J. Pharm. Pharmacol. 1979, 31: 277–283 Received July 5, 1978

Relative effects of different surfactants on intestinal absorption and the release of proteins and phospholipids from the tissue

D. A. WHITMORE, L. G. BROOKES* AND K. P. WHEELER†

*Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton, Sussex BN1 9QG, U.K. and *Upjohn Ltd., Fleming Way, Crawley, Sussex, U.K.*

The actions of anionic, cationic and non-ionic surfactants on the absorptive capability of rat jejunal tissue in vivo were compared with their effects on the amounts of protein and phospholipid released from the mucosal surface under the same conditions. Release of a comparatively small amount of protein was accompanied by large increases in the absorption rates of both L-valine and salicylate, whereas much larger quantities of phospholipid were released before any increase in absorption was observed. Much of the released material appeared to be derived from mucus which was partly degraded after exposure to the higher concentrations of surfactants. The liberation of cholesterol by high concentrations of anionic surfactants suggested that some disruption of the mucosal membrane occurred under those conditions. The relative potency of the surfactants in stimulating both absorption of the solutes and the release of polypeptides and lipids followed the order: anionic > non-ionic > cationic. The possible pharmaceutical relevance of these findings is discussed.

In principle a surfactant could affect the permeability of a membrane to a solute by interaction with either the membrane or the solute, or both, and such interactions could be either general or specific in nature. To distinguish among these possibilities would be difficult but it is possible that some general trends might be discernible and that these could be useful, for example, in attempts to modulate the absorption of drugs through biological membranes. Such pharmaceutical and physiological interest in the effects of surfactants on intestinal permeability have led to a number of studies in which permeability changes were shown to be caused by surfactants. However, the main interest has usually, although not exclusively, been in discovering whether or not any effect of surfactant on absorption could be demonstrated, rather than with the reason for the effect. Hence the work tended to concentrate on either the effects of an individual surfactant (Feldman & Gibaldi 1969; Cid et al 1971; Feldman et al 1973; Isomaa & Sjoblom 1975; Gouda et al 1977) or the permeation of a particular solute (Feldman & Gibaldi 1969; Cid et al 1971; Feldman et al 1973; Gillan & Florence 1973; Florence & Gillan 1975; Lovering & Black 1974; Sund 1975; Feldman & Reinhard 1976). It seemed worthwhile, therefore, to make a more comprehensive study of the effects of surfactants on intestinal absorption by examining several different surfactants, representing anionic, cationic and non-ionic types, together with several different types of solute, to see if any general patterns of effects could be established.

The everted sac preparation from rat small intestine (Wilson & Wiseman 1954) was selected for the studies in vitro and the following surfactants were chosen as representative examples of the different classes: taurodeoxycholate, laurylsulphate, cetyltrimethylammonium, Brij 35, 58 and 98. To try to distinguish any effects of the surfactants on the tissue from those caused by direct interaction between surfactant and solute, the everted sacs were first exposed to the surfactant during a preliminary incubation, after which that incubation medium was removed and replaced by a fresh medium containing the solute under study, without surfactant. Originally we planned to examine the absorption of salicylic acid, a neutral amino acid and a monosaccharide as a typical examples of solute of pharmaceutical or physiological interest; but the results we have obtained with salicylic acid and L-valine indicate some very clear general patterns, so that repetition of the experiments with a sugar seem unlikely to provide further useful information.

MATERIALS AND METHODS

Materials

Polyoxyethylene$_{23}$ lauryl ether (POE_{23} lauryl ether, Brij 35), POE_{20} cetylether (Brij 58) and POE_{20} oleyl ether (Brij 98) were used as received from Honeywill

† Correspondence.

Atlas Ltd, Carshalton. Sodium taurodeoxycholate* (STDC) was prepared from cholic acid (Maybridge Ltd., Tintagell) according to the method of Norman (1955). Sodium lauryl sulphate (SLS), cetyltrimethylammonium bromide (CTAB), salicylic acid, L-valine, acrylamide, *NN*-methylene bis acrylamide (bis acrylamide), ammonium persulphate, *NNNN*-tetramethylethylenediamine (TEMED), chloroform, methanol, acetone, light petroleum (b.p. 80–100 °C), glacial acetic acid and ammonia solution (35%) were obtained from BDH Ltd., Poole, and were of analytical reagent grade where possible. Lipid standards were obtained from Lipid Products Ltd., Nutfield, Surrey. [Carboxy-^{14}C]salicylic acid and L-[U-^{14}C]valine were obtained from Radiochemical Centre, Amersham. O_2/CO_2 (95/5%) and oxygen-free nitrogen gases were supplied by BOC Ltd., Crawley. Distilled water was used throughout.

Incubation medium

Krebs original Ringer phosphate buffer (Dawson et al 1969) was used as bathing solution during sac preparation and as the mucosal and serosal solutions during absorption studies. Note, however, that the pH was adjusted to 6·0, mainly to facilitate comparison with previous work (Feldman & Gibaldi 1969). The surface-active agent or solute was added to the mucosal solutions as described below.

Preparation and use of everted intestinal sacs for transport studies

Male Sprague-Dawley rats, 330–370 g, had free access to 'Expanded Lab diet No. 1' (Spratts Ltd., London).Sac preparation was at room temperature (20 °C) with the use of a modified version of the method of Wilson & Wiseman (1954) that provided 20 sacs of jejunal tissue from each rat small intestine. Rats were killed by exposure to ether vapour and tissue removed via a mid-line abdominal incision. Upper jejunum was defined as from 10 cm distal to the pyloric sphincter, and lower jejunum from 10 cm distal to the ileocoeliac valve. The small intestine was divided in half, each half was stretched by a 4 g weight for 30 s, and 30 cm removed to fresh buffer solution. The lengths of tissue were then stretched to 30 cm again and 9 ligatures tied at 3 cm intervals. The sacs were separated, everted and filled by tying over a blunt needle. The serosal volume was 0·4 ml in each sac. After the sacs had been rinsed, blotted and weighed, two upper and two lower jejunal sacs were transferred to 10 ml of mucosal solution in a 25 ml conical flask maintained at 37 °C. Four flasks contained a known concentration of a surfactant, whilst the remaining flask was a control. The solutions were continuously shaken at about 90 oscillations min^{-1} and saturated with a stream of O_2/CO_2 gas mixture. This preliminary incubation was carried out for 60 min. Each sac was then rinsed, blotted, weighed and transfered separately to a fresh 10 ml of mucosal incubation solution maintained at 37 °C. A solution of [^{14}C]-labelled salicylic acid or valine was added to this mucosal solution to provide a final concentration of 0·5mM. The air above the mucosal solution was displaced with O_2/CO_2 gas and sealed. After incubation with shaking for 10 min, each sac was removed, rinsed, blotted and weighed. The serosal volumes were collected by puncturing the sacs. Samples of serosal (0·1 ml) and mucosal (0·4 ml) solutions where assayed by scintillation spectroscopy.

* Gift from C. A. Hampson, Upjohn Ltd., Crawley.

Protein determination

The protein concentrations were determined according to Lowry et al (1951). The non-ionic and cationic surfactants interfered with the assay procedure but satisfactory standard curves for individual samples were obtained by addition of each surfactant at the appropriate concentration to the bovine serum albumin standards.

Gel electrophoresis

Polyacrylamide slab gels were used with a method based on that of Fairbanks et al (1971). A 12% running gel was overlaid with a 5% stacking gel. A ratio of 30:1 (w/w) acrylamide:bisacrylamide was mixed with 15 mg ammonium persulphate and 2·5 μl TEMED g^{-1} of acrylamide.

Samples for electrophoresis were lyophilized and extracted twice with chloroform–methanol (2:1 v/v). Between 40 and 50 g of protein was loaded into each well. A current of 17mA cm^{-2} was applied until the tracking dye reached the gel junction and then the current was raised to 29 mA cm^{-2}. Overall running time was about 4 h. The gels were stained in methanol–acetic acid–water (5:1:5) plus 1·1 g Coomassie Blue per 440 ml for 1 h and then excess stain was removed in methanol–acetic acid–water (2:3:35) with stirring for at least 60 h. Gels were stained for carbohydrate with the periodic acid-Schiff reagent according to Zacharius et al (1969).

Thin layer chromatography

Thin layer chromatography (t.l.c.) plates were prepared according to Rouser et al (1970) and washed

in acetone–light petroleum (3:1). The chloroform–methanol extracts of samples prepared for electrophoresis were pooled and heated at 37 °C under a stream of oxygen-free nitrogen. When dry, 0·2 ml chloroform–methanol (2:1) was added to each and the samples were stored under nitrogen at −20 °C. Samples of the extracts were chromatographed in the following solvent systems: chloroform–acetone–methanol–acetic acid–water, (5:2:1:1:0·5); chloroform–methanol–acetic acid–water, (25:15:4:2), chloroform–methanol–ammonia solution, (95:5:0·8).

Lipid standards and specific colourimetric detection tests (Lowenstein 1969) were used for lipid detection and identification.

Scintillation spectroscopy

Samples of the mucosal and serosal solutions were 'counted' in a Toluene-based scintillation fluid containing 33% Trition-X100, using either a Packard Tricarb model 3320 or a Beckman LS233 liquid scintillation counter. Counting efficiency was monitored by the channels ratio method. All radioactivity measurements were made to an accuracy of at least ±2%.

Lipid phosphorous determinations

Samples of the chloroform–methanol extracts were dried under a stream of nitrogen and analysed for lipid phosphorous by the method of Rouser et al (1970).

RESULTS

Conditions for measurement of initial rates of uptake

The rates of accumulation of both salicylate and valine in the sacs were determined by omitting the preliminary incubation and varying the incubation time. They were linear with time for at least 20 min (Fig. 1) and an incubation of 10 min was therefore used to measure initial rates of uptake.

Effects of preliminary incubation with surfactants on absorption rates

The effects of incubating the sacs with different surfactants at various concentrations on the subsequent absorption of salicylate and L-valine are shown in Figs 2 and 3, respectively. The overall absorption pattern was the same for both upper and lower jejunum, but the absolute uptake rates were often greater in the latter. The anionic STDC and SLS increased the rates of uptake of both salicylate and L-valine. STDC produced a maximal effect at 50 mM with both solutes. In contrast, the cationic

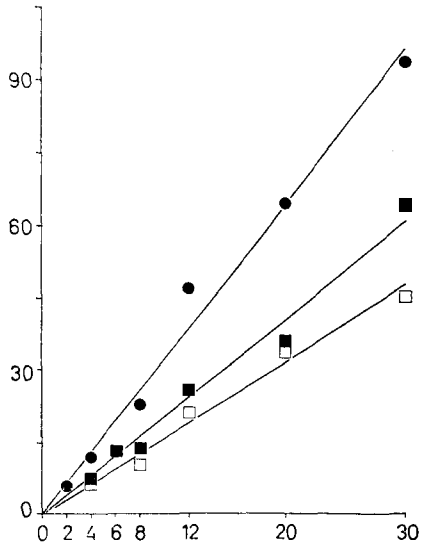

FIG. 1. Accumulation of salicylate and L-valine in sacs of everted jejunum from rat. The values for salicylate (●) were obtained from randomly assigned sacs, whereas those for valine were from either upper (□) or lower (■) jejunum. Mean values (± s.e.m.) of the wet and dry weights of these sacs were 195 ± 4 (20) mg and 33 ± 1 (20) mg, respectively. Ordinate: solute accumulation (nmol per sac). Abscissa: sac incubation time (min).

CTAB had no effect on valine absorption but decreased the absorption of salicylate. The non-ionic surfactants increased L-valine absorption but had no effect upon salicylate absorption, except for a small increase caused by the highest concentration of Brij 98. All changes in absorption rates caused by the surfactants were clearly related to the concentrations of surfactants used, the effects increasing with increasing concentration of surfactant.

Effects of exposure to surfactants on the intestinal tissue

The protein and lipid phosphorous concentrations of each mucosal solution at the end of the preliminary incubation are shown in Fig. 4. T.l.c. of the extracts from these solutions showed the presence of phosphatidylcholine, lysophosphatidylcholine, cholesterol, free fatty acids, sphingomyelin, sphingomyelin-sugar derivatives and amino acids or small polypeptides. Phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidyl serine, phosphatidylglycerol, phosphatidylinositol, and phosphatidic acid were not detected.

Polyacrylamide gel-electrophoresis of the solutions after the preliminary incubation revealed some general trends in the protein bands (Fig. 5a, b). The lowest concentration of each surfactant used, except

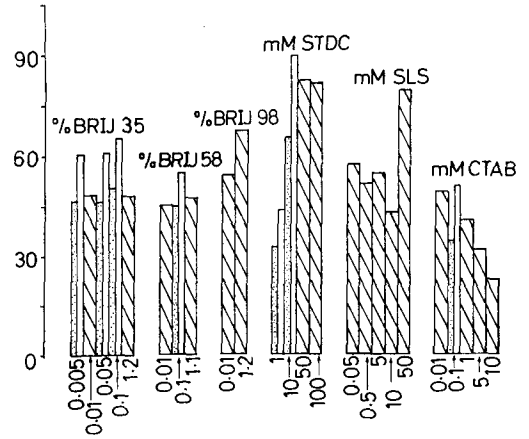

FIG. 2. Effects of some surfactants on the rate of absorption of salicylate by sacs of everted jejunum from rat. Sacs were first incubated for 60 min in the standard buffer solution with or without the addition of the surfactants at the indicated concentrations, as described in in the text. They were then rinsed, blotted, weighed and transferred to fresh incubation medium containing the salicylate. The mean values (± s.e.m.) of the wet and dry weights of the sacs were 116 ± 2 (140) mg and 20 ±0·4 (140) mg, respectively. The mean accumulation rates (±s.e.m.) in the control sacs were 45 ± 2 (12) nmol of salicylate/10 min per sac for the upper jejunum and 54 ± 3 (11) nmol of salicylate/10 min per sac for the lower jejunum. Cross hatched areas indicate that the accumulation rates for upper and lower jejunum were within 10% of their averaged values. Where the values were outside this range the rate for the upper jejunum are denoted by stippled lines and those for lower jejunum by outline only. All values are the means of duplicate or quadruplicate measurements. Ordinate: Rate of accumulation of salicylate (nmol per 10 min per sac). Abscissa: Preliminary incubation surfactant and concentration.

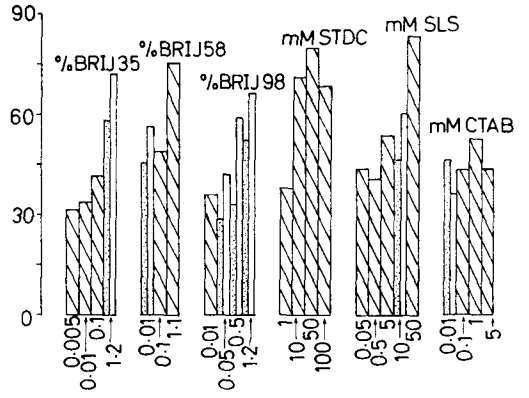

Fig. 3. Effects of some surfactants on the rate of absorption of L-valine by sacs of everted jejunum from rat. Details exactly as described in the legend to Fig. 2, except that L-valine replaced salicylate as the test solute. Mean values of the wet and dry weights of the sacs were exactly the same as those given in Fig. 2. The mean accumulations rates (± s.e.m.) in the control sacs were 39 ± 2 (12) nmol of valine/10 min per sac for the upper jejunum and 40 ± 2 (11) nmol of valine/10 min per sac for the lower jejunum. Ordinate: Rate of accumulation of L-valine (nmol per 10 min per sac). Abscissa: Preliminary incubation surfactant and concentration.

STDC, gave a banding pattern identical to that of the control. STDC was an exception in that all concentrations decreased the amount of protein in the higher molecular weight bands and increased that in the lower molecular weight bands. However, higher concentrations of most of the other surfactants brought about a similar change in the size distribution.

CTAB was the only surfactant to produce a distinctly different protein banding pattern. As the CTAB concentration was raised not only was there the progressive change in size distribution noted above, but also there was a marked reduction in intensity of the band corresponding to a protein of molecular weight of about 39 000.

The Schiff's periodate reagent showed the very high molecular weight protein band at the front of the stacking gel, and the low molecular weight bands, to have carbohydrate components. Electrophoresis of the material extracted by chloroform–methanol

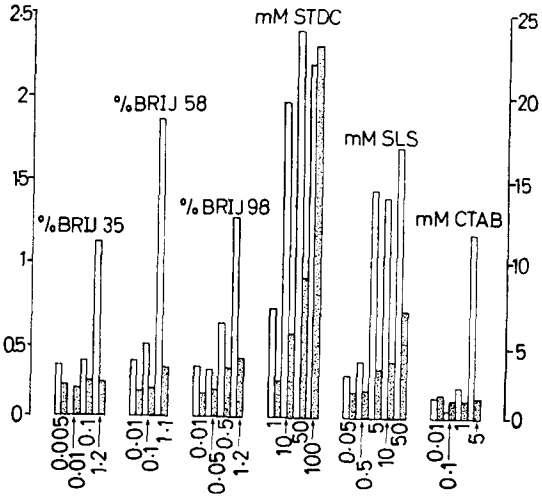

FIG. 4. The release of protein and lipid phosphorus from the mucosal surface of rat everted jejunal sacs by incubation in the presence of various surfactants. Protein concentrations are shown by stippled lines and lipid phosphorus in outline. Release values by control preincubation solutions were 0·22 μmol lipid phosphorus per 60 min incubation per 3 cm sac and 1·6 mg protein per 60 min incubation per 3 cm sac. The surfactants gave negligible or zero levels of phosphorus by the assay method. Left ordinate: Lipid phosphorous (μmol per 60 min per sac). Right ordinate: Protein (BSA equivalents, mg per 60 min per sac). Abscissa: Preliminary incubation: surfactant and concentration.

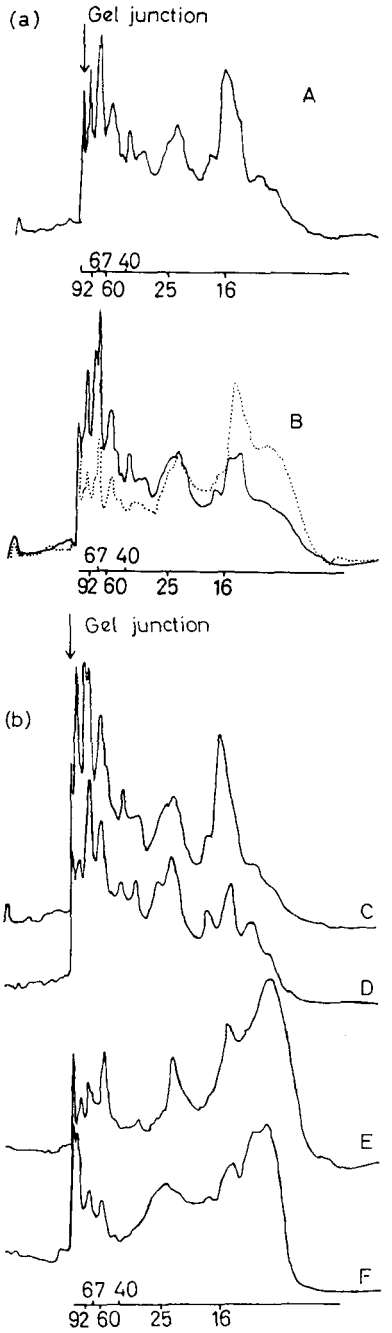

FIG. 5a,b. The protein banding patterns obtained with polyacrylamide slab electrophoresis of some mucosal incubation solutions. A. Control, B. 0·01% Brij 35 (solid line) and 1·2% Brij 35 (dotted line). C. 0·01 mM CTAB, D. 1 mM STDC, E. 5 mM CTAB, F. 50 mM STDC. The molecular weight scale is shown on the abscissa.

The protein bands were stained with Coomassie Blue and the gels scanned on a Joyce–Loebl double beam densitometer.

showed the presence of low levels of protein only when the preliminary incubation medium had contained surfactant at the higher concentrations.

DISCUSSION

Absolute rates of absorption

Larsen et al (1964) obtained values of 3 μmol/1·5 h per 100 mg dry weight for V_{max} and 3·3 mM for K_m in their study of the uptake of L-valine by rat small intestine. Hence for an initial mucosal concentration of 0·5mM L-valine, as used in our work, the expected rate of absorption would be 0·4 μmol/1·5 h per 100 mg dry weight. From Fig. 1 the initial rate of uptake by our control sacs was 0·5 μmol/1·5 h per 100 mg dry weight.

With an initial mucosal concentration of 2 mg ml^{-1}, Feldman & Gibaldi (1969) obtained an uptake of 30 μg salicylate min^{-1} per 10 cm sac, which is equivalent to about 2·8 nmol min^{-1} per 3 cm sac length for an initial mucosal concentration of 0·5mM, assuming that the rate is directly proportional to the concentration. Our control value (Fig. 1) was 3·3 nmol min^{-1} per 3 cm sac for an initial mucosal concentration of 0·5mM.

It seems, therefore, that our absolute rates of uptake of both L-valine and salicylate by the control sacs were in good agreement with those recorded in the literature, although exact comparison is hardly warranted because of slight differences in experimental technique.

Relation between permeability changes and release of proteins and phospholipids from the tissue

The overall similarities in the effects of the various surfactants on the absorption of both salicylate and L-valine suggest that the effects had a common origin. One possibility is that the preliminary exposure of the tissue to the surfactants caused a partial disruption of the tissue, as indicated by the release of proteins and phospholipids, and that was the underlying factor responsible. On the other hand the surfactants could simply have stimulated the release of mucous from the tissue, so that the increases in rates of absorption were not causally related to the release of the compounds detected in the mucosal solution. Quantitative comparison of the amounts of protein and phospholipid liberated by the surfactants with the rates of absorption of salicylate and valine is shown in Fig. 6, which again emphasizes the parallelism in response of the two solutes as far as their absorption was concerned. In addition they reveal that clear increases in absorption rates were apparent when the loss of phospholipid was about

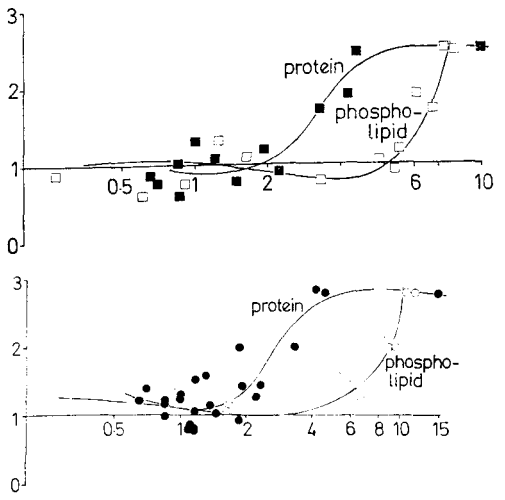

FIG. 6. Relationship between absorption of salicylate (upper) or L-valine and the release of protein and phospholipid in the presence of the various surfactants. Data from Figs 2–4, ignoring the nature and concentrations of the surfactants. ●, ■, protein; ○, □, phospholipid. (Values pertaining to salicylate and the non-ionic surfactants have been omitted because the absorption rates were largely unaffected—see Fig. 2). Ordinates: Final serosal concentration of salicylate or L-valine (upper and lower figure respectively) relative to control value; upper jejunal values. Abscissa: Protein or phospholipid release relative to control value.

6·7 times as great as in the control incubations, but when the loss of protein was only about twice that of the controls. Since the results in Fig. 4 also show that the release of proteins and phospholipids bear no obvious relation to one another, it seems unlikely that the compounds detected in the mucosal solutions arose simply from the secretion of differing amounts of mucus in control and test conditions. It therefore appears most likely that the surfactants caused some disruption of the mucosal surface, although not enough to be detected by simple visual inspection, or to produce drastic breakdown in permeability properties.

Analyses of materials released during the preliminary incubations

The main effect of the surfactants on the patterns of polypeptide bands recovered from the mucosal solutions was to increase the proportion of lower molecular weight material at the expense of the higher molecular weight compounds (Fig. 5), although this trend was obvious only when the surfactants were used at the higher concentrations. The very high molecular weight band shown in Fig. 5 was stained by the periodate-Schiff reaction and almost certainly contained a mucus macromolecule. It was absent from mucosal solutions containing high concentrations of surfactants. The latter could have disrupted the molecule directly (Martin et al 1976) but it is also possible that surfactant activation of endogenous proteases was responsible for the general degradation of large polypeptides to smaller ones (Fairbanks et al 1971).

Semi-quantitative analysis by t.l.c. of chloroform–methanol extracts of the mucosal solutions showed that sphingomyelin, sphingomyelin-sugar derivatives, phosphatidylcholine, lysophosphatidylcholine and trace amounts of free fatty acids were present after incubation with most concentrations of each surfactant. Cholesterol was found only in the solutions containing the anionic surfactants at 50 and 100 mM concentrations. Some ninhydrin-sensitive compounds, presumably lipo-peptides, were also detected. The presence of cholesterol was most significant because that almost certainly was released from the mucosal membrane itself, rather than mucus.

Effects of cetyltrimethylammonium bromide

Unlike the anionic and non-ionic surfactants, the cationic cetyltrimethyl-ammonium did not cause an increase in the rate of absorption of valine (Fig. 3) and actually elicited a decrease in the rate of absorption of salicylate (Fig. 2). The same surfactant was shown to decrease the absorption of glucose, methionine and butyrate (Nissim 1960) and acetylsalicylate (Cid et al 1971) when present at concentrations similar to or exceeding those used in this work. An increase in the absorption of tripalmitate was attributed to an increase in gastrointestinal motility in the presence of the detergent (Isomaa & Sjoblom 1975). However, Feldman & Reinhard (1976) found that concentrations of cetyltrimethylammonium similar to those used here caused an increase in the absorption of salicylate by rat everted intestinal sacs. There is no obvious explanation for this apparent discrepancy between their results and ours.

It may be significant that this surfactant did not produce an increase in the release of protein from the tissue during our studies, although it did increase the loss of phospholipids (Fig. 4). The data in Fig. 5 show that the cationic surfactant appeared to prevent the loss of a protein of molecular weight about 39 000, compared with the other surfactants, and it is possible that this was important.

Another possibility is that there was enough residual cetyltrimethylammonium associated with the tissue after the preliminary incubations to interact directly with the salicylate. These two com-

pounds have been shown to interact in solution to produce an increase in viscosity (Wan 1966, 1977) and any such interaction might account for the decreased rate of absorption of salicylate.

Conclusion

Evidence for both biochemical and histological changes in hamster small intestine after exposure to various anionic surfactants has recently been correlated with changes in secretion and permeability (Gullikson et al 1977) and an association between protein release and an increased absorption of salicylate by rat intestine has been reported by Feldman & Reinhard (1976). Our findings indicate that, bearing in mind the partially exceptional effects of cetyltrimethylammonium, all types of surfactant we tested tended to accelerate breakdown of the mucous layer covering the epithelium, and that some of them, particularly when present at high concentrations, probably began to interfere with the structure of the mucosal membrane itself. This interpretation is supported by recent histochemical evidence for epithelial desquamation and necrosis when rabbit intestinal mucosa were exposed to various anionic and non-ionic detergents (Yonezawa 1977). Such disruptive effects therefore appear to underlie the accompanying increases in permeability properties of the tissue, the order of potency of the surfactants being anionic > non-ionic > cationic for both effects. The surfactants must also have stimulated secretion of mucus (Yonezawa 1977), but that could not be expected to increase absorption of solutes.

We conclude, therefore, that surfactants of all classes are unlikely to enhance membrane permeability to solutes without causing membrane damage, unless very low concentrations are able to interact directly with a particular solute in such a way that its absorption is facilitated. These findings are important physiologically only in so far that surfactants are used to aid dispersion and absorption of some drugs. It is difficult to determine what concentrations of surfactant result, but they could be high for a time in a limited area.

Acknowledgements

We thank Upjohn Ltd. both for some financial assistance and for the use of materials and laboratory facilities during the initial stages of this work. The award of a CASE studentship to D. A. W. from the Science Research Council is also gratefully acknowledged.

REFERENCES

Cid, E., Dresse, A., Jaminet, Fr. (1971) Pharm. Acta Helv. 46: 377–382

Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M. (eds) (1969) Data for Biochemical Research 2nd edn, p 507

Fairbanks, G., Steck, T. L., Wallach, D. F. H. (1971) Biochem. 10: 2606–2617

Feldman, S., Gibaldi, M. (1969) J. Pharm. Sci. 58: 425–428

Feldman, S., Reinhard, M. (1976) Ibid. 65: 1460–1462

Feldman, S., Reinhard, M., Willson, C. (1973) Ibid. 62: 1961–1964

Florence, A. T., Gillan, J. M. N. (1975) J. Pharm. Pharmacol. 27: 152–159

Gillan, J. M. N., Florence, A. T. (1973) Ibid. 25: Suppl. 136P–137P

Gouda, M. W., Khalafalah, N., Khalil, S. A. (1977) J. Pharm. Sci. 66: 727–728

Gullikson, G. W., Cline, W. S., Lorenzson, V., Benz, L., Oleson, W. A., Bass, P. (1977) Gastroenterology 73: 501–511

Isomaa, B., Sjoblom, G. (1975) Food Cosmet. Toxicol. 13: 517–520.

Larsen, P. R., Ross, J. E., Tapley, D. F. (1964) Biochim. Biophys. Acta 88: 570–577

Lovering, E. G., Black, D. B. (1974) J. Pharm. Sci. 63: 671–676

Lowenstein, J. M. (ed) (1969) Methods in Enzymology, 14: pp 544–548

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Martin, G. P., Marriott, C., Kellaway, I. W. (1976) J. Pharm. Pharmacol. 28: Suppl. 76P

Nissim, J. A. (1960) Nature, 187: 308–310

Norman, A. (1955) Ark. Kemi 8: 331–342

Rouser, G., Fleischer, S., Yamamoto, A. (1970) Lipids 5: 494–496

Sund, R. B. (1975) Acta Pharmacol. Toxicol. 37: 297–308

Wan, L. S. C. (1966) J. Pharm. Sci. 55: 1395–1399

Wan, L. S. C. (1977) Ibid. 66: 1779–1780

Wilson, T. H., Wiseman, G. (1954) J. Physiol. 123: 116–125

Yonezawa, M. (1977) Nihon. Univ. J. Med. 19: 125–141

Zacharius, R. M., Zell, T. E., Morrison, J. H., Woodcock, J. J. (1969) Anal. Biochem. 30: 148–152