but epithelial restitution occurred by a similar, but slower, process to that described elsewhere in the alimentary tract. The difference between the rate of epithelial restitution

in the proximal and distal parts of the gastrointestinal tract may reflect differences in the formation and maintenance of a mucoid layer following insult.

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