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# HISTOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE INTESTINE OF THE RAT EXPOSED TO SOLUTIONS OF MYRJ 52 AND PEG 2000

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## SUMMARY

The closed gut segment technique was used to investigate the effects of various concentrations of polyoxyethylene (40) stearate (Myrj 52) and Polyethylene glycol 2000 (PEG 2000) solutions on the histology of the intestinal mucosa and on fluid movements across the intestinal barrier. The results show that the higher concentrations of Myrj 52 and PEG 2000 induced cell loss and epithelial damage, and in addition solutions of PEG 2000 caused an unequal loss of enterocytes and goblet cells. Cell loss is likely to be a transient phenomena, with normal cell proliferation in the intestinal crypt being responsible for the restoration of villus architecture. Solutions of Myrj 52 caused little movement of fluid across the intestinal barrier but PEG 2000 at similar concentrations induced a considerable fluid loss from the mucosa to the lumen. The mechanism of this effect appears to be related to the anomalous osmotic behaviour of the PEG 2000 solutions, which dramatically changes the normal osmotic gradient across the intestinal barrier.

## INTRODUCTION

The absorption of an orally administered drug is dependent upon the physicochemical properties of the drug, its formulation and the physiological and morphological characteristics of the intestinal barrier. The formulation is particularly important in the presentation of compounds which have a poor dissolution in aqueous media. Griseofulvin is such a compound (Katchen and Symchowicz, 1967; Marvel et al., 1964) and its formulation in polyethylene glycol solutions greatly increases its uptake (Marvel et al., 1964; Chiou and Riegelman, 1970), while the solvent itself may be regarded as almost inert when taken orally (Smyth et al., 1945, 1955). However, a number of studies have shown that PEG causes cellular damage to the intestinal mucosa (Kameda et al., 1968; Clarke and Kobayashi, 1975).

Initial experiments with the surfactant polyoxyethylene (40) stearate (Myrj 52) have

shown that the dissolution rates and dispersion of a variety of drugs, including griseofulvin, were improved when formulated with Myrj 52 (Kaur et al., 1979). The early work on the toxicology and dietary effects of Myrj 52 was summarized by the WHO (1974); it is poorly absorbed and at low levels has no adverse effects in a number of different species. In rats the compound has a laxative effect which is severe when the level reaches 25%. It is possible that there may be other intestinal changes since Yonezawa (1977) observed hypersecretion of mucus, desquamation and necrosis in intestinal preparations exposed to a number of different non-ionic surfactants.

The present study was undertaken to compare the effects of polyethylene glycol 2000 (PEG 2000) and Myrj 52 on the morphological integrity of the small intestine of the rat using a closed gut segment preparation, as a prelude to a comparison of these polymers as excipients of griseofulvin. In addition, the effects of these compounds on fluid movements across the intestinal barrier were assessed since Kitazawa et al. (1977) have shown that a number of surfactants influence transmucosal fluid movement and drug uptake.

## MATERIAL AND METHODS

Adult male WAG Wistar rats weighing 150–400 g and fed on a standard Pilsbury 41B diet with water ad libitum were used in this study. The animals were anaesthetized with intraperitoneal pentobarbitone (1.2 ml/kg body weight) and the abdominal cavity opened with a midline incision. Two closed gut segments were prepared by the application of circumferential ties to a portion of the ileum free of intestinal contents. To ensure a competent vascular supply to each closed segment the ties were made at the site of anastomosis between arcades of adjacent mesenteric branch vessels. A test solution was introduced into a closed segment via a 22-gauge needle passed through one of the ties and as the needle was withdrawn the tie was tightened and the segment sealed. The intestine was returned to the abdominal cavity and the abdominal wall clipped for the duration of the experiment.

Polyoxyethylene (40) stearate (Myrj 52) ($C_{17}H_{35}COO(C_2H_4O)_{40}H$ mean molecular weight 2044) and polyethylene glycol 2000 (PEG 2000) ($HO(CH_2CH_2)_{45}H$ mean molecular weight 2000) were supplied by Hoechst, U.K., Pharmaceuticals Division. Solutions of these compounds were prepared at the following concentrations: 1%, 10%, 20%, and 30% in 0.9% saline and introduced into closed segments for either 30 or 60 min (Table 1). Similar segments were left unfilled (sham segments) or filled with 0.9% saline as controls.

TABLE 1

NUMBERS OF SEGMENTS SAMPLED FOR HISTOLOGY AND ELECTRON MICROSCOPY

| | Myrj 52 | | | | PEG 2000 | | | | Saline segments | Sham segments |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1% | 10% | 20% | 30% | 1% | 10% | 20% | 30% | | |
| 30 min | 2 | 2 | 2 | 4 | – | 2 | 2 | 4 | 5 | 5 |
| 60 min | 3 | 4 | 5 | 5 | 3 | 5 | 5 | 4 | 5 | 5 |

At the termination of the experiment the experimental and contro segments together with a portion of untreated ileum were removed and the tissue samples divided. One half, destined for light microscope examination, was fixed in Bouins Hollande fluid and embedded in paraffin wax. The other half was fixed for 3 h in a 50 : 50 mixture of 3% glutaraldehyde and 3% paraformaldehyde in cacodylate buffer at pH 7.2, washed overnight in buffer and post-fixed in osmium tetroxide. Thereafter, the samples were dehydrated through an ascending series of alcohols and then further subdivided. One portion was embedded in Araldite for transmission electron microscopy (TEM) and the remaining tissue critically point-dried with liquid carbon dioxide, sputter-coated with gold and examined in a Joel JSM 35 scanning electron microscope (SEM). For light microscopy wax-embedded specimens were sectioned at 6 $\mu$m and stained with either haematoxylin and eosin or haematoxylin and alcian blue, while Araldite sections sectioned at 1 $\mu$m were stained with toluidine blue or PAS after removal of the resin. For TEM ultrathin sections were louble-stained with uranyl acetate and lead citrate, and examined in a Philips 300 electron microscope.

To assess fluid movements across the intestinal barrier, closed segments (two per animal) were prepared as previously described except that a fine cannula attached to a 1-ml syringe via a 3-way tap was used to introduce 1 ml of the test solutions and then left in place. Three segments at each concentration (10%, 20% and 30%) of Myrj 52 and PEG 2000 in 0.9% saline containing 16.5 mg/l of phenol red were sampled, and 3 control segments were prepared containing saline and phenol red alone. Samples of luminal fluid were taken at 10-min intervals throughout a 60-min experiment. Luminal fluid was taken up into the 1-ml syringe and sampled via the 3-way tap using a 50-$\mu$l pipette; fluid not used for the sample was returned to the segment. The phenol red concentration was measured in alkali by mixing standard samples with 4.0 ml standard 0.1 M sodium hydroxide and then measured in a Unicam SP 1700 spectrophotometer at its $\lambda_{max}$ of 558 nm. The concentration of phenol red was calculated from a standard curve and from this figure the net gain or loss of fluid from the initial 1 ml luminal fluid calculated and plotted against time. The osmotic pressures of the test solutions of Myrj 52 and PEG 2000 were measured by freezing point depression using an Osmette S Automatic osmometer.

## RESULTS AND DISCUSSION

No histological differences were detected between the untreated ileum and the sham segments, and in the latter there was no evidence of ischemic damage comparable to that described in a number of studies of the intestinal mucosa following vascular stress (Brown et al., 1970, Chiu et al., 1970; Wagner et al., 1979a and b; Várkonyi et al., 1977). Saline segments showed some evidence of cell loss from the epithelium as judged by the number of detached cells free in the lumen of sectioned segments, but in the SEM the villus architecture (Fig. 12) resembled that of the untreated tissue (Fig. 11). The dilation of the lateral intercellular spaces of the epithelial cells over the apices of the villi was a typical feature of the saline segments (Fig. 7), reflecting the absorption of intestinal fluid. In the phenol red experiments with saline segments, fluid absorption reduced the luminal volume by 50 min below a value which could be measured (Fig. 1A).

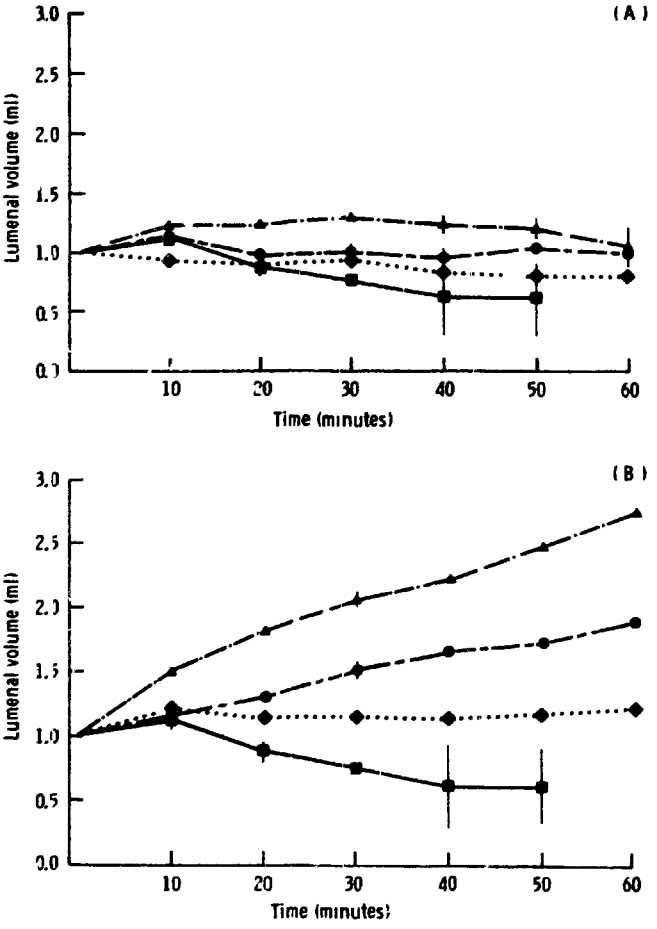



Fig. 1. A 10% (♦), 20% (●) and 30% (▲) w/v Myrj 52 in 0.9% saline for 60 min. B. 10% (♦), 20% (●) and 30% (▲) w/v PEG 2000 in 0.9% saline for 60 min. Saline control (■). Starting volume 1 ml. Means ± S.E.M. for 3 segments per treatment group.

The histology of segments exposed to 1% and 10% solutions of both Myrj 52 and PEG 2000 appeared unchanged; there was a slight reduction in the luminal volume of the 10% Myrj 52 segments and a slight increase in the luminal volume of the 10% PEG 2000 segments (Fig. 1A and B). The lumina of segments exposed to the higher concentration (20% and 30%) of Myrj 52 and PEG 2000 contained many free cells which had been detached from the extrusion zones over the highest parts of the villi. Many villi in these segments were covered with a mass of rounded cells which were in the process of extrusion (Figs. 13 and 14); the cells had lost their attachment to the basement membrane and their cytoplasm showed some evidence of a changed ultrastructure (Fig. 4). In the Myrj 52 segments the extrusion masses were more commonly observed after 60 min exposure (Figs. 4, 6, and 13), but similar concentrations of extruded cells were present in the 20% PEG 2000 segments after 60 min (Figs. 8 and 14) and the 30% PEG 2000 segments after 30 min. The ultrastructure of cells undergoing extrusion and also of cells immediately adjacent to the extrusion zone particularly in the Myrj 52 segments (Fig. 4) was different

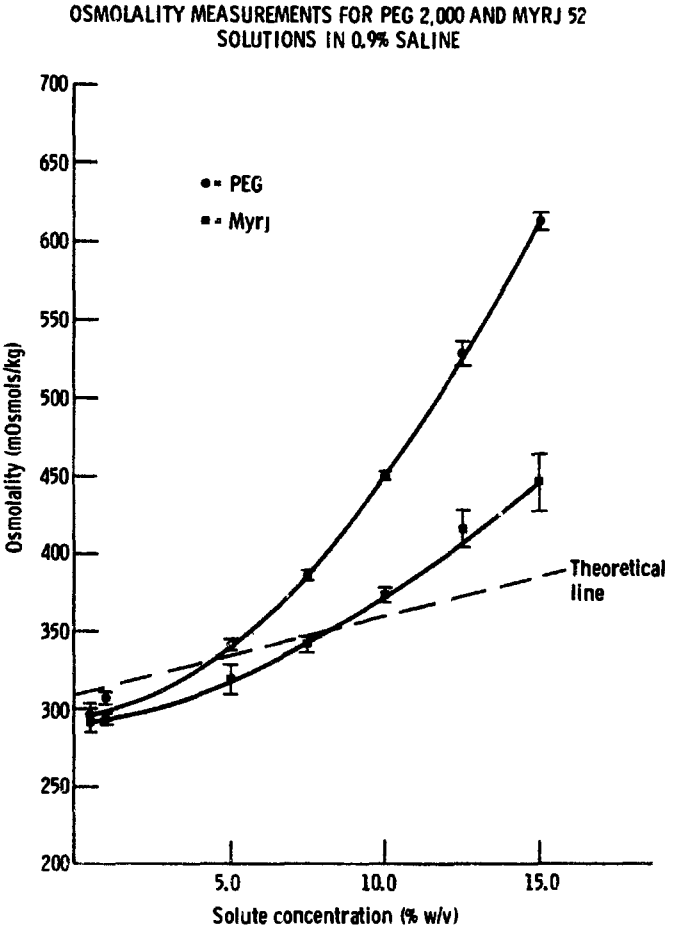
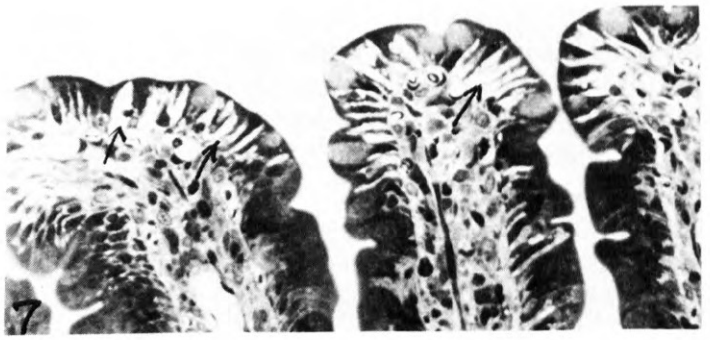



Fig. 2. Osmolality of solutions containing PEG 2000 (•) and Myrj 52 (▪) in 0.9% saline. Theoretical line for a perfect solution of molecular weight 2000 (– – –) shown for comparison.

from that of enterocytes away from the villi apices of experimental segments, and also from the ultrastructure of cells at the normal extrusion zone in untreated tissue (Fig. 3). These changes in ultrastructure do not mirror those described by Potton and Allen (1977) for epithelial extrusion in the normal gut, nor do they compare with the events preceding the formation of a "Grünhagen space" typical of cell loss following vascular stress (Wagner et al., 1979a and b).

No intestinal changes, other than a slight caecal enlargement (Fitzhugh et al., 1959) were observed in a number of dietary studies in rats using Myrj 52 in concentrations up to 25% (chow et al., 1953; Brush et al., 1957; Oser and Oser, 1957a and b; Fitzhugh et al., 1959). Diarrhoea was not present using diets with 15% Myrj 52 (Chow et al., 1953) but was present in rats fed a 25% diet (Fitzhugh et al., 1959). Oser and Oser (1957a) proposed that the laxative effect of 20% Myrj 52 and other emulsifiers in rats resulted from the concentration and molecular weight of the particular polyol moiety released upon hydrolysis within the intestine. Incubation of solutions of Myrj 52 in vitro with porcine



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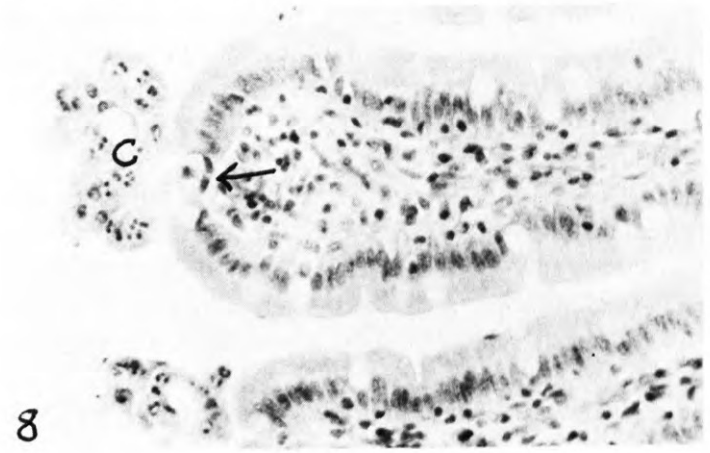


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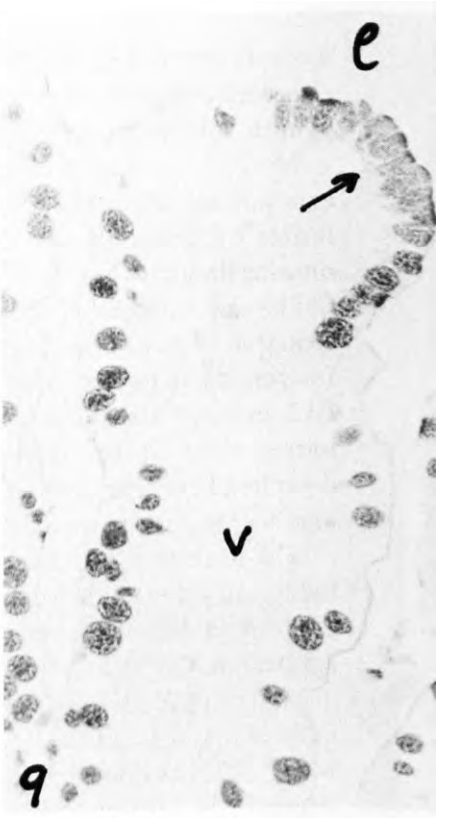


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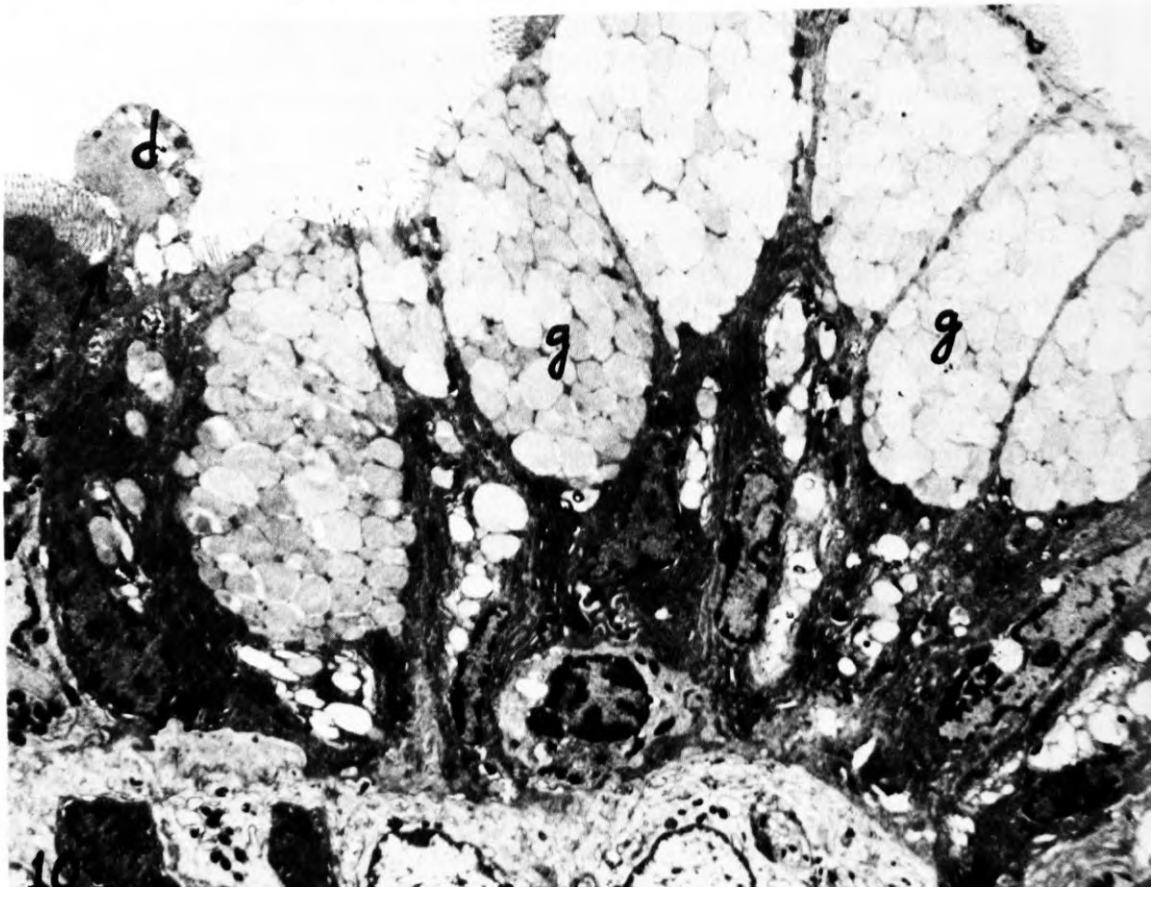

lipase failed to degrade the excipient (Kaur, unpublished observation) and the laxative effect may well result from the osmotic characteristics of the Myrj solution since the luminal volume in both 20% and 30% segments remained essentially unchanged (Fig. 1A).

Most of the masses of extruded cells were detached from the villi following 60 min exposure to 30% PEG 2000 (Figs. 15 and 16) and the sites of extrusion were then characterized by groups of goblet cells (Fig. 10) containing alcian blue-positive and PAS-positive mucous droplets (Fig. 9). This phenomenon of goblet cell capping was first described by Clarke and Kobayashi (1975) who employed a continuous infusion technique for the presentation of isosmotic (16.8%) PEG 4000 to a jejunal sac in conscious unrestrained rats for periods up to 72 h. They observed the formation of a cap of goblet cells typically after 24 h infusion and the subsequent loss of these goblet cells after 48 h. Our observations mirror some of the changes described by these authors; however, the experiments described here terminated after 60 min for fear of hypothermia inducing additional histological changes, and we did not observe any extrusion of goblet cells at this time.

The formation of a cap of goblet cells at the apex of a villus must reflect the differential loss of other epithelial cells from the upper part of the villus and the apposition of the retained goblet cells as migration pressure from the crypts maintains the integrity of the epithelium. Clarke and Kobayashi (1975) suggested that PEG exerted its greatest damaging effect once absorbed by the enterocytes although most studies have indicated high molecular weight PEGs are unabsorbed or poorly absorbed (Smyth et al., 1945, 1955; Wade, 1977). Although both Myrj 52 and PEG 2000 solutions induced cell loss, the ultrastructure of cells undergoing extrusion were not identical and goblet cell capping was not observed in the Myrj 52 segments after 60 min (Figs. 4 and 5). Thise findings may reflect the differences in the osmolalities of the experimental solution (Fig. 2) with the more extensive changes in the PEG segments resulting from the extreme osmotic imbalance across the intestinal barrier. The retention of goblet cells presumably reflects their greater resistance to, or protection from dramatic changes in osmotic gradients.

The increase in the luminal volume of segments containing 20% and 30% PEG solutions was considerable (Fig. 1B) and in the case of the higher concentration gave rise to a clearly visible distension of the intestinal segment. The villi in these segments were never denuded of epithelium and fluid gain to the lumen did not reflect the loss of tissue fluid from ulcerated villi. Hallbäck et al. (1979) have calculated a villous tip osmolality of

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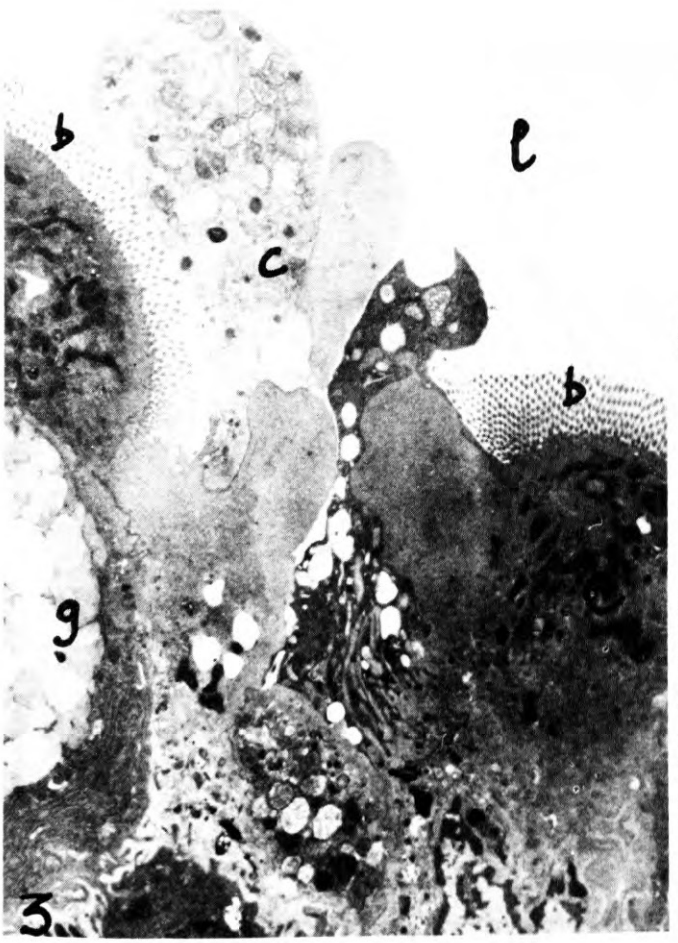
Fig. 3. Transmission electron micrograph of the extrusion zone of a villus from an untreated sample of ileum showing cells (c) in the process of detachment l, lumen; g, goblet cell, e, enterocyte, b, brush border. ×4350.

Fig. 4. Transmission electron micrograph of the extrusion zone of a segment exposed to 20% Myrj 52 for 60 min showing the detachment of an enterocyte (e) and a goblet cell (g). The ultrastructure of enterocytes at, and adjacent to, the extrusion zone are different from those in untreated tissue (Fig. 3). l, lumen, b, brush border ×4350.

Fig. 5. Light micrograph of a plastic section stained with PAS to illustrate the distribution of goblet cells (arrows) over villi exposed to 30% Myrj 52 for 50 min. Note the absence of goblet cell capping compared with Fig. 9. ×300.

Fig. 6. Light micrograph from a plastic section of a villus exposure to 20% Myrj 52 for 60 min showing cells undergoing detachment (arrow) and the vacuolated cytoplasm of enterocytes over the upper part of the villus. ×600.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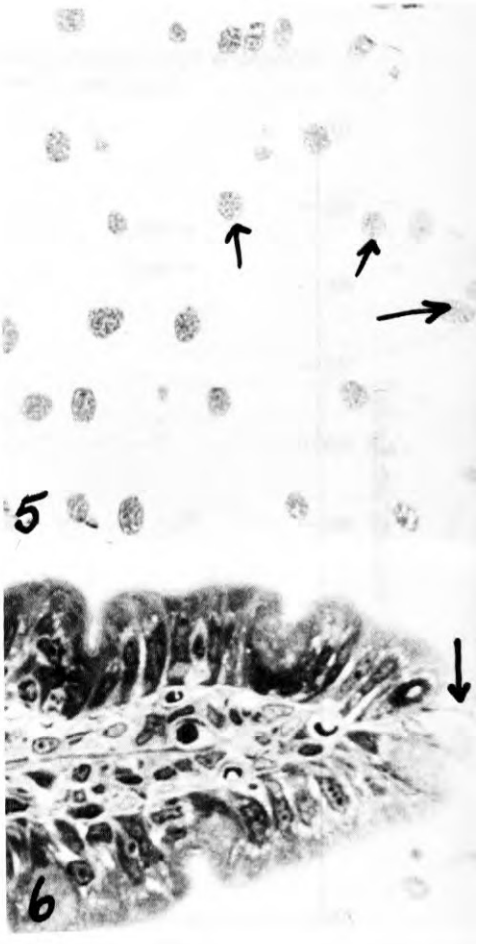
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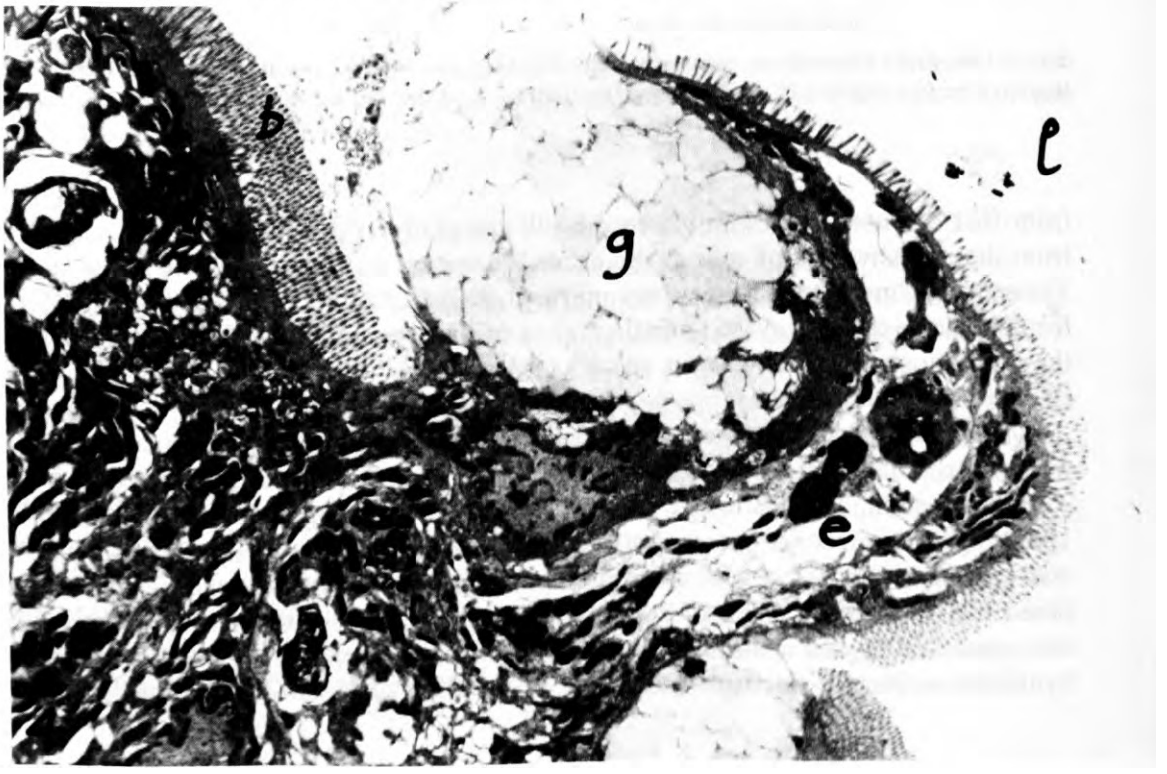
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around 500 mOsm/kg $H_2O$ on the cat. These authors propose that this hyperosmolar compartment is maintained by a countercurrent multiplier mechanism which according to their hypothesis, exists in most villous structures. For both Myrj 52 and PEG 2000, the experimentally determined plot of osmolality versus concentrations deviates from the theoretical line (Fig. 2). This deviation is much greater for the PEG 2000 solutions and accounts for the scale of fluid gain into the lumen (Fig. 1B). Kameda et al. (1968) found a similar difference between the calculated and measured values for solutions of PEG 550 and attributed the intestinal flattening he observed to the osmotic activity of the PEG solutions. A solution of 20% Myrj 52 would have an osmolality approximately equal to 500 mOsm/kg $H_2O$ at which Hallbäck et al. (1979) found the net luminal water flux was zero. A zero water flux was observed in our studies for the 10% PEG solution (Fig. 1B) and the 10% and 20% Myrj 52 solutions (Fig. 1A) which suggests that the osmolality of the rat villous tip is of the same order as that observed in the cat.

Yonezawa (1977) evaluated the effects of 10% solution of 7 surface-active compounds on closed intestinal segments in male rabbits. He found that hypersecretion of mucus, epithelial desquamation and epithelial necrosis were the 3 histopathological lesions produced. Each surfactant differed in the range and extent of the 3 lesions but not one induced goblet cell capping, and although he observed the dilation of the segments with time he attributed this effect to the accumulation of a mucoid substance.

We conclude that concentrated preparations of surface-active polymers cause morphological damage to the intestinal epithelium but the damage is transient for when the hypertonic agents are removed, normal morphology returns (Norris, 1963; Clarke and Kobayashi, 1975) because of the natural proliferation of crypt cells. However, in the evaluation of any preparations containing significant amounts of surfactant, consideration should be given to the possible disturbance of the osmotic gradient across the intestinal barrier with its concomitant effects on drug dissolution and absorption.

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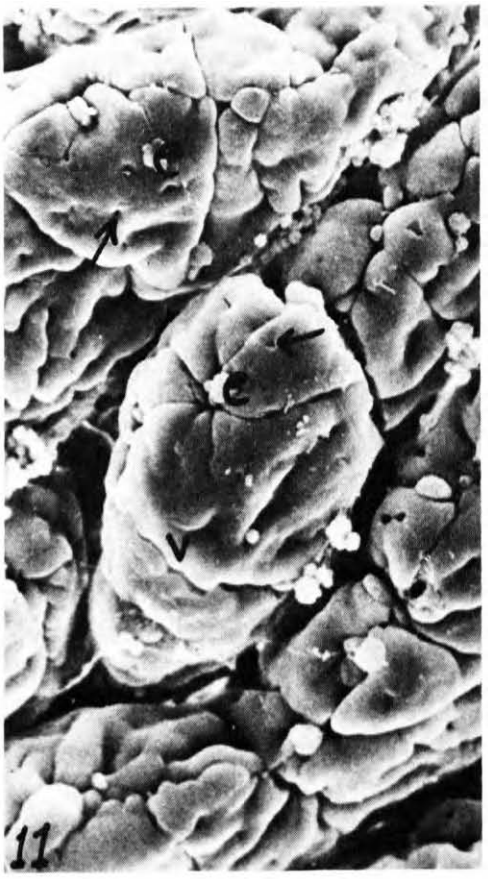
Fig. 7. Light micrograph from a plastic section from a segment exposed to 0.9% saline for 60 min showing the typical dilation of the lateral intercellular spaces (arrow) over the highest part of the villi. ×300.

Fig. 8. Paraffin section of a villus exposed to 20% PEG 2000 for 60 min showing a group of extruded cells (c) still attached to the apex of the villus and the first stage in the formation of a cap of goblet cells (arrow). ×300.

Fig. 9. Light micrograph of a plastic section stained with PAS to illustrate the distribution of goblet cells and the formation of a cap of goblet cells (arrow) after exposure to 30% PEG 2000 for 60 min. l, lumen; v, villus. ×300.

Fig. 10. Transmission electron micrograph of the apex of a villus exposed to 30% PEG 2000 for 60 min showing the morphology of the collection of goblet cells (g) over the apex of the villus and a portion of a detached cell (d) at the junction of the goblet cell cap and surrounding enterocytes (arrow). Note the smooth lining of the brush border stops at this junction; compare with Fig 16. ×4350.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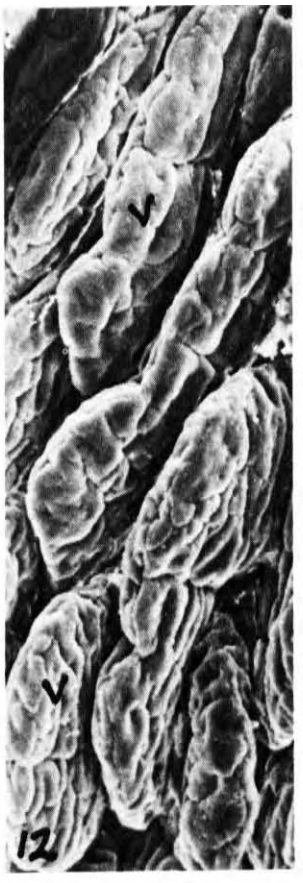
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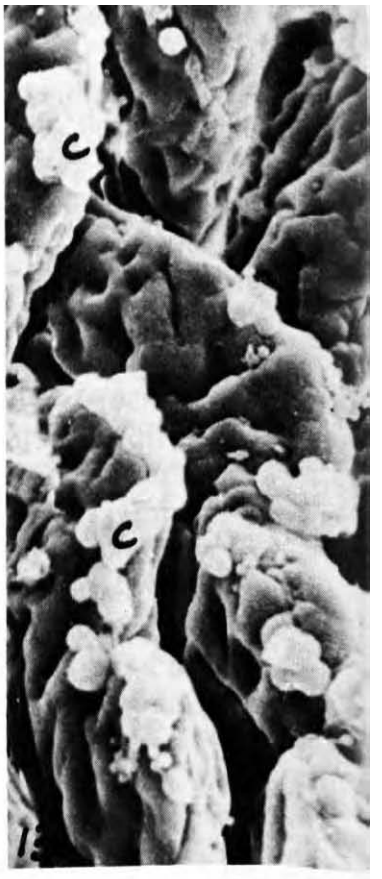
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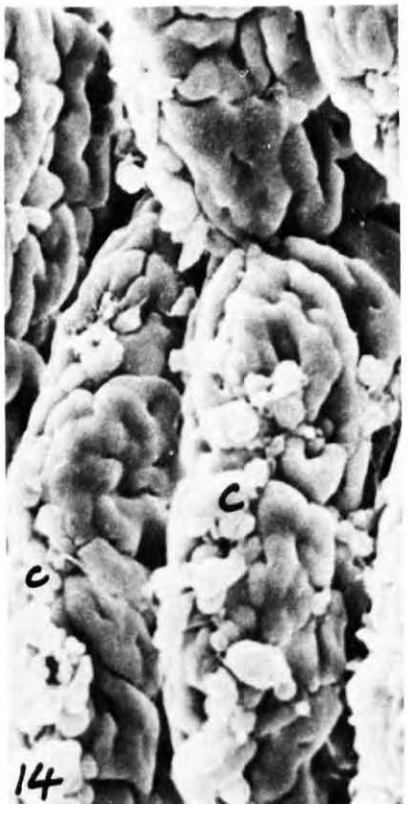
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Figs. 11–16. Scanning electron micrographs.

Fig. 11. Untreated ileum showing villi (v) with normal extrusion of enterocytes (e) and pits (arrow) showing the sites of discharge of mucus from the goblet cells. ×690.

Fig. 12. Tissue treated with saline for 60 min showing relatively normal leaf-like villi (v), extruded cells have been detached to the lumen. ×220.

Fig. 13. Villi from a segment treated with 30% Myrj 52 for 60 min showing the typical appearance of cells (c) in the process of detachment extending in a linear manner across the highest part of the villi. ×625.

Fig. 14. Villi for a segment treated with 20% PEG 2000 for 60 min showing a similar distribution of cells (c) in the process of detachment as seen in Fig. 13. ×625.

Fig. 15. Villus from a segment exposed to 30% PEG 2000 for 60 min showing fewer detached cells across the crest of the villus, their position being occupied by a region less smooth in appearance (r) than the rest of the villus, this region is the luminal surface of the cap of goblet cells. ×625.

Fig. 16. Apex of a villus showing the appearance of a cap of goblet cells; compare the image with the profile in Fig. 10 and note the junction of goblet cells and brush border of enterocytes (arrow), the irregular surface of the apex of the goblet cells with results in its rough appearance, and the outline of enterocytes (e) visible in one part of the micrograph. ×2330.

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