International Journal of Pharmaceutics, 85 (1992) 129–140

IJP 02825

Mechanisms of penetration enhancement for transbuccal delivery of salicylic acid

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(Received 29 April 1991)
(Modified version received 16 December 1991)
(Accepted 28 February 1992)

Key words: Penetration enhancer; Salicylic acid; Buccal delivery; Sodium deoxycholate; Sodium lauryl sulfate; Electrical resistance; Protein transition; Lipid transition

Summary

The process of in vitro permeation across rabbit buccal mucosa was examined using salicylic acid as a model compound. The promoting action of penetration enhancers on salicylic acid flux was studied using differential scanning calorimetry, flux, electrophysiology, and microscopy techniques. The enhancers, sodium deoxycholate and sodium lauryl sulfate, decrease the electrical resistance and increase the permeability of salicylic acid across rabbit buccal mucosa. The results of these techniques appear to support the premise that the superficial layers and protein domain of the epithelium may be responsible for maintaining the barrier function of the buccal mucosa. This work provides additional insight in identification of the permeability barrier and the mechanism of enhancer activity.

Introduction

Transmucosal delivery, other than via the oral route, e.g., nasal, rectal, buccal, and sublingual, is often a useful alternative pathway for the administration of those drugs with poor oral bioavailability and relatively low dose. The major limitation to transmucosal delivery is frequently the low flux through the attendant tissue, coupled with short contact time and, occasionally, metabolism at the absorption site. Candidate drugs for these alternative routes of administration are commonly charged hydrophilic compounds or those with high molecular weight, e.g., peptides and proteins.

Penetration enhancers like sodium deoxycholate (Pontiroli et al., 1982; Mosses et al., 1983) and sodium lauryl sulfate (Hirai et al., 1981) have been shown to be effective promoting agents for transmucosal drug delivery. However, sufficient work to explain the mechanisms of these enhancers in specific mucosal locations such as the buccal area has been lacking. A specific objective of the present study was to identify the location of the permeability barrier and to investigate the mechanism of penetration enhancement in buccal mucosa using calorimetric, electrophysiologic, and

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flux techniques. In order to monitor morphological and ultrastructural changes as a result of treatment with enhancers, light and electron microscopy studies were also performed.

Experimental

Materials and Methods

Tissue collection

Male albino New Zealand rabbits (Bakkom's Rabbitry, Viroqua, WI) weighing between 2.5 and 3.0 kg were used for all studies. Rabbits were killed by an intravenous injection of an overdose of sodium pentobarbital given via a marginal ear vein. Two incisions were made on each side of the head, one along the upper and the other along the lower boundaries of the buccal cavity. The underlying connective tissue was separated and the remaining muscle and underlying submucosal tissue were then separated and the epithelium cleaned.

Chemicals and solutions

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as received. Radiolabel ([^{14}C]salicylic acid) was purchased from Amersham (Arlington Heights, IL).

Differential scanning calorimetry (DSC)

DSC studies were performed using a Mettler TA 3000 DSC (Mettler Instrument Corp., Heightstown, NJ). Special stainless-steel pans were used which can withstand pressures up to 20 bar. In order to study thermotropic transitions, freshly excised tissue was equilibrated at 97% R.H. in a closed chamber containing a saturated solution of potassium sulfate for 24 h at 30°C. Approx. 50 mg of the sample were weighed and sealed in the stainless-steel pans. The empty pans were preheated to 200°C in an oven (Blue M Electric Co., Blue Island, IL) and cooled to room temperature prior to sample loading. Sample and reference pans were then heated at 3°C/min from 40 to 180°C.

Permeability studies

All permeability determinations were performed in 25 mM Hepes buffer, pH 7.4. In all experiments the solutions were made isotonic using NaCl. Isotonicity was adjusted to 290–310 mOsm, as measured by a Wescor 55D vapor pressure osmometer (Logan, UT). The pH of the solution was adjusted to 7.4 using sodium hydroxide. Radiolabelled salicylic acid was dissolved in Hepes buffer (25 mM) for the permeability studies. Buccal tissue was obtained and cleaned as described above (under Tissue collection). The tissue was mounted in the diffusion cell with the epithelial side facing the donor chamber of the diffusion cell. All permeability studies were performed over a 4 h period. Earlier work in this laboratory had examined tissue integrity and found it to be viable post excision for up to 4 h (Longer, 1988). The diffusion cell used was a modification of that described previously (Schoenwald and Huang, 1983). This cell has been well characterized with respect to its hydrodynamics (Edelhauser et al., 1965). The total volume of each half cell was 7 ml and the cross-sectional area of the exposed tissue was 0.785 cm^2. The permeability of salicylic acid was evaluated in the presence of penetration enhancers, namely, sodium deoxycholate and sodium lauryl sulfate in the above-described in vitro diffusion cell. The studies were performed using 0.5 and 1% w/v of sodium deoxycholate and 0.1, 0.5 and 1% w/v of sodium lauryl sulfate. The enhancers were added with the radiolabelled drug solution on the donor side, while the receiver compartment contained only Hepes buffer.

Resistance measurements

Resistance measurements were carried out as described earlier (Rojanasakul and Robinson, 1989). The membrane was bathed on the epithelial side with various concentrations of both penetration enhancers, viz., 0.1 and 0.5% sodium deoxycholate and 0.1, 0.5 and 1% w/v sodium lauryl sulfate. The serosal side contained only Hepes buffer.

Transmission electron microscopy

Samples of buccal mucosa were taken after incubation in Hepes buffer or Hepes buffer con-

taining sodium deoxycholate (0.5% w/v) or sodium lauryl sulfate (0.1% w/v) for 4 h. These were then placed in primary fixative solution (4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3) for 2 h. Subsequently, the tissue was washed several times with phosphate buffer, pH 7.3, before terminal fixing. The tissue was then post fixed in 2% osmium tetroxide.

Following post fixation, the tissue was dehydrated using a graded series of ethanol solutions at 35, 50, 70, 90, and 100%. The dehydrated samples were then embedded in Durcupan ACM embedding medium (Fluka Chemical Corp., Ronkonkoma, NY) and thin sections of tissue, 700–800 nm, were taken using a Sorvall MT 2B ultramicrotome with a diamond knife. These sections were subsequently stained with uranyl acetate and lead citrate and viewed with a transmission electron microscope (Hitachi H-600).

Light microscopy

Samples of buccal mucosa were taken after incubation either in Hepes buffer or Hepes buffer containing sodium deoxycholate (0.5% w/v) or sodium lauryl sulfate (0.1% w/v) for 4 h. 10–20 μm sections were obtained from these tissues employing a Sorvall MT 2B ultramicrotome using a glass knife, stained with toluidine blue 0.5%, and photomicrographs taken with a camera attachment on the microscope (Orthoplan large field microscope, Leitz).

Results and Discussion

DSC profiles show the lipid and protein transitions in buccal mucosa. Fig. 1 (trace I) presents the DSC profile of buccal mucosa when it was heated from 40 to 90°C. A single peak was seen at 69°C. When the same sample was cooled and reheated to 180°C, four peaks were seen as shown in Fig. 1 (trace II). This technique of phased heating offers advantages, as described by Potts (1990). In addition to endotherm A, three endotherms B–D having transition temperatures of 105, 154 and 166°C, respectively, were observed. The thermal profile obtained upon again cooling and reheating the same sample as described in

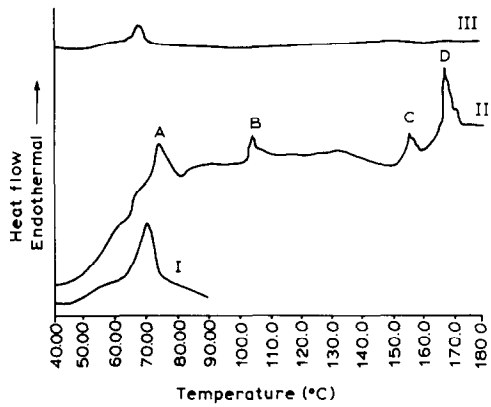

Fig. 1. DSC thermal profiles obtained using rabbit buccal mucosa all hydrated at 30°C and 97% RH. (Trace I) Intact samples from 40 to 90°C; (trace II) same sample heated from 40 to 180°C; (trace III) same sample reheated from 40 to 180°C.

trace II is illustrated in the upper panel (Fig. 1; trace III). The thermally induced transition near 69°C reappeared during the reheating of the sample. In contrast, the higher temperature transitions disappeared on reheating.

Endotherm A is completely reversible and may be due to a lipid transition. Like all lipid transitions (Chapman, 1985), this one is thermally reversible. Endotherms B–D, which occur at high temperature, are thermally irreversible and may be due to thermal denaturation of proteins or lipoproteins. The assignments of the thermal transitions of lipid and proteins are in accordance with those observed in other mucosal membranes of rabbit (Corbo et al., 1990) and skin (Golden et al., 1987).

Fig. 2 demonstrates a DSC thermogram of tissue after treatment with various concentrations of sodium deoxycholate. Sodium deoxycholate 0.5% w/v caused the disappearance of endotherm C and shifted the lipid transition temperature of endotherm A. It is possible that the intercellular content of superficial cells consisting mostly of proteins might be extracted by enhancer, resulting in the disappearance of endotherm C. Alternatively, denaturation or unfolding of the protein may occur, creating a straighter vs tortuous path, thereby decreasing resistance and increasing permeability. Treatment with 1%

sodium deoxycholate probably denatures and extracts all proteins and leads to the disappearance of endotherms B–D. It also broadens lipid transitions.

Fig. 3 presents the DSC profiles recorded with various concentrations of sodium lauryl sulfate. At the lowest concentration of sodium lauryl sulfate, endotherms C and D are significantly affected. As the concentration is increased endotherms C and D are reduced, and finally at 1% sodium lauryl sulfate both endotherms completely disappear with broadening of the lipid endotherm at a lower temperature.

With both sodium lauryl sulfate and sodium deoxycholate, the lowest concentration of enhancer appears to significantly affect the protein domain of the tissue. In transdermal studies, compounds like ozone (Goodman and Barry, 1986) and oleic acid (Francoeur et al., 1990) affect lipid fluidity and are of potential use for the enhancement of drug permeability. In contrast, for buccal delivery, enhancers which increase permeability appear to influence predominantly the protein domain of the tissue.

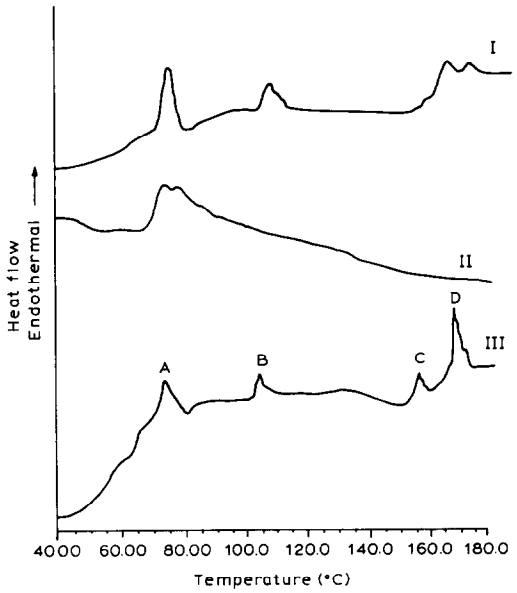

Fig. 2. DSC thermal profiles obtained from rabbit buccal mucosa when treated with sodium deoxycholate as penetration enhancer on the epithelial side for 4 h. (Trace I) 0.5% w/v sodium deoxycholate; (trace II) 1.0% w/v sodium deoxycholate; (trace III) untreated tissue.

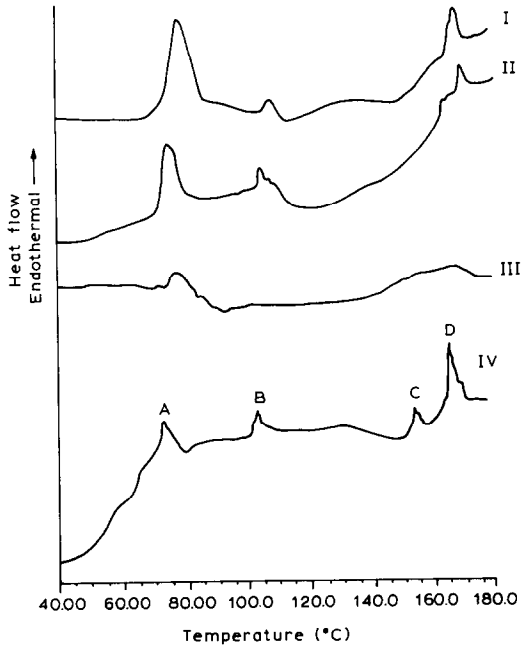

Fig. 3. DSC thermal profiles obtained from rabbit buccal mucosa when treated with sodium lauryl sulfate as penetration enhancer on epithelial side for 4 h. (Trace I) 0.1% w/v sodium lauryl sulfate; (trace II) 0.5% w/v sodium lauryl sulfate; (trace III) 1.0% w/v sodium lauryl sulfate; (trace IV) untreated tissue.

Permeability experiments were performed to determine the changes in flux of salicylic acid in the presence of enhancers. As shown in Fig. 4, the permeability of salicylic acid was increased significantly in the presence of 0.5% w/v sodium deoxycholate and 0.1% w/v sodium lauryl sulfate. The steady-state fluxes, calculated from the slope of percent permeated vs time plots, indicate that the value of the permeability coefficient in the presence of 0.5% w/v sodium deoxycholate was 2.2×10^{-5} cm/s and that in the presence of 0.1% w/v sodium lauryl sulfate amounted to 4.36×10^{-6} cm/s. The permeability coefficient of the control in Hepes buffer without any enhancer was 0.93×10^{-6} cm/s. The extent of enhancement can be expressed as a parameter, designated as the enhancement factor (EF), which can

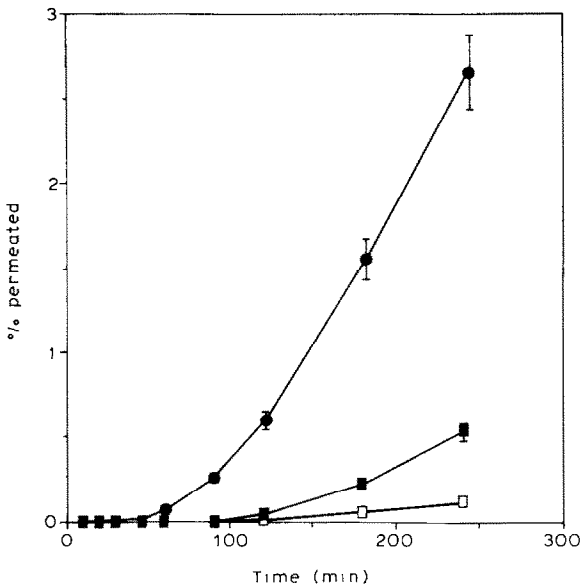

Fig. 4. In vitro permeability profiles of salicylic acid across buccal mucosa in the presence of penetration enhancers in Hepes buffer on epithelial side. (□ ——— □) Salicylic acid; (◆ ——— ◆) salicylic acid + 0.5 % w/v sodium deoxycholate; (■ ——— ■) salicylic acid + 1% w/v sodium lauryl sulfate. Error bars represent S.E.; $n = 5$.

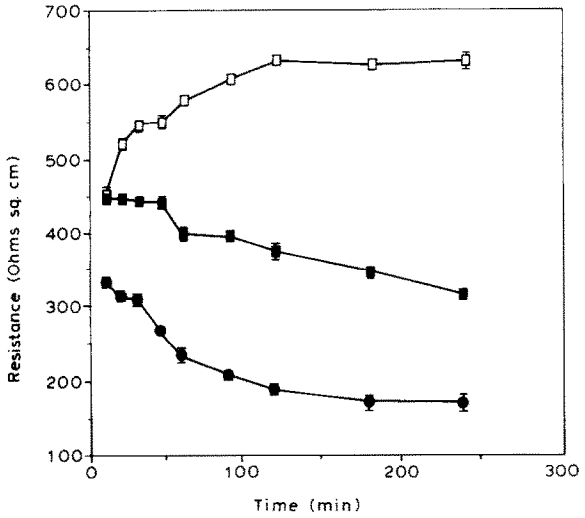

Fig. 5. Profiles showing resistance of rabbit buccal mucosa in the presence of penetration enhancers on the epithelial side. (□ ——— □) Hepes buffer; (■ ——— ■) 0.1% w/v sodium lauryl sulfate; (◆ ——— ◆) 0.5% w/v sodium deoxycholate. Error bars represent S.E.; $n = 5$.

be calculated according to the following expression.

$$EF = [\text{permeation rate}]_{\text{enhancer}} / [\text{permeation rate}]_{\text{control}}$$

Table 1 lists the enhancement factors determined after treatment with enhancers. It is worth noting that the permeability of buccal tissue increased in the presence of 0.5% w/v sodium deoxycholate with an EF of 23.5 and by a factor of 4.7 with 0.1% w/v sodium lauryl sulfate. From Figs 2 and 3 it appears that sodium deoxycholate has a more profound effect on proteins than does sodium lauryl sulfate. The major effect of penetration enhancers on the protein domain indicates that the possible mechanism of action of the above enhancers may involve the uncoiling and extending of the protein helices, thereby opening up the polar pathway.

TABLE 1

Effect of penetration enhancers on permeability coefficient of salicylic acid across rabbit buccal mucosa

Treatment	p ($\times 10^6$) (cm/s)	EF
Salicylic acid	0.93 (0.09)	1.0
Salicylic acid + 0.5% sodium deoxycholate	22.15 (0.87)	23.5
Salicylic acid + 0.1% sodium lauryl sulfate	4.36 (0.07)	4.7

P, permeability coefficient; EF, enhancement factor; $n = 5$ for all determinations. Values in parentheses represent S.E.

Fig. 5 shows plots of tissue electrical resistance as a function of time in the presence of penetration enhancers. It is seen that there was a significant reduction in resistance in the presence of enhancers. In the presence of 0.5% w/v sodium deoxycholate, resistance decreased from 628 to 170 Ω and in the presence of 0.1% w/v sodium lauryl sulfate from 628 to 313 Ω. The decrease in electrical resistance is proportional to the increase in permeability. The decrease in resistance may be due to removal of the superficial layer from the epithelium and also to the removal of desmosomes and actin filaments as demonstrated in electron microscopy studies. The enhancement in permeability and decrease in resistance de-

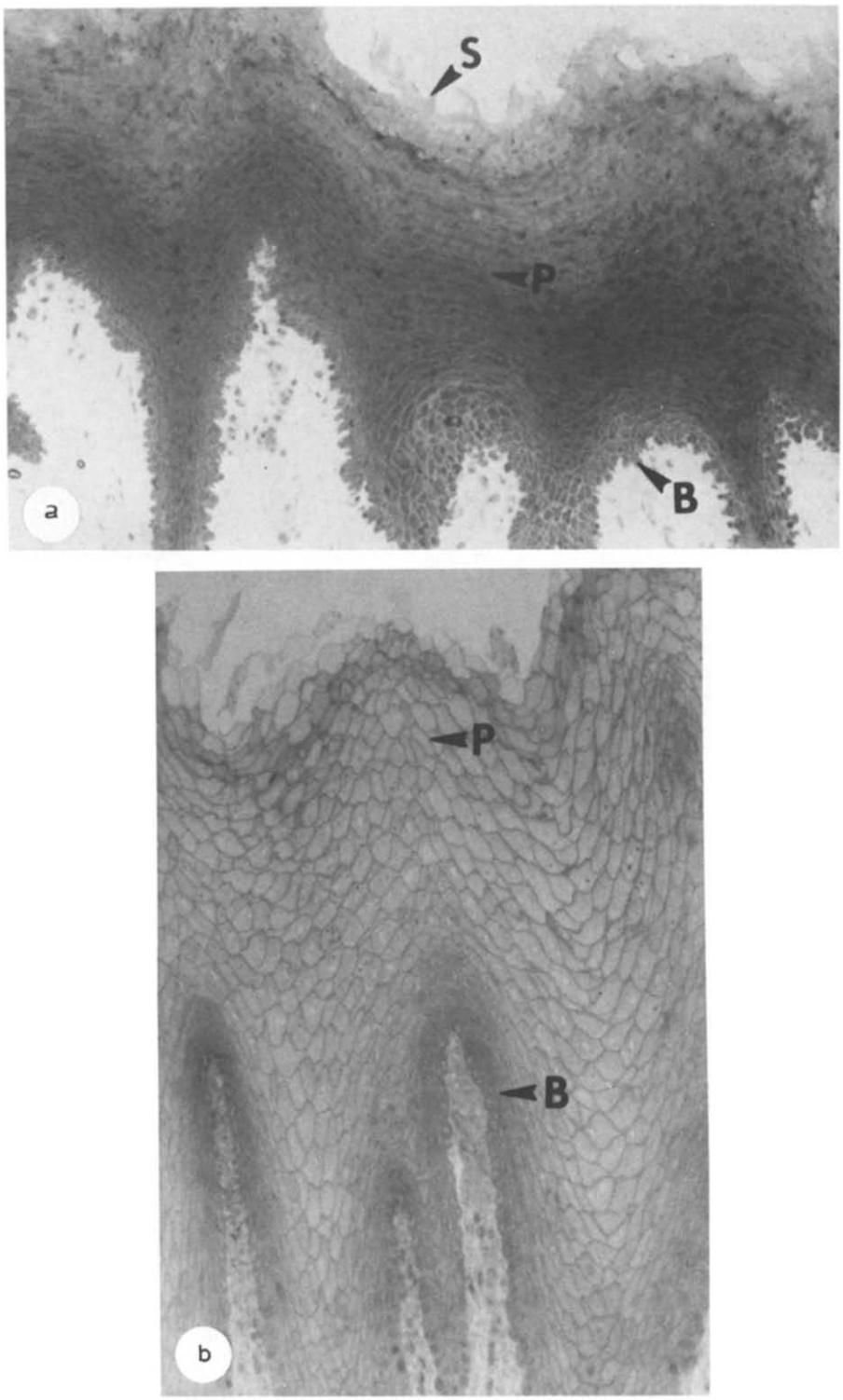

Fig. 6. (a) Light microscopy ot rabbit buccal mucosa incubated in Hepes buffer for 4 h. (S) Superficial cell; (P) prickle cell; (B) basal cell. (b) Light microscopy of rabbit buccal mucosa incubated for 4 h with 0.5% sodium deoxycholate in Hepes buffer. Note the absence of superficial layers. (P) Prickle cell, (B) basal cell. (c) Light microscopy of rabbit buccal mucosa incubated for 4 h with 0.1% w/v sodium lauryl sulfate in Hepes buffer. Note the absence of superficial layers. (P) Prickle cell; (B) basal cell.

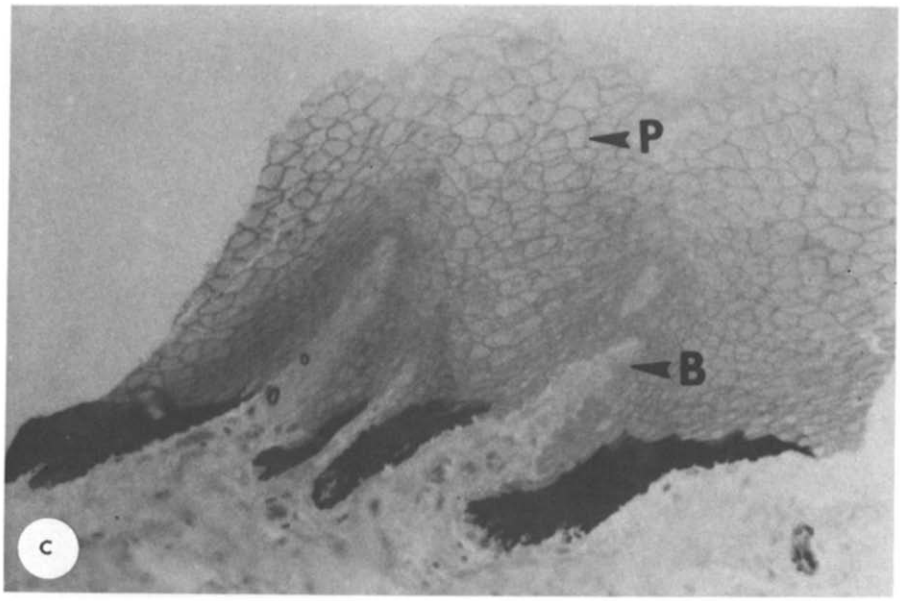

Fig. 6c.

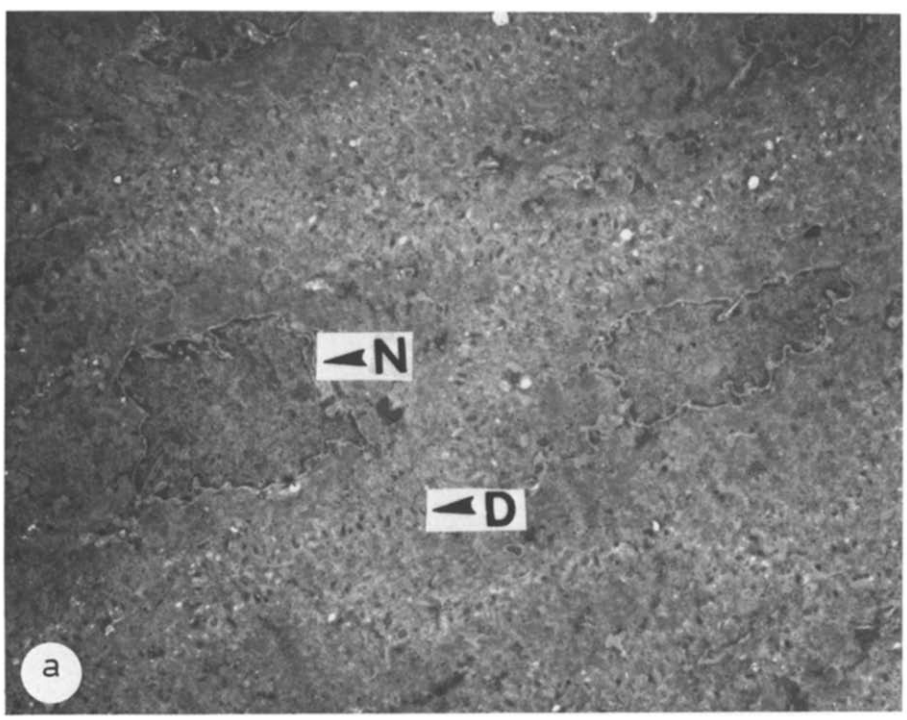

Fig. 7. (a) TEM of rabbit buccal mucosa incubated in Hepes buffer for 4 h (magnification, 3500 ×) showing prickle cells. (N) Nucleus; (D) desmosomes. (b) TEM of rabbit buccal mucosa after treatment with 0.5% sodium deoxycholate in Hepes buffer for 4 h (3500 ×) showing prickle cells. (N) Nucleus; (I) intercellular space. (c) TEM of rabbit buccal mucosa after treatment with 0.1% w/v sodium lauryl sulfate in Hepes buffer for 4 h (3500 ×) showing prickle cells.

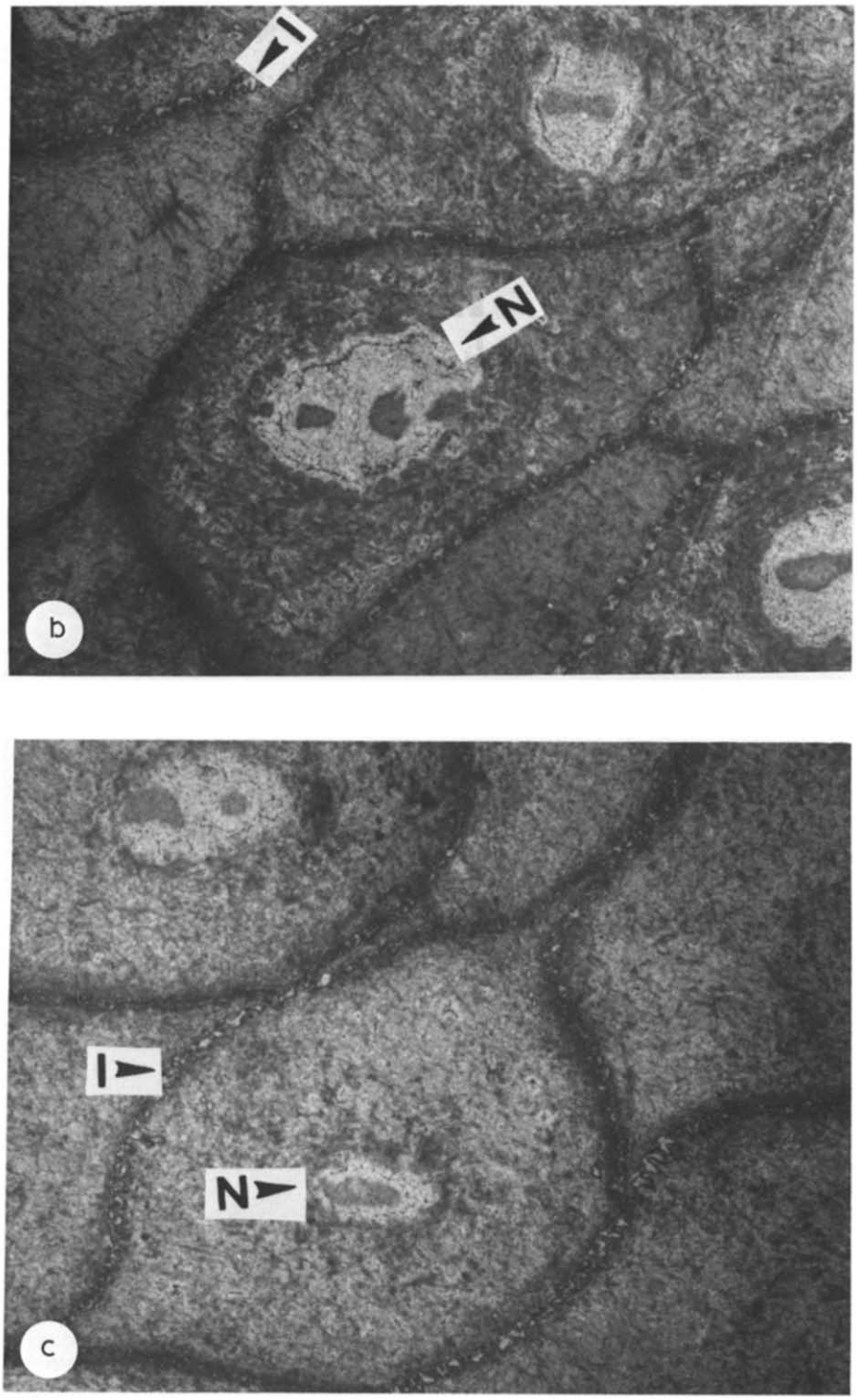

Fig. 7b, c.

pend not only on the specific penetration enhancer used and its concentration but also on the time of exposure. Hence, one can optimize concentration and time of exposure to achieve a sufficient increase in permeability without causing gross damage to the tissue.

Fig. 6 shows a section of tissue at the light microscope level after incubation with Hepes buffer, pH 7.4 for 4 h. In the case of the control, one observes the presence of superficial cells which are markedly flattened, followed by prickle cells and finally cuboidal basal cells. Fig. 6b displays a section of tissue at the light microscope level after treatment for 4 h with 0.5% w/v sodium deoxycholate on the epithelial side. Fig. 6c shows a section of tissue incubated with 0.1% w/v sodium lauryl sulfate on the epithelial side for 4 h. Compared to the control the treated tissues show extensive loss of the surface epithelial layers. Assuming the epithelial cells constitute a major component of the permeability barrier, the loss of cells could readily account for the increase in permeability. The light microscope pictures clearly show that the superficial cell layer has been removed. Since this treatment with enhancer increases permeability and decreases resistance, it appears that the superficial layer is a major barrier to permeation of drugs across the buccal epithelium. These findings are in accordance with the observation of Squier and coworkers (Squier, 1973; Squier and Rooney, 1976). In their work they found that the transport of horseradish peroxidase and lanthanum, both of which are hydrophilic, did not penetrate deeper than one to three layers. When introduced subepithelially they penetrated through the intercellular spaces into and up to the prickle cell layer. Kaaber (1974) observed keratinized and non-

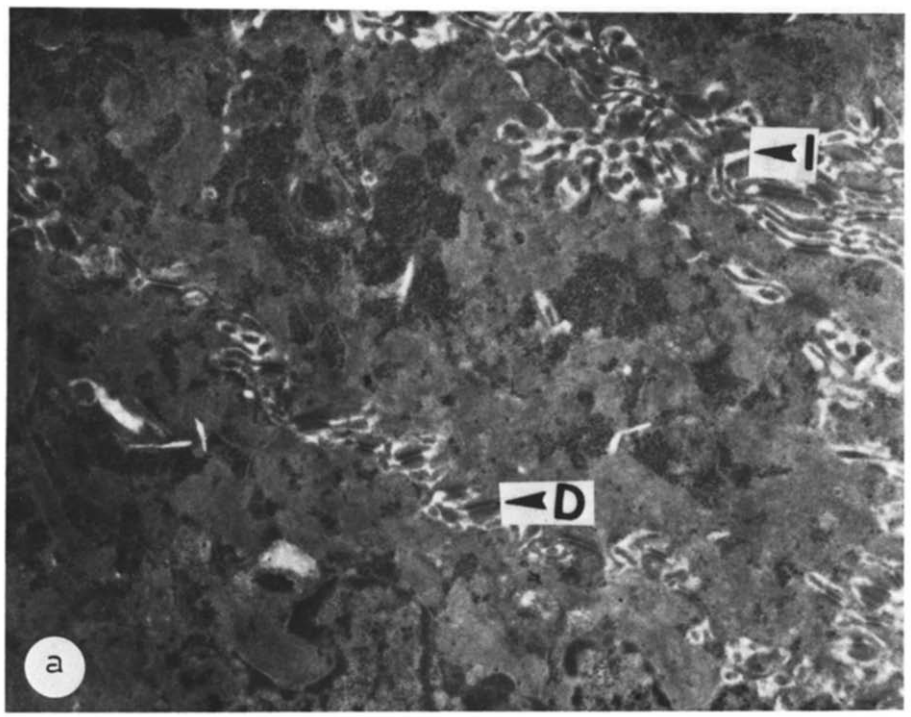

Fig. 8. (a) TEM of rabbit buccal mucosa incubated in Hepes buffer for 4 h (magnification 8000 ×) showing prickle cells. (I) Intercellular space; (D) desmosomes. (b) TEM of rabbit buccal mucosa after treatment with 0.5% sodium deoxycholate in Hepes buffer for 4 h (8000 ×) showing prickle cells. Note the increase in intercellular space (I) and absence of desmosomes. (c) TEM of rabbit buccal mucosa after treatment with 0.1% sodium lauryl sulfate in Hepes buffer for 4 h (8000 ×) showing prickle cells. Note the increase in intercellular space (I) and absence of desmosomes.

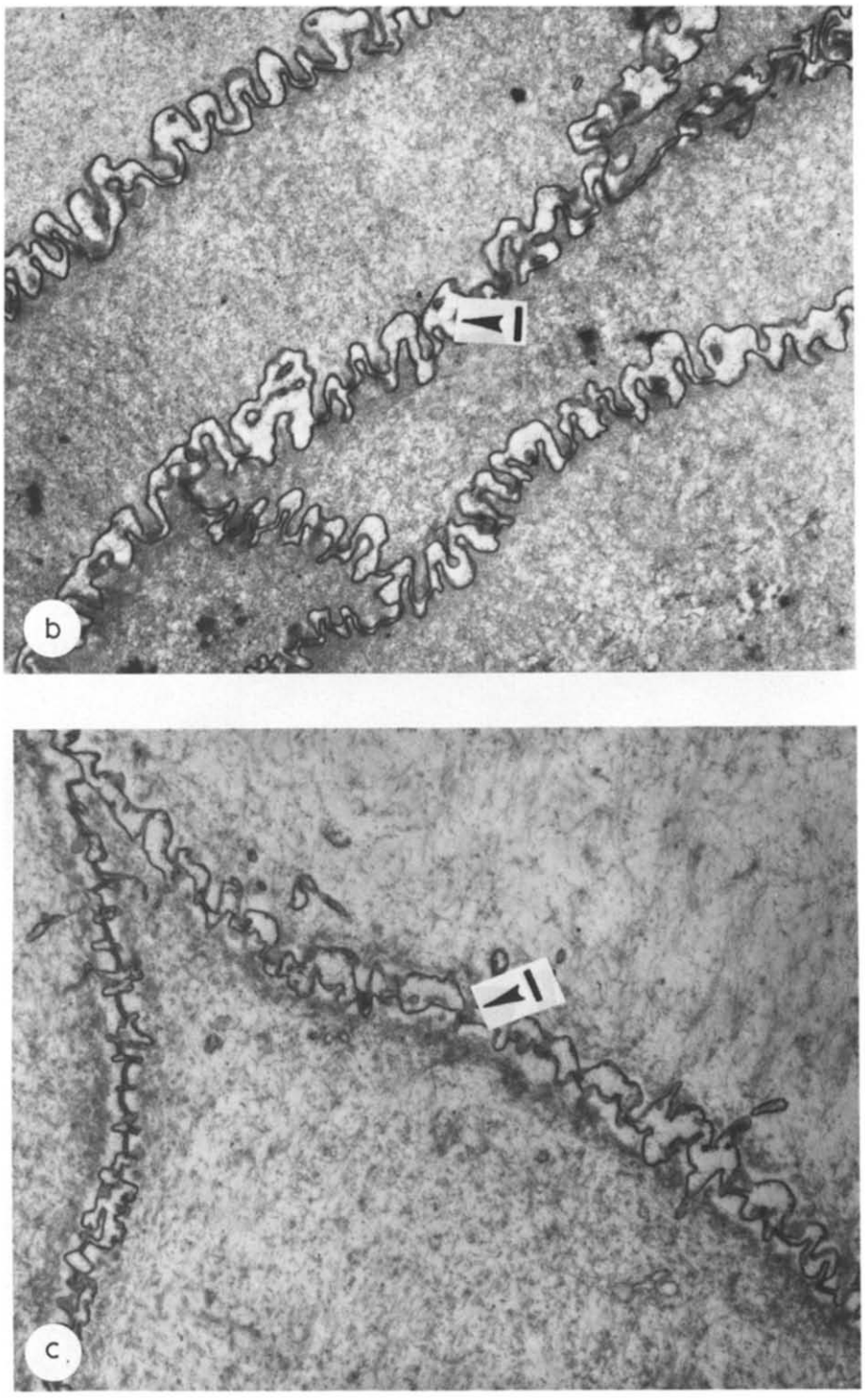

Fig. 8b, c.

keratinized epithelia to be equally impermeable to electrolytes which, like peroxidase, tend to pass through intercellular spaces (Middleton, 1969). This intercellular route, especially in deeper cell layers, is essentially an aqueous channel. Although the intercellular space is only a minor component of the tissue volume, it may nevertheless provide the pathway by which electrolytes and lipid-insoluble substances traverse the tissue (Tregear, 1966) provided they can overcome the intercellular barrier in the superficial layer.

Fig. 7a and c shows electron micrographs of prickle cells of control and penetration enhancer treated tissue. From these pictures together with those at the light microscopy level, it appears that the treatment causes gross swelling of the cells. Also the nuclei and cytoplasm in the treated cells appear less electron dense compared to controls, suggesting the extraction of proteins. This is in agreement with the results of the DSC studies, where the protein endotherms disappear. Fig. 8a–c displays electron micrographs of the same tissue at higher magnification. It is clear that both treatments with enhancers increase the intercellular space significantly. Furthermore, it is evident from these electron micrographs that the penetration enhancers affect junctional elements like desmosomes. In addition, association of the actin filaments may also increase paracellular permeability (Madara et al., 1986).

The observation that these penetration enhancers can damage the superficial cells at the concentrations used indicates that the use of these enhancers to increase permeability is not without risk, since it is likely that the superficial layer serves as a defense against any infecting organism. While repair of minor damage to the mucosa by reepithelization may be expected in vivo, it might not be a very fast process. Hence, the use of these enhancers must be optimized in terms of applied location, concentration and time as well as the associated recovery time for transbuccal administration of therapeutic agents.

Conclusions

Treatment of buccal epithelium with penetration enhancers such as 0.5% w/v sodium deoxycholate and 0.1% w/v sodium lauryl sulfate decreased the electrical resistance and increased the flux of salicylic acid across buccal mucosa. The results of DSC studies suggest that these penetration enhancers affect the protein domain of the tissue under these experimental conditions. Light and electron microscopy studies appear to support the hypothesis that the superficial layer may be a major barrier to penetration of salicylic acid, and further that the influence of effective penetration enhancers is on the superficial part of the tissue. The mechanism of action of penetration enhancers is mostly due to tissue swelling and an increase in the intercellular space causing uncoiling and extension of protein helices, thereby opening up the polar pathway.

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