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Localization of the permeability barrier inside porcine buccal mucosa: a combined in vitro study of drug permeability, electrical resistance and tissue morphology

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Summary

In vitro drug transport and morphological studies were performed on porcine buccal mucosa, in order to localize the permeability barrier inside this tissue. As model compounds, two β-blocking agents (acebutolol and bupranolol) were used with similar pK_a and molecular weight values, but with varying hydrophilicity. Porcine buccal mucosa was used, from which the connective tissue was removed by mechanical separation. The splitting procedure permitted the use of epithelial tissue layers of different thicknesses for diffusion experiments and measurements of electrical resistance. Both series of experiments showed that the major barrier to drug transport is not the buccal epithelial layer itself, but rather a deeper tissue layer. Morphological data showed that only the least permeable tissue samples, cut at maximal thickness (0.8 mm), included the entire basal lamina. A similar set of experiments has been performed using EDTA-split epithelium. The permeability and electrical resistance of these epithelia were much greater than for dermatomed tissue. Immunofluorescence microscopy revealed that upon EDTA splitting, the basal lamina remained attached to the connective tissue layer. In all experiments, a semiquantitative correlation was observed between permeability and electrical resistance of the buccal mucosa on the one hand and the degree of integrity of the basal lamina on the other. From the data it was concluded that most likely the basal lamina determines buccal permeability.

Introduction

In order to investigate alternative routes of delivery of metabolically unstable drugs, for instance, peptides, studies are being performed on the permeability of buccal mucosa (Merkle et al., 1986; Nagai and Konishi, 1987; Veillard et al., 1987). In a cross-section of buccal mucosa, the following strata can be distinguished: an upper layer of squamous epithelium, covered with mucus and, at the proximal end, bound to a connective tissue layer, called the lamina propria, through the so-called basal lamina, which sepa-

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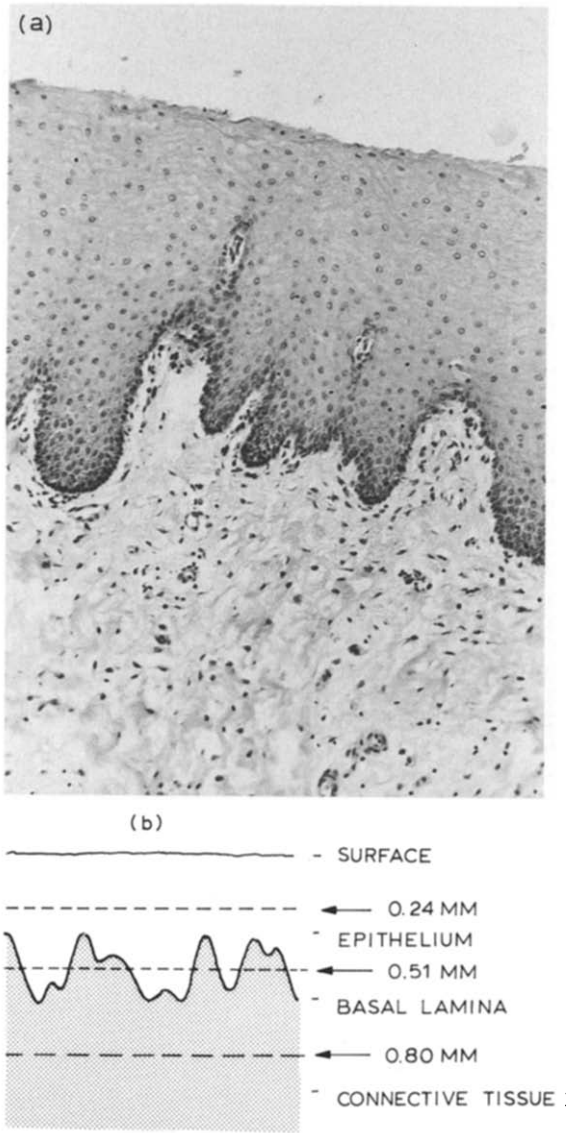

Fig. 1. (a) Photomicrograph of a cross-section of porcine buccal mucosa. (b) Schematic cross-section of porcine buccal mucosa, in which the effect of dermatomizing at various depths is illustrated.

rates the two layers (Fig. 1a). The epithelium serves as a barrier protecting the underlying tissues, whereas the lamina propria acts as a mechanical support and also carries blood vessels and nerves.

For studying drug absorption in non-keratinized tissue, monkeys (Nagai and Konishi, 1987), rabbits (Eggerth et al., 1987) and pigs (Squier, 1986) have been used. In contrast to keratinized tissue, in which the keratinized upper layer has been proven to be the major barrier to drug absorption (Eggerth et al., 1987), it is difficult, and it might be even incorrect, to pinpoint a single element of the non-keratinized buccal tissue as being the major determinant of its barrier properties. On the basis of present knowledge, the entire epithelium, part of it, or the basal lamina come into consideration.

In order to localize the absorption barrier within the buccal mucosa, Squier and Hall (1985a,b) visualized the presence of absorbed horse radish peroxidase (HRPO) in porcine oral mucosae. HRPO appeared to be present in the upper third part of the epithelium, corresponding to the presence of intercellular glycoproteinaceous material, extruded by membrane coating granules. They therefore concluded that these organelles play a major role as a barrier system. However, it should be noted that this approach merely revealed HRPO binding tissue components rather than a (major) tissue permeation barrier. Furthermore, they did not study the absorption vs time.

Recently, many investigations on the localization of the transport barrier have been performed using rabbit buccal mucosa, which is supposed to be non-keratinized. The results also suggest that the barrier is found in the upper third part of the epithelium, and corresponds with the presence of membrane coating granules (Squier, 1973; Squier and Rooney, 1976; Dowty et al., 1990; Knuth et al., 1990). However, in evaluating these results, it should be borne in mind that, according to Squier (1973), 'frequently the non-keratinized epithelium presented a histological appearance tending towards parakeratinization, particularly in rabbit cheek'!

The basal lamina may act as a barrier to the passage of immune complexes (Brandtzaeg and Tolo, 1977) and of endotoxin (Alfano et al., 1975). In vitro studies utilizing organ culture techniques (Alfano et al., 1977) have demonstrated that the basal lamina is an important barrier to the trans-

port of inulin (MW 5000) and of dextran 70 (MW 70 000), but not to that of dextran 20 (MW 20 000).

For in vitro absorption studies two compartment diffusion cells, with buccal tissue clamped inside, are being used. The advantage of such cells is that the amount of drug actually transported through the tissue can be determined. Various types of diffusion cells exist: Eggerth et al. (1987) used standard Franz^R diffusion cells. Le Brun et al. (1989) used a two-chambered flow-through diffusion cell, developed by Tiemessen et al. (1988) and made of teflon. Probably the most suitable diffusion cells for these studies are Ussing chambers, in which both the donor and acceptor phases can be provided with (carbogen) gas which ensures circulation of the solutions and — in combination with Krebs-Ringer solution — viability of the tissue (Grass and Sweetana, 1988).

In the present article, an attempt was made to localize the major barrier tissue structure within porcine buccal mucosa. In earlier experiments the epithelial layer of porcine buccal tissue was separated from the underlying tissue by a chemical splitting procedure. In the present study it was decided to separate the epithelium from the underlying tissue mechanically using a dermatome; this mechanical slicing method made it possible to use epithelial tissue layers with varying thicknesses and structures (see Fig. 1b). As diffusion cells Ussing chambers were used. Morphological studies using light microscopy and immunofluorescence microscopy were performed in order to evaluate the tissue structure. As model drugs for permeation experiments, acebutolol · HCl and bupranolol · HCl were used, respectively. These are respectively the least and most lipophilic compounds out of a range of β-blockers used in a previous study (Le Brun et al., 1989). Their molecular weights and pK_a values are comparable. In addition to diffusion experiments, electrical resistance measurements on similar tissue samples were performed to investigate a possible correlation between these values and the permeability data. Moreover, the electrical resistance across EDTA-split epithelium was measured in order to determine to what extent the splitting procedure damages the barrier. Immunofluorescence microscopy was used to study the fate of the basal lamina as a result of this splitting procedure.

Materials and Methods

Tissue preparation

The buccal tissue of castrated male Great Yorkshire × Dutch Landrace pigs (approx. 40–70 kg; provided by Research Institutes) was excised directly after slaughter and kept in Krebs buffer solution during transport. Most of the underlying tissue was removed from the mucosa with a surgical scalpel. With an electrodermatome (Padgett Dermatome, Div. of Kansas City Ass. Co., Kansas City, U.S.A.), the epithelium was separated from the rest of the underlying tissue. The tissue was dermatomed at 0.24, 0.51 and 0.80 mm. These mucosal preparations were used within 3 h upon slaughter. Krebs buffer solution (pH 7.4) consisted of 6.75 g NaCl, 0.31 g KCl, 1.84 g $NaHCO_3$, 2.2 g dextrose · H_2O, 0.95 g Hepes, 0.286 g $MgSO_4 \cdot H_2O$, 0.368 g $CaCl_2$ and 0.249 g $NaH_2PO_4 \cdot 2H_2O$ per l and was saturated with carbogen gas. All ingredients were analytical grade.

For electrical resistance measurements, similarly dermatomed tissue layers as well as chemically split epithelium were used. For chemical splitting, samples were treated using 0.02 M EDTA in phosphate-buffered saline (PBS), pH 7.4, for 50 min at 60 °C as in former studies (Le Brun et al., 1989) or in a modified version for only 25 min. Alternatively, some epithelial samples were split by thermal treatment only (25 min in Krebs buffer at 60 °C).

Morphological studies

For morphological studies, dermatomed tissue samples of 1 mm^2 were initially fixed in Bouien buffer solution and subsequently embedded in Spurr's resin. From the samples 2–4 μm sections were cut using a microtome (LKB), equipped with a glass knife. The sections were stained with toluidine blue, and examined under a light microscope (Olympus) (see Fig. 2a–c). From the 0.51

mm sample, a horizontal section was also cut (Fig. 2d).

1 mm^2 chemically split epithelia and matching connective tissue samples were cryofixed in Tissue Tek (Miles Inc.) at $-70\,^{\circ}C$; 5 μm sections were excised using a cryomicrotome. Subsequently, the sections were thawed, treated with acetone, rinsed with PBS, and incubated with human collagen VII antibody. Fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse antibody was used as the second antibody (Leigh et al., 1987). Subsequently, the sections were mounted in FA mounting fluid (Difco) and examined under a fluorescence microscope (Leitz photomicroscope).

Diffusion experiments

The mucosal preparations were placed in an Ussing chamber (diffusion area 1.77 cm^2). Krebs buffer solution was used both as donor and acceptor medium. Acebutolol · HCl and bupranolol · HCl were gifts from Schwarz Pharma AG (Monheim, Germany). The donor concentrations were 4 and 2 mg/ml, respectively. The system was equilibrated with Krebs buffer for at least 1 h prior to a diffusion experiment. The buffer was replaced before the donor and acceptor solutions were added. The diffusion cells were placed in a water bath at $35\,^{\circ}C$. Samples of 800 μl were taken manually from the acceptor fluid at 30-min intervals, each time replenishing the acceptor medium with the same amount of fresh buffer solution.

Drug analysis

The fractions were analyzed as follows (Duchateau et al., 1986): to 800 μl acceptor solution 400 μl methanol p.a. (Merck) was added.

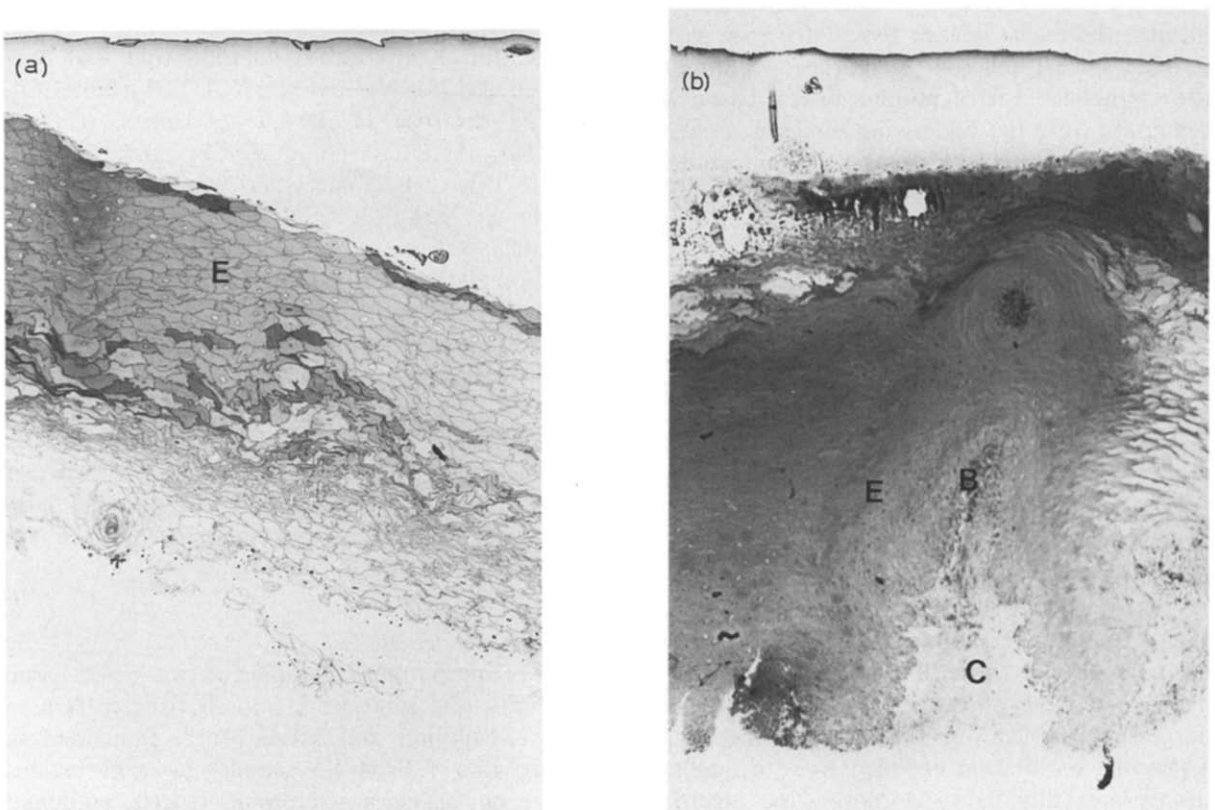

Fig. 2. Photomicrographs of cross-sections of porcine buccal mucosa that has been dermatomed at 0.24 mm (a), 0.5 mm (b) and 0.8 mm (c) showing the epithelial layer (E), the basal lamina (B) and connective tissue (C). (d) Photomicrograph of a horizontal section of porcine buccal mucosa which has been cut at 0.5 mm, showing cross-sectioned papillae (180 ×).

The mixture was vortexed and centrifuged to precipitate the Hepes salt from the Krebs buffer. The supernatant was injected on a C-18 reversed-phase HPLC column and UV detection performed: [eluent (0.7 ml/min)] acebutolol: 85:15 v/v buffer/acetonitrile, 235 nm; bupranolol: 70:30 v/v buffer/acetonitrile, 220 nm; [buffer] 1.33% triethylamine + conc. phosphoric acid at pH 5.

Calculation of diffusion resistance

If the epithelium were a homogeneous barrier to drug diffusion, the permeability coefficient (P) would be inversely proportional to the thickness of the membrane (h).

$$P = \text{constant} \times h^{-1} \quad (1)$$

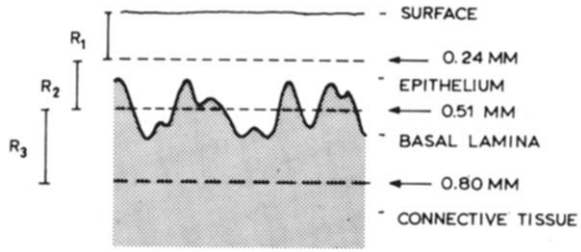

Fig. 3. Representation of R as the sum of R_1, R_2 and R_3.

Alternatively, a tissue layer can be considered to act as a diffusion resistance R. Bearing in mind that the tissue permeability coefficient P is inversely proportional to the tissue diffusion resistance R, it is evident that R is proportional to tissue thickness (h).

$$R = \text{constant} \times h \quad (2)$$

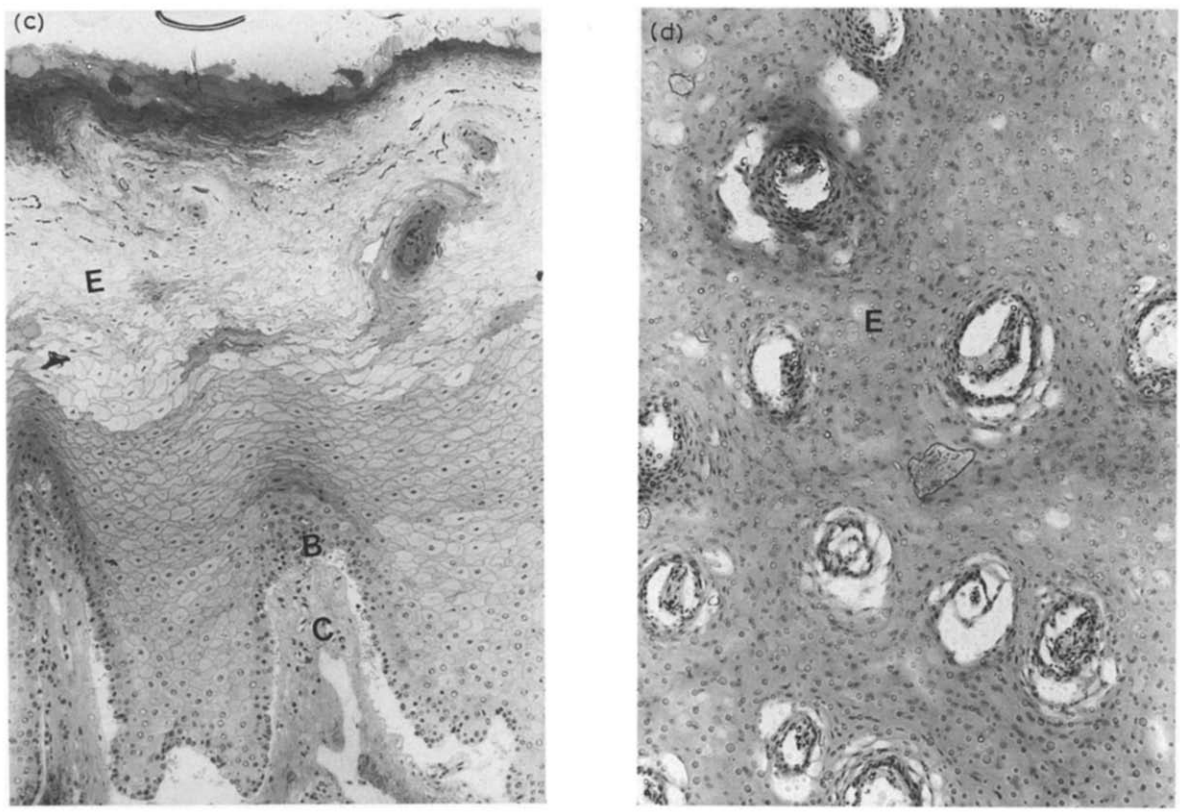

Fig. 2 (c,d).

Since, in principle, diffusional resistances are additive

$$R = R_1 + R_2 + R_3 \qquad (3)$$

separate diffusion resistances can be calculated for each additional tissue layer (see Fig. 3). All results from the diffusion experiments are expressed as diffusion resistance data.

Electrical resistance measurements

Tissue layers were clamped into Ussing chambers. The chambers were filled with Krebs buffer (pH 7.4) and provided with Ag-AgCl electrodes, positioned on either side of the mucosal slab. The electrical resistance across the membranes was measured using a pulsed current source in combination with a computer-controlled resistance measurement unit (developed in the Central Electronic Department of our laboratory). The block-shaped current had a duty cycle of 50%, a frequency of 100 Hz, and a mean current density of 0.0028 mA/cm^2. The duration of each measurement was less than 5 s so as to minimize any effect of the current on the integrity of the tissue and hence on its electrical resistance. The frequency was sufficiently low to allow the resulting potential drop across the membranes to reach its maximal level. The resistance was calculated as the quotient of the voltage-amplitude and current-amplitude.

Results and Discussion

Dermatomed tissue

Diffusion experiments — acebutolol

A set of flux curves of acebutolol is shown in Fig. 4. It is apparent that the flux decreases with increasing tissue thickness. Results from different pigs show a similar correlation but the variability in the absolute values is rather large. Presumably, this is due to the fact that the tissue is no longer completely viable. Although the tissue was kept in Krebs buffer and provided with oxygen, the viability of different tissue samples must have decreased to a different extent.

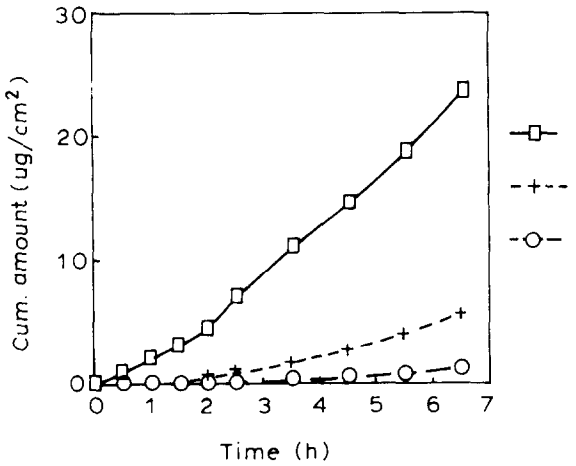

Fig. 4. Results of an acebutolol diffusion experiment: fluxes across tissue of one animal dermatomed at 0.24 (□), 0.51 (+) and 0.8 (◇) mm.

Therefore, it was decided to calculate the relative permeability coefficient, separately, for each pair of tissue samples from the same animal, and hence, the relative diffusion resistance (R_{rel}):

$$R_{rel} = \frac{R_{0.24}}{R_{0.8}} \qquad (4)$$

where $R_{0.8}$ and $R_{0.24}$ represent the diffusion resistances of epithelial tissue membranes that have been dermatomed at 0.8 and 0.24 mm thickness, respectively, from the same animal. In this way, results from different pigs could be compared. The results of these calculations are plotted vs tissue thickness in Fig. 5a, from which it is evi-

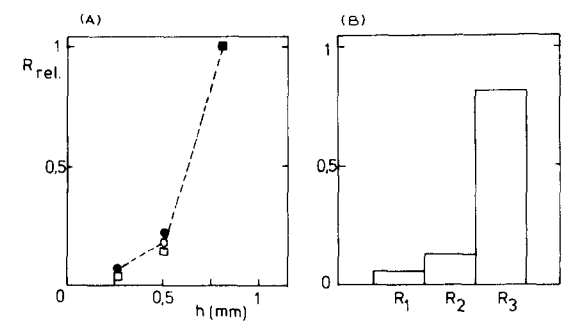

Fig. 5. (a) Plot of R_{rel} vs tissue thickness for three pigs for acebutolol. (b) Plot of R_1, R_2 and R_3 for acebutolol; mean values, calculated from panel a (see text for details).

dent that the effect of increasing the tissue layer thickness is similar for different animals. It is noteworthy that the increase in the diffusional resistance is non-linear with increasing tissue thickness. Fig. 5b shows the separately calculated diffusion resistances R_1, R_2 and R_3.

From Fig. 5a, it can be seen that reducing the thickness of the diffusion membrane by a factor of 2 does not result in a reduction of the diffusion resistance by the same factor. Hence, a relatively large absorption barrier must be present in the thickest dermatomed tissue. This point is illustrated more clearly when the separate diffusion resistances are considered (see Fig. 5b). Once again, it is evident that R_3, representing the contribution of the 'deepest' tissue layer as a diffusion barrier, is much larger than R_1 or R_2, underlining the aforementioned non-linearity.

Diffusion experiments — bupranolol

In Fig. 6, a set of flux curves of bupranolol is shown. The absolute permeability for bupranolol was larger than for acebutolol. Moreover, a non-linear relationship between diffusion resistance and tissue thickness was observed, as was the case for acebutolol. However, when the diffusion resistances for acebutolol are compared to those for bupranolol by calculating their ratio

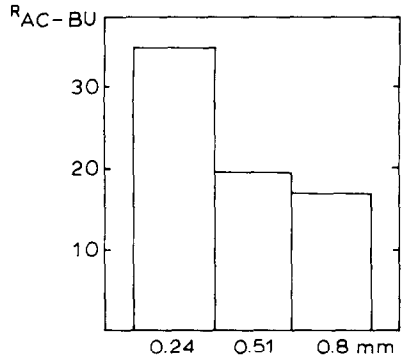

Fig. 7. Ratio of diffusion resistances of acebutolol and bupranolol ($R_{Ac\text{-}Bu}$) at various tissue thicknesses.

('$R_{Ac\text{-}Bu}$' = R_{Ac}/R_{Bu}) at various tissue thicknesses, a remarkable difference is noticed (Fig. 7). Apart from the observation that the diffusion resistance for bupranolol is much lower than for acebutolol, indicating that the buccal mucosa is more permeable for lipophilic compounds, the ratio R_{Ac}/R_{Bu} appears to decrease to values around 16–18 for the 0.5 and 0.8 mm dermatomized tissue, whereas the ratio exceeds 30 for the 0.24 mm cut tissue. This observation suggests that a relatively hydrophilic barrier is present in the deeper tissue layers as compared to the upper mucosal layer.

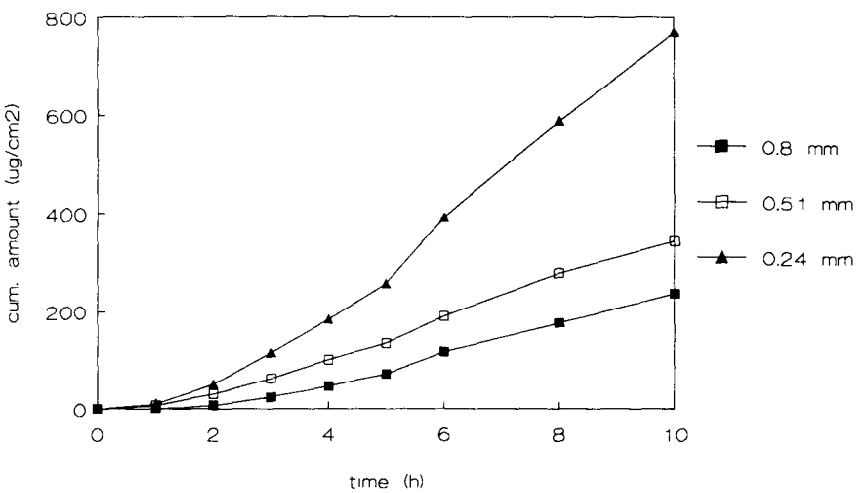

Fig. 6. Results of a bupranolol diffusion experiment: fluxes across tissue of one animal dermatomed at 0.24 (▲), 0.51 (□) and 0.8 (■) mm.

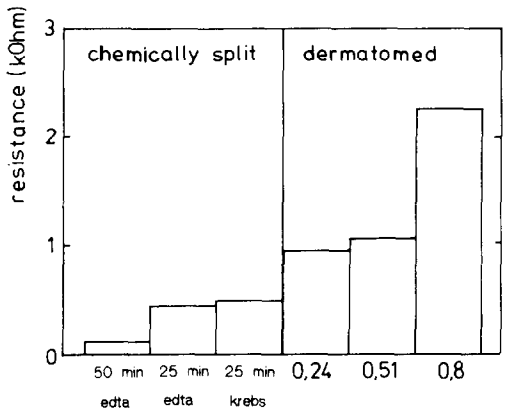

Fig. 8. Electrical resistance of epithelial slabs from one animal plotted vs dermatoming depth and splitting procedure.

Electrical resistance

The results of the electrical resistance measurements are shown in Fig. 8. From the right-hand part of the plot, it appears that the thickest tissue corresponds to a relatively high resistance and that resistance is not linearly proportional to tissue thickness (i.e. dermatomizing depth).

The results of the electrical resistance measurements on dermatomed tissue layers show a non-linearity with increasing tissue thickness similar to those of the diffusion experiments: a relatively large resistance shows up in the thickest membrane, indicating that the deeper tissue layers make a relatively large contribution to the total resistance. This result indicates that the major absorption barrier for ionic species might be the same as for the β-blockers (which are also ionic at pH 7.4). Since ion transport takes place along the intercellular hydrophilic pathways while β-blocker transport is presumed to be (at least partly) transcellular, this particular barrier might be the basal lamina which forms a similar proteinaceous barrier to both types of permeants.

Morphological studies

Fig. 2a–c shows photomicrographs of dermatomed buccal mucosae, cut at 0.24, 0.5 and 0.8 mm, respectively. As is evident, tissue layers of different compositions have been obtained. Dermatoming at 0.24 mm yields samples which consist of epithelial cells only. Tissue cut at 0.5 mm shows cross-sectioned papillae and some connective tissue whereas the thickest tissue samples contain the entire epithelium with intact papillae and thus the complete basal lamina. Fig. 2d represents a horizontal section of such a sample made at 0.5 mm; the relatively tight epithelial cells surround 'holes' of loose connective tissue at the sites where the papillae have been cross-sectioned. Thus, only by dermatoming porcine buccal mucosa at 0.8 mm are tissue slabs obtained that contain both the complete epithelium and the intact (i.e. not cross-sectioned) basal lamina, which separates the epithelium from the underlying connective tissue. Dermatoming at 0.24 or 0.5 mm yields slabs containing epithelium only or epithelium with a cross-sectioned (i.e. damaged) basal lamina as well, respectively.

Chemically split epithelium

In the left-hand part of Fig. 8, the results of the electrical resistance measurements are plotted vs splitting procedure: irrespective of the splitting method used, the resistance of the residual epithelium is much lower than of intact (i.e. untreated) tissue. Apparently, all splitting procedures, even the 'mild' thermal splitting procedure without EDTA, affect the barrier function of buccal tissue.

Fig. 9a shows a light-microscopic image of an FITC-immunolabelled cross-section of intact buccal mucosa. The deposition of collagen VII reflects the location of the basal lamina. Fig. 9b shows an uninterrupted fluorescent lining along the upper surface of the lamina propria, indicating the presence of the basal lamina. The image was taken after the lamina propria had been split chemically (EDTA) from the epithelium; the remaining epithelium did not show any fluorescence (image not shown).

Obviously, upon chemically splitting the tissue the basal lamina has been removed from the epithelium, together with the connective tissue. Therefore, the epithelial slabs used for in vitro diffusion experiments can be considered to contain only lipoidal barriers. Moreover, the epithelial desmosomes will no longer function properly due to chelation of Ca^{2+} and hence cause the

epithelium to be a cellular membrane with enlarged hydrophilic intercellular pathways. Previous penetration data from experiments with β-blockers performed in our laboratory (Le Brun et al., 1989) showed that chemically split porcine buccal epithelium is more permeable to β-blocking agents than dermatomed tissue. Obviously, the treatment with EDTA facilitates the penetration of β-blockers by damaging or removing an important part of the absorption barrier.

Comparison of the diffusion resistances for acebutolol and bupranolol suggests that there must be a relatively hydrophilic barrier in the deeper tissue layers; since it is unlikely that the loose, hydrophilic connective tissue acts as a barrier, these findings together with the results of the electrical resistance measurements and morphological studies strongly suggest that the basal lamina, being mainly proteinaceous (Odland et al. 1967), presents a major barrier to absorption. Although the experiments reported by Squier and Hall (1985a,b) and Squier (1973) showed that the water-soluble HRPO, when injected subepithelially, passes the basal lamina easily, our results correlate well with their findings in stripping experiments. Upon stripping porcine buccal mucosa, the permeability of the tissue did not increase, indicating that the tissue barrier remained intact.

In earlier investigations (Le Brun et al., 1989) a log-log linearity was observed for permeability and partition coefficients within a range of β-blockers that led to the conclusion that the epithelial membrane behaves as a lipoidal barrier. This conclusion is completely in agreement with the present finding that upon chemical splitting

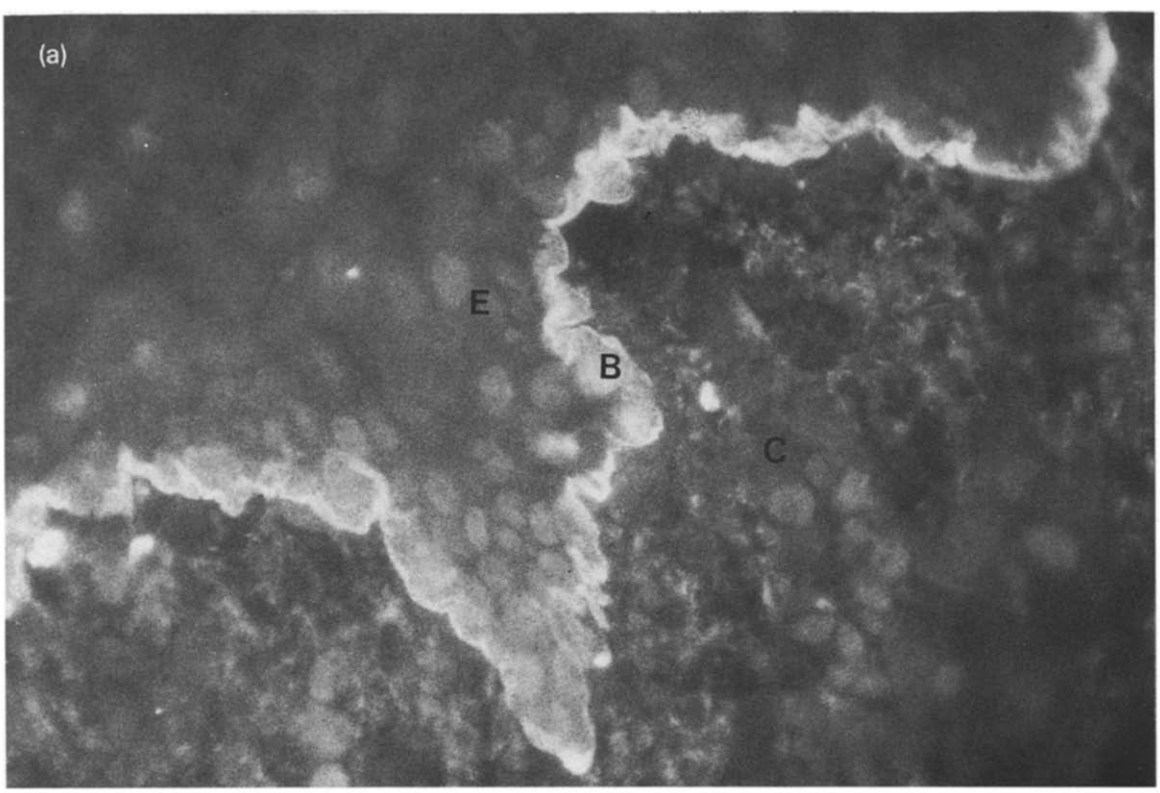

Fig. 9. Photomicrograph of FITC-collagen VII antibody labeled porcine buccal mucosa, showing the location of the basal lamina in intact mucosa (a) and in chemically split connective tissue (b) (E, epithelium; B, basal lamina; C, connective tissue).

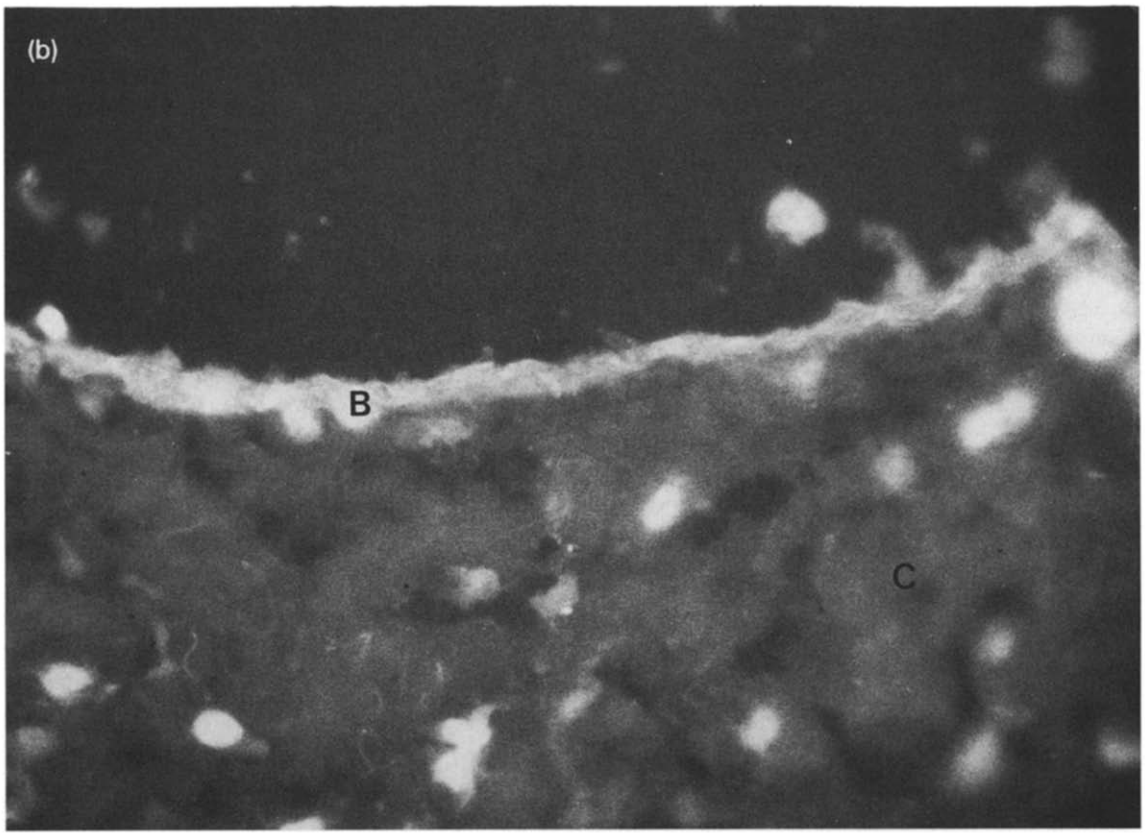

Fig. 9 (b).

the epithelium is reduced to a hydrophobic barrier after having lost its hydrophilic basal lamina.

Conclusions

The most important conclusion from this work is that, on combining the permeabilities, electrical resistances and morphologies of porcine buccal mucosae, sliced or split in various ways, results were obtained which strongly indicate that the basal lamina presents a major (proteinaceous) transport barrier. The basal lamina does not entirely determine the barrier function of the buccal mucosa; the lipoidal barrier, formed by the upper layer epithelium acts in conjunction with it. The relative contribution of either of these two most likely depends on the molecular structure of the permeant, as well as on the degree of integrity of the tissue.

Furthermore, we conclude that the chemical splitting procedure and the milder thermal splitting procedure, without EDTA, are convenient methods to obtain epithelial slabs, but that both appear to damage the barrier function of the mucosa, partly due to the fact that the basal lamina is being removed from the epithelial layer.

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References

Alfano, M.C., Chasens, A.I. and Masi, C.W., Autoradiographic study of the penetration of radiolabeled dextrans and inulin through non-keratinized oral mucosa in vitro. *J. Periodont. Res.*, 12 (1977) 368–377.

Alfano, M.C., Drummond, J.F. and Miller, S.A., Localization of rate-limiting barrier to penetration of endotoxin through non-keratinized oral mucosa in vitro. *J. Dent. Res.*, 54 (1975) 1143–1148.

Brandtzaeg, P. and Tolo, K., Mucosal penetrability enhanced by serum-derived antibodies. *Nature*, 266 (1977) 262–263.

Dowty, M.E., Irons, B.K., Knuth, K.E. and Robinson, J.R., Characterization of transport pathways of thyrotropin releasing hormone in rabbit buccal mucosa. *Proc. Int. Symp. Control. Rel. Bioact. Mater. (Reno)*, 17 (1990) 226–227.

Duchateau, G.S.M.J.E., Zuiderma, J., Albers, W.M. and Merkus, F.W.H.M., Nasal absorption of alprenolol and metoprolol. *Int. J. Pharm.*, 34 (1986) 131–136.

Eggerth, R.M., Rashidbaigi, Z.A., Mahjour, M., Goodhart, F.W. and Fawzi, M.B., Evaluation of hamster cheek pouch as a model for buccal absorption. *Proc. Int. Symp. Control. Rel. Bioact. Mater. (Toronto)* (1987) 14.

Grass, G.M. and Sweetana, S.A., In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.*, 5 (1988) 372–376.

Knuth, K.E., Dowty, M.E. and Robinson, J.R., Biochemical characterization of rabbit buccal mucosa. *Proc. Int. Symp. Control. Rel. Bioact. Mater. (Reno)* 17 (1990) 297–298.

Le Brun, P.P.H., Fox, P.L.A., De Vries, M.E. and Boddé, H.E., In vitro penetration of some β-adrenoreceptor blocking drugs through porcine buccal mucosa. *Int. J. Pharm.*, 49 (1989) 141–145.

Leigh, I.N., Purkis, P.E. and Bruckner-Tuderman, L., LH-7.2 monoclonal antibody detects tight-VII collagen in the sublamina densa zone of ectodermally-derived epithelia, including skin. *Epithelia*, 1 (1987) 17–29.

Merkle, H.P., Anders, R., Sandow, J. and Schurr, W. Drug delivery of peptides: the buccal route. In Tomlinson, E. and Davis S.S. (Eds) '*Delivery Systems for Peptide Drugs*', Plenum, New York (1986) pp. 159–175.

Nagai, T. and Konishi, R., Buccal/gingival drug delivery systems. *J. Control. Rel.*, 6 (1987) 353–360.

Odland, G.F. and Reed, T.H., Epidermis. In Zelickson, A.S. (Ed.), *Ultrastructure of Normal and Abnormal skin*, Lea and Febiger, Philadelphia (1967) p. 61–63.

Squier, C.A., The permeability of keratinized and non-keratinized oral epithelium to horseradish peroxidase. *J. Ultrastruct. Res.*, 43 (1973) 160–177.

Squier, C.A., Penetration of nicotine and nitrosonornicotine across porcine oral mucosa. *J. Appl. Toxicol.*, 6 (1986) 123–128.

Squier, C.A. and Hall, B.K., The permeability of skin and oral mucosa to water and horseradish peroxidase as related to the thickness of the permeability barrier. *J. Invest. Dermatol.*, 84 (1985a) 176–179.

Squier, C.A. and Hall, B.K., In-vitro permeability of porcine oral mucosa after epithelial separation, stripping and hydration. *Arch. Oral Biol.*, 30 (1985b) 485–491.

Squier, C.A. and Rooney, L., The permeability of keratinized and non-keratinized oral epithelium to lanthanum in vivo. *J. Ultrastruct. Res.*, 54 (1976) 286–295.

Tiemessen, H.L.G.M., Boddé, H.E., van Koppen, M., Bauer, W.C. and Junginger, H.E., A two-chambered diffusion cell with improved flow-through characteristics for studying the drug permeability of biological membranes. *Acta Pharm. Technol.*, 34 (1988) 99–101.

Veillard, M.M., Longer, M.A., Martens, T.W. and Robinson, J.R. Preliminary studies of oral mucosal delivery of peptide drugs. *J. Control. Rel.*, 6 (1987) 123–131.