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TR146 cells grown on filters as a model for human buccal epithelium: I. Morphology, growth, barrier properties, and permeability

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Abstract

The characterization and functional application of filter-grown TR146 cells are presented. TR146 is a continued cell line of human buccal epithelial origin. Growth parameters indicated a saturation density in the order of 2×10^5 cells/cm^2 and a doubling time ranging from 33 to 49 h dependent on type of support. Phase-contrast microscopy of the filter-grown TR146 cells showed morphologically epithelial-like cells. Transmission electron microscopy (TEM) of the TR146 cells, grown for 10 days (day of confluence) and until day 50 in culture, revealed a stratified epithelium of 4–7 cell layers. The surface cells were flattened and clearly distinct from cells in the lower layers. Filter-grown TR146 cells express ultrastructural characteristics of normal human buccal epithelium, e.g., intermediate filaments, microvilli-like processes, no tight junctions, multilaminar bodies considered equivalent to membrane coating granules, and absence of complete keratinization. The TEM of TR146 cells delineated an increasing number of organelles and desmosomes with the advancing age of the culture. The maximum integrity of the cell layers was reached at around day 30 in culture, and was assessed by measuring the transepithelial electrical resistance (TEER) (55–120 Ω cm^2, $n = 92$), and by conducting permeability studies. The apparent permeability coefficient (P_{app}) of mannitol, a hydrophilic marker of the paracellular transport pathway, declined until about day 30 in culture ($P_{app} \sim 4 \times 10^{-6}$ cm/s). The permeability of the lipophilic marker testosterone did not alter from the time of confluence to day 50 in culture ($P_{app} \sim 2 \times 10^{-5}$ cm/s). An estimation of the thickness of the unstirred water layer (h_{aq}) adjacent to the filter with and without TR146 cells was conducted using testosterone and various agitation rates. At the highest agitation rate, 163 rpm, the h_{aq} was approx. 1450 μm (cellular permeability coefficient (P_c) of testosterone $\sim 4 \times 10^{-4}$ cm/s). The TEER and P_{app} of mannitol and testosterone showed significant inter-passage variations ($p < 0.05$). The P_{app} values of testosterone and mannitol were not related to postfeeding time, were independent of the initial drug concentration, and were slightly dependent on the transport direction. The functional application of the filter-grown TR146 cells testing the permeability of various β-adrenoceptor antagonists showed a

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positive correlation between the P_c and the lipophilicity. The findings of this study indicate that filter-grown TR146 cells has a potential to model the human buccal epithelial permeation of drugs.

Keywords: Human buccal epithelium; Filter-grown cell line TR146; Morphology; Unstirred water layer; Permeability; β-Adrenoceptor antagonist; Lipophilicity

1. Introduction

Non-invasive administration of drugs as an alternative to parenteral delivery is currently attracting interest. New drug delivery systems are continuously being developed in order to optimize the administration of metabolically unstable drug substances, e.g. peptides and nucleic acids produced by advancing biotechnology.

The buccal administration of drugs intended for systemic circulation has attracted attention. The buccal route of delivery offers advantages to peroral administration, e.g., reduced first-pass metabolism, less drug decomposition and adverse effects, and often a more rapid onset of therapeutic effect. The advantages of transbuccal delivery compared to parenteral administration are the ease of administration and the convenience for the patient (De Vries et al., 1991; Rathbone and Hadgraft, 1991; Rogerson, 1991).

A knowledge of permeability barriers, transport pathways, and transport mechanisms of the human buccal mucosa is important for the development and optimization of drug and drug delivery systems intended for buccal administration.

Studies of transport across buccal epithelium have been carried out by different methodologies. In 1967, an in vivo human buccal absorption test was advised by Beckett and Triggs (1967) (reviewed by Rathbone and Hadgraft, 1991). The test is performed by swirling a drug solution around the mouth for a fixed period of time. The expelled solution and the post-rinse solution are analysed for the content of drug. A disadvantage conferred to this test is that the amount of drug disappearing from the swirled solution may not necessarily be equivalent to the actual amount of drug reaching the systemic circulation.

The oral cavity is lined by several types of epithelia with different permeability characteristics. In vivo investigations of drug delivery in a definite oral region or in a specific oral area have employed a filter paper disk (Pimlott and Addy, 1985), a perfusion chamber mechanically attached to the buccal mucosa (Barsuhn et al., 1988; Rathbone, 1991), and different buccal-adhesive formulations (reviewed by Smart, 1993). The results obtained with these methods suffer from the same disadvantage as Beckett and Triggs' buccal absorption test, i.e., they provide information regarding the disappearance of a drug from the oral cavity or the attached delivery device, while the actual amount of parent drug or metabolites permeating the epithelium and reaching the systemic circulation is not directly measured.

In vitro studies of drug transport across human buccal epithelium comprise studies using either biopsied material or primary cell cultures. 24–72 h post-mortem, human buccal biopsied material was mounted in a diffusion chamber (Adams, 1974). The drawbacks of this method are the use of non-viable material, interindividual variations, and variations due to the isolation of different strata. Primary cultures of human buccal cells initiated from explanted biopsies have been reported (Arenholt-Bindslev et al., 1987; Southgate et al., 1987). However, the disadvantages of using primary cultures are the dependence on and the shortage of human biopsies, interindividual variations, and the short life time of the cells.

Sparse availability of human buccal tissue for experimental use led to the application of animal tissue based on histological criteria (reviewed by Hoogstraate and Boddé, 1993). In vitro diffusion of a series of substituted acetanilides across excised hamster cheek pouch epithelium has been compared to in vivo data obtained in humans using a buccal absorption test (Garren and Repta, 1989). The logarithmic epithelial permeability constant derived from the hamster cheek pouch epithelium vs the percent buccal absorption in 5 min showed a positive correlation ($r = 0.878$). The utility of the excised hamster cheek pouch as a

model of the human buccal mucosa may be limited due to keratinization of the hamster tissue (Garren and Repta, 1989). A primary cell culture derived from the hamster cheek pouch epithelium has been developed as a system for studying drug transport and metabolism (Tavakoli-Saberi and Audus, 1989a). Morphological, enzymatical, and permeability characteristics were investigated, revealing a non-keratinized stratified epithelium. However, differences between species have to be considered.

A cell culture model based on a human buccal epithelial cell line might be a valuable model for studying transport of drugs across the human buccal epithelium. The objective of this paper is to present the potential usefulness of the continued cell line TR146 grown on filters as such a model.

2. Materials and methods

2.1. Materials

The continued cell line TR146, derived from a human neck metastasis originating from a buccal carcinoma (Rupniak et al., 1985), was kindly provided by Imperial Cancer Research Technology (London, UK) with generous help from Professor E.B. Lane (University of Dundee, UK). Information on the passage number was not available from the supplier, consequently, the obtained passage was designated number one. Heat-inactivated foetal calf serum (FCS) was obtained from Sera-Lab (Sussex, UK). Dulbecco's modified Eagle medium (DMEM; containing pyruvate, 4.5 g glucose/l, and glutamax-1), gentamicin, trypsin-EDTA and other culture media were purchased from Gibco BRL (Paisley, UK). The antimycoticum *p*-hydroxybenzoic acid *n*-butyl ester and Hoechst No. 33258 (cell culture tested) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Nunclon 25 cm^2 T-flasks (tissue culture treated, polystyrene), Nunc tissue culture inserts (inorganic membrane, 10^9 pores/cm^2, pore diameter 0.2 μm, growth area 4.15 cm^2), 6-well culture plates (tissue culture treated, polystyrene) were obtained from A/S Nunc (Roskilde, Denmark). Falcon cell culture inserts (polyethylene terephthalate, 1.6×10^6 pores/cm^2, pore diameter 0.45 μm, growth area 4.6 cm^2) and Falcon 6-well culture plates (tissue culture treated, polystyrene) were obtained from Becton Dickinson Labware (NJ, USA). D-[1-^{14}C]Mannitol (spec. act. 49.3 mCi/mmol) and [1,2,6,7-^{3}H]testosterone (spec. act. 92.5 Ci/mmol) were obtained from New England Nuclear (Boston, MA, USA). DL-[4-^{3}H]Propranolol hydrochloride (spec. act. 27 Ci/mmol) was obtained from Amersham Life Science (Little Chalfont, UK). [^{3}H]Atenolol (spec. act. 0.49 Ci/mmol) and [^{3}H]metoprolol (spec. act. 1.34 Ci/mmol) were generous gifts from Dr Kurt-Jörgen Hoffmann (Astra Hässle, Mölndal, Sweden). Atenolol, DL-metoprolol D-tartrate salt, oxprenolol hydrochloride, and DL-propranolol hydrochloride were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Ultima Gold MV scintillation cocktail was obtained from Packard Instrument B.V. (Groningen, The Netherlands). Glutaraldehyde (EM grade) was purchased from Bie and Berntsen (Rødovre, Denmark). Papanicolaou's solution 1b) and trichloroacetic acid were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

A Nikon Diaphot-TMD inverted microscope equipped with phase-contrast optics, Epi-Fluorescence, UV-1A filter box for *Mycoplasma* tests, and graticule NE11a were obtained from Nikon (Tokyo, Japan). An epithelial voltohmmeter (EVOM) and resistance measurement chamber (Endohm-24) were purchased from World Precision Instruments (Sarasota, FL, USA). A horizontal orbital plate shaker (Swip KL-2) was obtained from Edmund Bühler (Bodelshausen, Germany). A Minaxi Tri-Carb® 4000 Series scintillation counter was purchased from Packard Instrument B.V. (Groningen, The Netherlands).

A Hitachi HPLC system consisting of a L-6000 pump, a L-4000 variable UV detector, a Rheodyne model 7125 injection valve (CA, USA) equipped with a 100 μl loop, a (4 mm × 125 mm) Lichrospher 100 RP18 column (5 μm), and a (4 mm × 10 mm) Lichrosorb RP18 guard column were purchased from E. Merck (Darmstadt, Germany), and a Goertz Metrawatt SE 120 recorder obtained from CKI Claus Kettel (Rødovre, Denmark).

2.3. Cell culture

TR146 cells were maintained in 25 cm^2 T-flasks and incubated at 37°C in a 98% relative humidity (RH) atmosphere of 5% CO_2/95% air. Culture medium consisted of DMEM supplemented with 10% FCS, 50 μg/ml gentamicin, and 0.2 μg/ml *p*-hydroxybenzoic acid *n*-butyl ester. Cells of passage number 1–15 were used. At approx. 70% confluence, the medium was aspirated, and the cell culture was rinsed with 5 ml DMEM. The rinse was discarded, 2 ml trypsin-EDTA was added per flask and decanted after 30 s, and the flask was incubated with residual trypsin-EDTA for approx. 9 min at 37°C. The cells were suspended in culture medium and centrifuged at $250 \times g$ for 10 min. The pellet was suspended in a known volume of culture medium and the cell concentration was estimated using a haemocytometer. After appropriate dilution, the cells were either seeded in T-flasks (1×10^6 cells/flask, i.e. 4×10^4 cells/cm^2) or on filters (2.4×10^4 cells/cm^2), adding 2.5 ml of the culture medium to the apical chambers and adding 2.0 ml of the culture medium to the basolateral chambers. Cells were grown on filters until day 50 after seeding. If not stated otherwise, the culture medium was changed three times a week starting on day 1 of seeding when cells were thawed or on day 3 of passage. TR146 cells were stored at −80°C in a freeze medium consisting of DMEM supplemented with 5% glycerol and 15% FCS (1×10^6 cells/ml).

Daily, the cultures of TR146 cells were controlled for bacterial or fungous contamination by phase-contrast microscopy.The cultures were monitored routinely for *Mycoplasma* contamination by Hoechst 33258 staining using a method described by Chen (1977) and slightly modified by Lind (1993).

2.4. Transepithelial electrical resistance (TEER)

The integrity of the TR146 cell layers was monitored before and after each transport study by measuring the resistance (R; Ω) and calculating the transepithelial electrical resistance (TEER; Ω cm^2). The measurement of R was performed at 37°C using an Endohm-24 connected to an epithelial voltohmmeter (EVOM). Culture medium or transport medium was decanted prior to transferring an insert to the chamber of the Endohm-24. At 37°C DMEM 1.4 ml was added to the apical chamber of the insert and 4.5 ml to the chamber of the Endohm-24, respectively. R was recorded, and TEER calculated according to:

$$\text{TEER} = \left(R_{(\text{insert with TR146})} - R_{(\text{insert without TR146})}\right) \times A \quad (1)$$

where A is the surface area (cm^2) of the filter. $R_{(\text{insert without TR146})}$ signifies the resistance of cell-free inserts, incubated for 4 days in culture medium.

2.5. Cell density of filter-grown TR146 cells

The cell density was estimated by means of a graticule counting the number of fixed Papanicolaou-stained cell nuclei at various times in culture: The culture medium was aspirated and the insert ($n = 3$) with cells was washed twice with DMEM. Fixation was performed at room temperature for two 5-min periods by adding 2 ml of fixative solution to the apical and basolateral chambers. The cell layers were rinsed five times in distilled water, stained for 15 min with 2 ml Papanicolaou's solution 1b added apically and basolaterally, and finally washed six times in distilled water. Cells grown on Nunc tissue culture inserts were fixed in 100% methanol, the fixative was decanted, the inserts were washed with 5% trichloroacetic acid, and postfixed in 5% trichloroacetic acid. The cells grown on Falcon tissue culture inserts were fixed in 100% methanol: glacial acidic acid (3:1), the fixative was decanted, and the cells were postfixed using a similar solution for another 5 min. The average number of cell nuclei was estimated immediately after fixation employing a graticule. Five squares of the graticule were counted per filter.

The doubling time was derived from the slope of the growth curve, a semilogarithmic plot of the cell number/growth area (cm^2) vs time in culture, and calculated according to

$$\text{Doubling time} = \ln 2/\text{slope} \quad (2)$$

2.6. Permeability study

The permeability studies were performed under a 98% RH atmosphere of 5% CO_2/95% air and at 37°C in DMEM. Agitation was accomplished on a plate shaker. The speed control of the plate shaker was conducted with a photocell and a digital tachometer. Initially, DMEM 2.50 ml was added to the basolateral chamber and drug solution 2.50 ml was added to the apical chamber or vice versa. A 50–200 μl sample ($n = 2$) was removed from the receiver chamber at regular time intervals and immediately replaced by transport medium. At the end of a transport study a 50 μl sample ($n = 2$) was withdrawn from the donor chamber, the insert was washed twice in distilled water, and the filter was removed from the insert.

The test solutions consisted of radiolabeled isotopes and the corresponding unlabeled substances in DMEM (final concentration = 1×10^{-4} M; 600 nCi/ml), except [^{14}C]mannitol (final concentration = 60 nCi/ml) and oxprenolol (2×10^{-4} M). Unlabelled testosterone and unlabeled β-adrenoceptor antagonists were added to obtain a high concentration in order to overcome if any metabolism (Buur and Mørk, 1991) and adsorption to filters (Artursson, 1990).

The permeability studies of mannitol and testosterone were performed using filter-grown TR146 cells aged 9–50 days old, and the permeability experiments with the four β-adrenoceptor antagonists were conducted using filter-grown TR146 cells aged 30 days old.

The amount of radiolabeled substance and oxprenolol transported into the receiver chamber vs time was calculated, and the apparent permeability coefficient (P_{app}; cm/s) was determined according to the following equation using values above lag time

$$P_{app} = \frac{Q}{t \times 60 \times A \times C_0} \tag{3}$$

Lag time is defined as the period of time elapsing between application of a test substance and a linear increase of the amount of test substance in the receiver chamber. Q denotes the quantity of substance (cpm or μg) in the receiver chamber at time t (s), A signifies the surface area (cm^2) of the filter, and C_0 is the initial concentration (cpm or μg/ml) in the donor chamber. The permeability study was carried out under sink conditions, i.e., at the end of a study, the donor concentration remained above 90% of the concentration in the receiver chamber.

Cellular permeability (P_c; cm/s), was calculated by means of P_{app} values obtained at different agitation rates, see below.

Percent recovery was calculated according to:

$$\text{Recovery}(\%) = \frac{(X_{\text{Receiver}} + X_{\text{Donor}} + X_{\text{Filter}}) \times 100}{X_0} \tag{4}$$

where X_{Receiver} is the amount (cpm) of test substance in the receiver chamber including amounts of substance withdrawn during the study, X_{Donor} indicates the amount (cpm) of test substance in the donor chamber at the end of an experiment, X_{Filter} denotes the amount (cpm) of test substance associated with the filter at the end of an experiment, and X_0 is the initial amount (cpm) of test substance in the donor chamber. Values of X_{Receiver}, X_{Donor}, X_{Filter}, and X_0 were each corrected for background activity (50 μl transport medium in 3.0 ml scintillation cocktail ($n = 3$)). Radiolabeled oxprenolol was not available, hence, the amount of oxprenolol adsorbed at the filter was not analysable.

2.7. Thickness of the unstirred water layer (h_{aq})

The thickness (h_{aq}; μm) of the unstirred water layer (UWL) adjacent to the surface and the basal side of the culture and/or the filter was estimated at various agitation rates (V; rpm) using the previously described solution of radiolabeled testosterone. The transport studies were conducted at 37°C in a 98% RH atmosphere of 5% CO_2/95% air.

The h_{aq} is given by (Komiya et al., 1980; Karlsson and Artursson, 1991):

$$h_{aq} = \frac{D_{aq}}{P_{aq}} = \frac{D_{aq}}{KV^m} \tag{5}$$

where D_{aq} (cm^2/s) is the aqueous diffusion coefficient (7.84×10^{-6} cm^2/s; Karlsson and Arturs-

son, 1991), P_{aq} (cm/s) denotes the permeability coefficient of the UWL adjacent to the surface of the cell culture or filter and the underside of the filter, K (cm rpm s^{-1}) is a constant related to the diffusivity of [^{3}H]testosterone and the shape of the filter and the well, and m is an exponent of which $0 < m < 1$.

The P_{aq} is calculated according to the following equation (Karlsson and Artursson, 1991):

$$\frac{1}{P_{app}} = \frac{1}{P_{aq}} + \frac{1}{P_c} + \frac{1}{P_f} \quad (6)$$

substituting $P_{aq} = KV^m$ and rearranging Eq. 6

$$\frac{V^m}{P_{app}} = \frac{1}{K} + \left(\frac{1}{P_c} + \frac{1}{P_f}\right)V^m \quad (7)$$

P_c (cm/s) denotes the combined permeability coefficient of the para-and transcellular pathway, P_f (cm/s) is the permeability coefficient of the cell free filter estimated experimentally in the absence of the cell layer, i.e. $P_c = 0$.

An apparent thickness of UWL ($h_{aq\,(app)}$; μm) can be related to P_{app} using the following equation (Karlsson and Artursson, 1991; Hidalgo et al., 1992):

$$h_{aq(app)} = \frac{D_{aq}}{P_{app}} \quad (8)$$

2.8. Analysis

The radioactivity was determined by liquid scintillation counting. The radiolabeled samples were pipetted into a 3.0 ml scintillation cocktail and the filters were likewise placed into a 3.0 ml scintillation cocktail. The vials were counted maximally 20 min or counting was stopped when $2\sigma = 1.0$ was reached.

The concentration of oxprenolol in the samples was analysed by a high-performance liquid chromatography (HPLC) method. The mobile phase consisted of 20% methanol, 80% 0.08 M acetic acid buffer pH 4.0, and 100 μl n-nonylamine/l. The flow rate was 1.5 ml/min, wavelength at 224 nm, and the injection volume was 200 μl. The retention time was just below 5 min. Quantitation of oxprenolol was carried out on the basis of peak heights relative to standards (standard curve, linear regression, $r = 0.9993$). Limit of quantitation was 1 μg/ml. Standard error of mean (SE) at 9.0 μg/ml was $\pm 1.1\%$ ($n = 4$).

2.9. Transmission electron microscopy

Cells were prepared for transmission electron microscopy (TEM) on day 10 (day of confluence), and on days 16, 23, 30, and 50 in culture. Falcon tissue culture inserts ($n = 2$) with cultures of TR146 cells were rinsed twice in Dulbecco's phosphate-buffered saline and thereafter fixed with 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.2) for 30 min at room temperature. The filters were then removed from the plastic inserts and washed three times for 10 min each with 0.1 M sodium phosphate buffer (pH 7.2). The filters were stored in a 0.1 M sodium phosphate buffer (pH 7.2) at 4°C until further preparation for electron microscopy as previously described (Van Deurs et al., 1990). Additionally, cultures grown for 30 days ($n = 2$) were fixed from the surface side by replacing the medium with 2.5% glutaraldehyde and 0.5 mg/ml ruthenium red in 0.2 M sodium cacodylate buffer for 60 min followed by postfixation in 2% OsO_4 with ruthenium red (from the basal side fixatives without ruthenium red were used) before further processing for electron microscopy.

2.10. Statistical analysis

Data are expressed as mean $\pm$ standard error of mean (SE) of replications (n), $n = 3$–26. Differences between the passage numbers or permeability coefficients were tested for significance using Student's t-test, and considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Selection of cell line

To the author's knowledge, no continued human buccal cell line is available from any official cell bank. This was one of the motives for selecting the human buccal cell line TR146 for the

development of an in vitro model of the human buccal epithelium. TR146 was reported to form stratified cultures and to possess an epithelial-like morphology (Rupniak et al., 1985). Further, the keratins 4, 5, 6, 8, 10, 13, 14, 16, 17, and 18 were detected in extracts of TR146 cells (Purkis et al., 1990). This keratin pattern indicates cells of a stratified epithelial cell origin (keratins 5 + 14), non-cornifying epithelium (keratins 4 + 13) such as the buccal epithelium, and fast turnover (keratins 6 + 16) (Morgan et al., 1987; Purkis et al., 1990). A weakly positive reaction for keratins 8 and 18 was shown for TR146 cells (Rupniak et al., 1985), thus these keratins are considered as markers for simple epithelia they are also present in the small Merkle cell population in the attached gingiva and the hard palate (Morgan et al., 1987). Overall, TR146 cells express keratins characteristic of normal human buccal mucosa (Clausen et al., 1986).

TR146 cells express a protein/enzyme, involucrin/transglutaminase, which is precursor for formation of thickening of the cell membrane (Regnier et al., 1988). This protein/enzyme is seen in keratinized as well as non-keratinized oral epithelia (Squier and Hill, 1994).

The cell line KB (CCL17 KB, American Type Culture Collection (ATCC)), derived from a human male oral epidermoid carcinoma, has been proposed as an oral epithelial cell line (Royce and Baum, 1991). KB cells cultured at our laboratory were subjected to a chromosome analysis. This revealed no normal Y chromosomes in the tested metaphases ($n = 20$). Unfortunately, KB cells cannot be taken as a representative of the oral mucosa. HeLa (human cervical cells) cross

Fig. 1. Phase-contrast photomicrograph showing a confluent TR146 cell culture indicating morphologically epithelial-like cells. Cells were grown in culture flasks (support similar to a 6-well culture plate) for 7 days. Bar, 50 μm.

contamination of KB cells has been reported (Lavappa et al., 1976; Lavappa, 1978; Hay et al., 1988).

3.2. Growth parameters of TR146

In order to characterize and quantify the growth of TR146 cells (Fig. 1 and Table 1), a stock suspension of TR146 cells was thawed and seeded in 6-well culture plates. Confluence was reached within 7 days in culture. A doubling time of 33 h was calculated. Using other culture conditions and passage numbers, a doubling time of 22 h was reported (Rupniak et al., 1985).

3.3. Selection of filters

The adhesion and growth of TR146 cells using two different optically transparent filters were studied (Table 1). The growth curves (data not shown) were almost parallel showing a log phase and a plateau phase, and quantitatively 25% more cells were attached to Falcon tissue culture inserts at the plateau phase compared to cells grown on Nunc tissue culture inserts. A detachment of cells from Nunc tissue culture inserts were seen on day 20 of culture and forward. Falcon tissue culture inserts were chosen for the further experiments.

3.4. Ultrastructural characterization of filter-grown TR146 cells

The ultrastructure of TR146 cells grown on Falcon tissue culture inserts was studied on the day of confluence (day 10 in culture), and on day 16, 23, 30, and 50 in culture. After 10 days TR146 formed 2–3 layers, and after 16 days 4–6 cell layers (Fig. 2). Moreover, the cells appeared more differentiated after 16 days than after 10 days. Thus, after 16 days the surface cells were evidently more flattened than cells in the deeper layers, including the basal cells resting on the filter. The cells were provided with long, slender processes which were sometimes connected by small desmosomes (Fig. 2). Organelles of the biosynthetic apparatus (endoplasmic reticulum (ER), and Golgi complexes) were also more abundant after 16 days than after 10 days; intermediate filaments were, however, not well developed.

After 23 days, TR146 formed 4–7 layers and the surface layer consisted of flattened cells. The cells were connected by abundant desmosomes, while hemi-desmosomes between the filter and the basal cells were never observed (Fig. 3). Also, cytoplasmic organelles were well developed (Fig. 3). A few granules with a content of densely packed membrane-like material, which will be referred to as multilaminar bodies (MLB), were noticed. Although the nature of these multilaminar bodies remain uncertain, we consider them equivalent to the membrane coating granules of human oral stratified epithelia (Squier, 1977; Schroeder, 1981; Squier and Hill, 1994). It has been proposed that these membrane coating granules discharge their contents into the intercellular space and form a possible lipophilic permeability barrier (Squier, 1977; Schroeder, 1981; Squier and Hill, 1994).

Table 1
Growth characteristics of TR146 cells cultured on various supports

Support [a]	Seeding density (cells/cm^2)	Slope of log phase (day^{-1})	Doubling time (h)	Saturation density (cells/cm^2)	Time of confluence (day) [b]
Nunc 6-well tissue culture plate	4×10^4	0.51 ($r = 0.9833$)	33	1.65×10^5	within 7
Falcon tissue culture insert	2.4×10^4	0.52 ($r = 0.7952$)	32	2.34×10^5	9–12
Nunc tissue culture insert	2.4×10^4	0.65 ($r = 0.9358$)	25	1.88×10^5	within 12

[a] For further specification, see section 2.
[b] Estimated by means of phase-contrast microscopy.

After 30–50 days in culture, 4–7 cell layers were still present. Desmosomes and bundles of intermediate filaments were frequent and characteristic features (Fig. 4). The surface cells were flattened and distinct from the cells in the lower layers. They were often very tightly apposed and provided with short microvilli-like processes (Fig. 5). Experiments with ruthenium red, however, revealed that tight junctions were not present between the surface cells. The absence of tight junctions is in agreement with ultrastructural studies of normal human buccal epithelium (Squier and Hill, 1994). Desmosomes bind cells together, however, in opposition to tight junctions, the desmosomes are not considered to provide an effective permeability barrier (Powell, 1981), hence the paracellular transport pathway may contribute considerable to the total permeability of the buccal epithelium. The cells, in particular those in the middle layers just below the surface cells contained very well-developed ER and Golgi complexes as well as large amounts of multilaminar bodies (Fig. 6).

Fig. 2. Survey electron micrograph of TR146 cells grown for 16 days on a filter (Fil, Falcon tissue culture insert). Basal cells (BC), surface cells (SC). Note the many cellular processes which are sometimes connected by small desmosomes (arrows). Bar, 2 μm.

The present ultrastructural results showed that TR146 cells were readily grown on permeable supports. As early as 10–16 days on filters, a distinct multilayered organization was evident, although the differentiation of the cells was not complete. Finally, after day 23 onwards, the surface layer was comprised of very large, flattened and often tightly apposed epithelial cells differing from the more basal cells in morphology. Altogether, these findings indicate a certain degree of stratified epithelial differentiation which is more pronounced than that reported in the original publication concerning TR146 cells grown on a solid support (Rupniak et al., 1985). Filter-grown TR146 cells express ultrastructural characteristics of the normal human buccal mucosa such as desmosomes, intermediate filaments, multilaminar bodies considered equivalent to membrane coating granules, and microvilli-like processes. In addition, tight junctions are absence.

3.5. Integrity of TR146 cell layers as a function of time in culture

Assaying the integrity of cell layers can be done by measuring TEER as well as by determining the permeability of macromolecules/transport pathway marker molecules (Milton and Knutson, 1990; Anderberg et al., 1992). The integrity of confluent TR146 cell layers was monitored until day 50 in culture by TEER (Fig. 7), and the permeability of [^{14}C]mannitol, a hydrophilic marker of the paracellular pathway

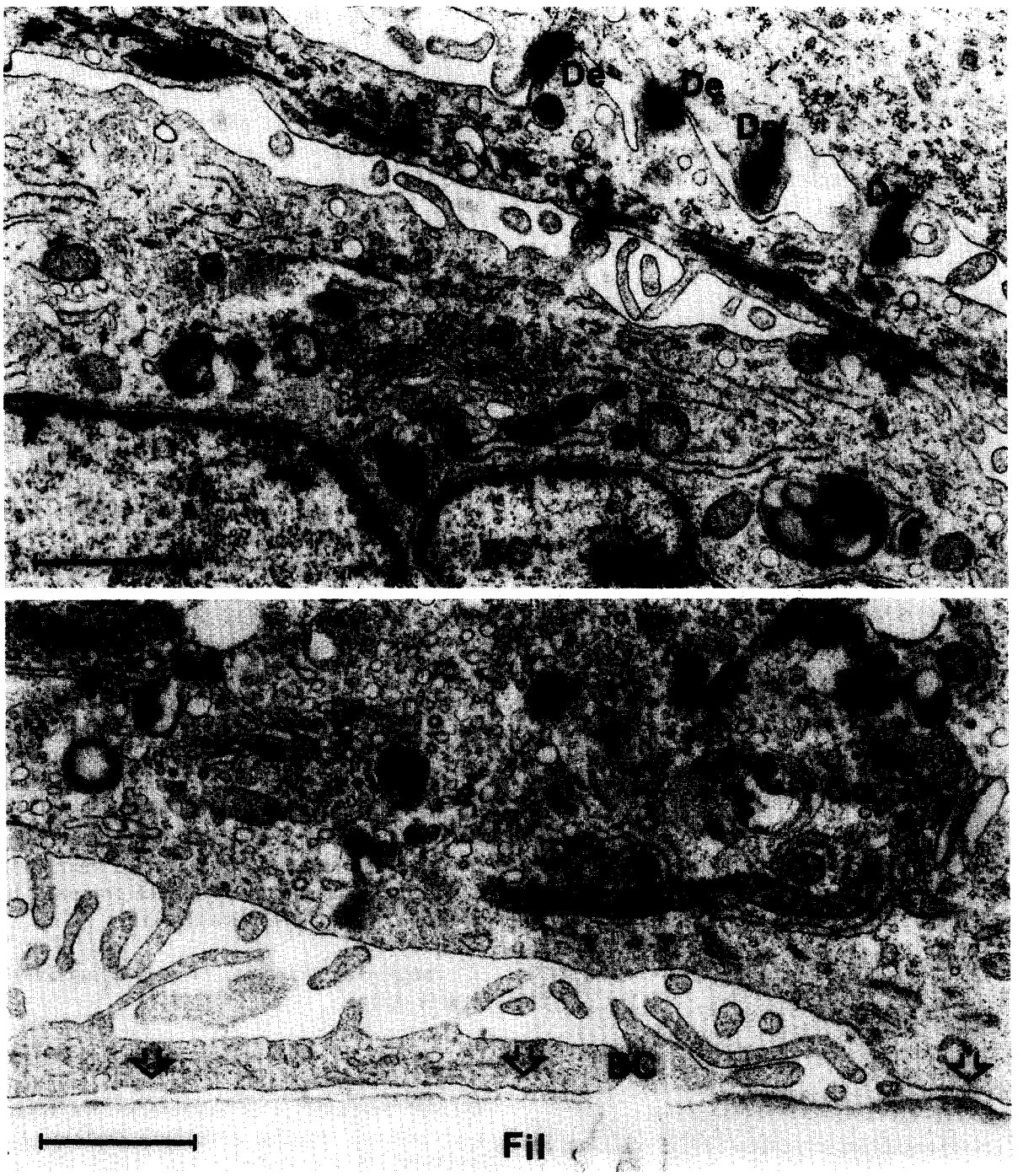

Fig. 3. TR146 cells grown for 23 days on a filter. (A) Shows a basal cell (BC) and two other cell layers above, connected by desmosomes (De). Also, note the intermediate filaments (IF) and the large Golgi complex (Go). (B) Demonstrates portions of two basal cells (BC) with well-developed Golgi complexes (Go). Note that no hemi-desmosomes are present between the BC and the filter below (arrows). Bars, 1 μm.

(Karlsson and Artursson, 1991), and of [^{3}H]testosterone, a lipophilic marker, was estimated (Fig. 8). TEER increased whereas the permeability of [^{14}C]mannitol declined until about day 30 in culture. The maximum of TEER, 68.2 $\pm$ 2.3 Ωcm^2 (mean $\pm$ SE, $n = 12$), was reached on day 29 of seeding. The value of TEER for cell free Falcon tissue culture inserts was 19.3 $\pm$ 0.8 Ω cm^2 (mean $\pm$ SE, $n = 9$). P_{app} of mannitol decreased from 1.1×10^{-5} cm/s on day 9 to 3×10^{-6} cm/s on day 50 of seeding. The decline of mannitol permeability could be due to the increased number of cell layers, the increased number of desmosomes, and modification of the intercellular material. The permeability of testosterone across the cell layers exhibited no alteration from time of confluence to day 50 in culture ($P_{app} \sim 2 \times 10^{-5}$ cm/s).

The results indicate maximal integrity of the cell layers around day 30 in culture, which was approx. 20 days after cell density had plateaued. The monolayers of the intestinal cell line, Caco-2, cultured on permeable supports, showed a parallel increase in cell density and TEER, and both parameters reached a plateau at the same time in culture (Artursson, 1990). The absence of tight junctions may explain the lower magnitude of TEER compared to other in vitro cell culture models known for forming tight junctions, e.g. Caco-2 approx. 260 Ω cm^2 (Artursson, 1990), 480

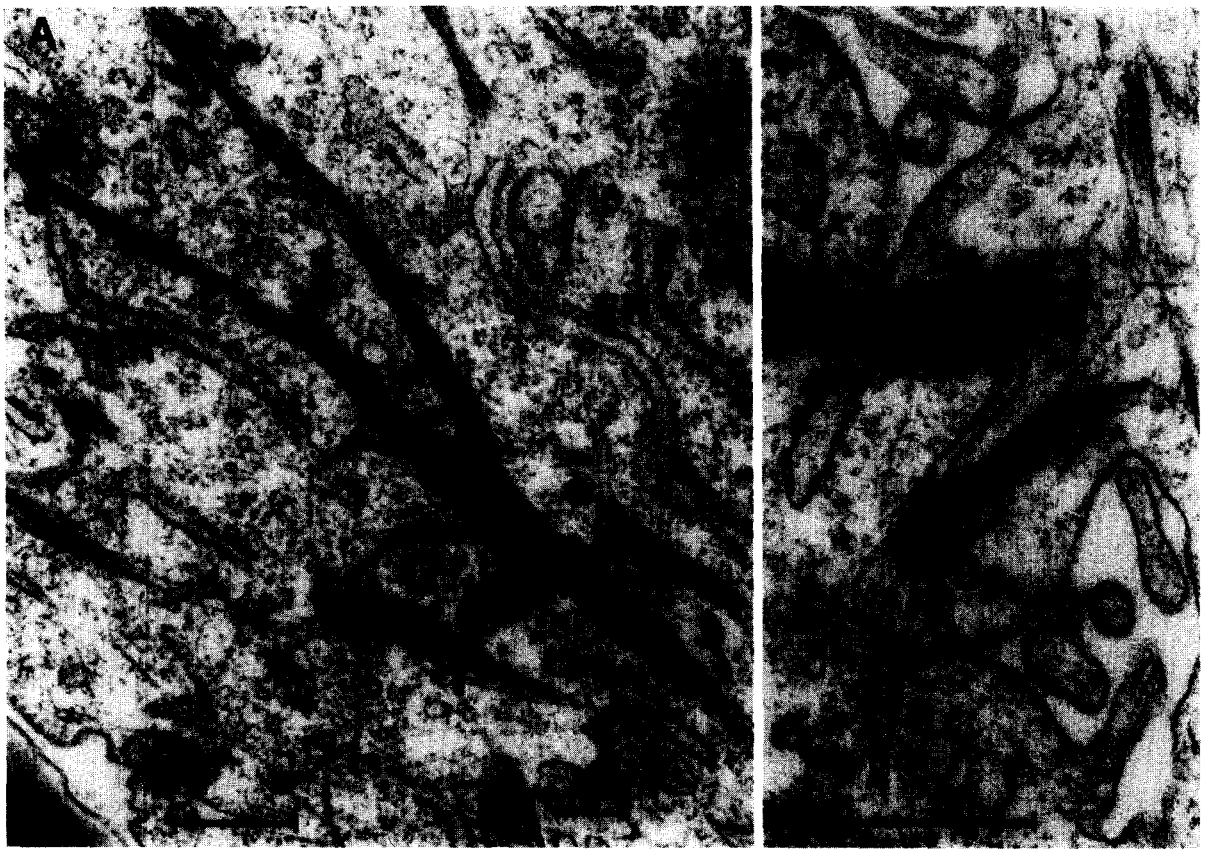

Fig. 4. TR146 cells grown for 30 days on a filter. Note the distinct bundles of intermediate filaments (IF) in (A) and the well-developed desmosomes (De) in (B). Endoplasmatic reticulum (ER), filter (Fil). Bars, 0.5 μm.

Ω cm^2 (Anderberg et al., 1992) or MDCK (stain I) at least in the order of 1500 Ω cm^2 (Cho et al., 1989; Van Deurs et al., 1990).

3.6. Inter-passage variation

TR146 cells demonstrated a wide inter-passage variation of TEER (Fig. 9). The results indicate no obvious relation between TEER and passage numbers. Further, the inter-passage variation is greater than the intra-passage variation ($n = 3$, SE < 10%). The latter observation has also been reported regarding monolayers of the cell line Caco-2 grown for 10–30 days (Artursson, 1990).

The inter-passage variation of P_{app} values for [^{3}H]testosterone and [^{14}C]mannitol across the cell layers of TR146, grown for 30–31 days in culture, are presented in Figs. 10 and 11. Regarding the transport of [^{3}H]testosterone and the increasing passage numbers, a decreasing tendency of P_{app} was shown. The mannitol experiments indicated a random distribution of P_{app}. Testing various passage numbers, ranging from 1 to 15, differences in the P_{app} values between some of the passages

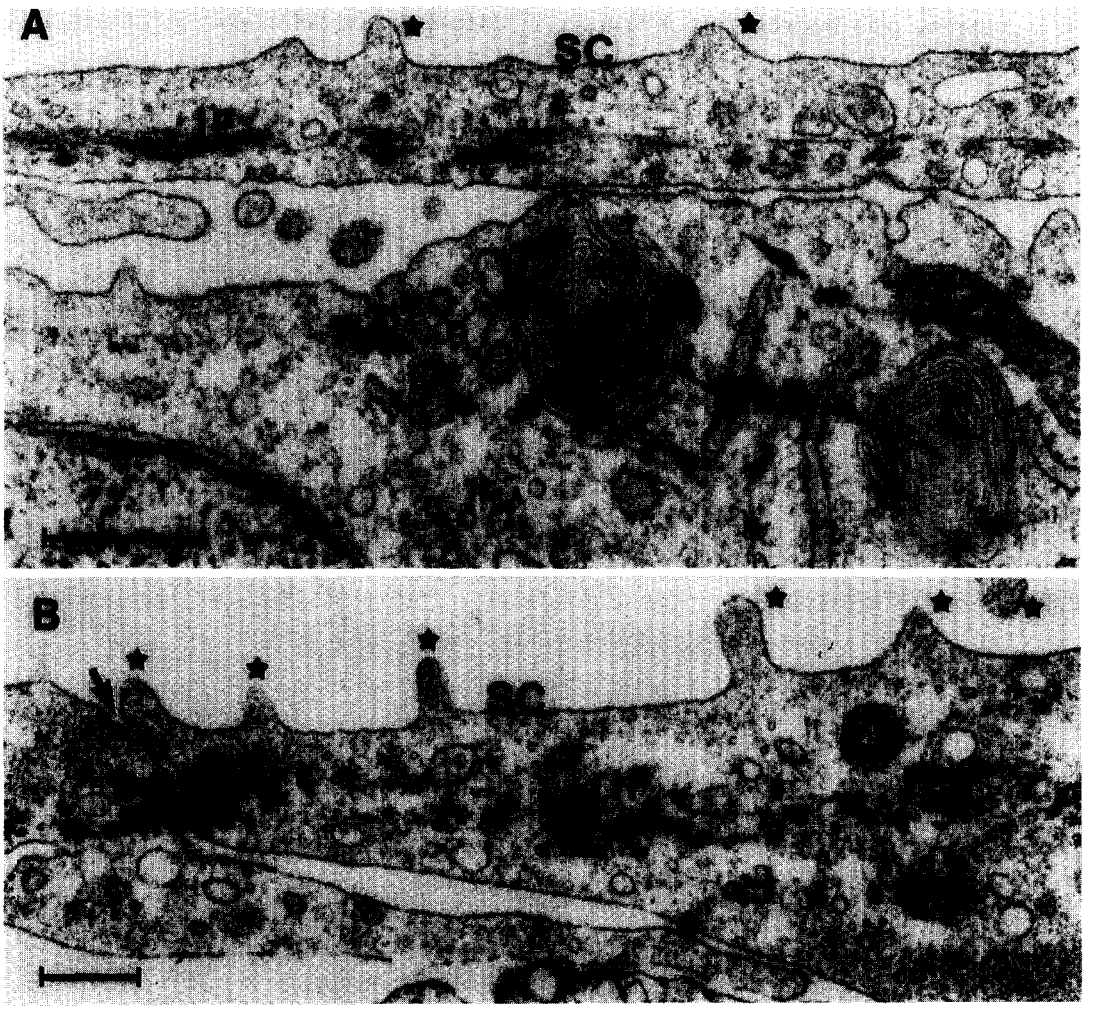

Fig. 5. TR146 cells grown for 50 days on a filter. Note the flattened surface cells (SC) provided with microvilli-like processes (asterisks). The surface cells are often tightly connected (arrow in (B)). Desmosome (De), intermediate filaments (IF), multilaminar body (MLB). Bars, 0.5 μm.

were calculated statistically significant. The TEER and P_{app} results point out the importance of performing comparative studies using only one passage. This circumstance has been observed in the present study.

3.7. Recovery and filter adsorption

In order to verify the fate of the compounds during a transport study, the percent recovery and the filter adsorption were calculated for all the test substances, see Table 2. Nearly 100% recovery was achieved for cell-free filters and 70–100% recovery was obtained for filters with TR146 cells. Data regarding the β-adrenoceptor antagonists showed the highest percent filter adsorption for the most lipophilic compound propranolol.

3.8. Postfeeding time

Carrying out a transport study, a possible influence of postfeeding time (i.e., the time interval between the last change of culture medium and

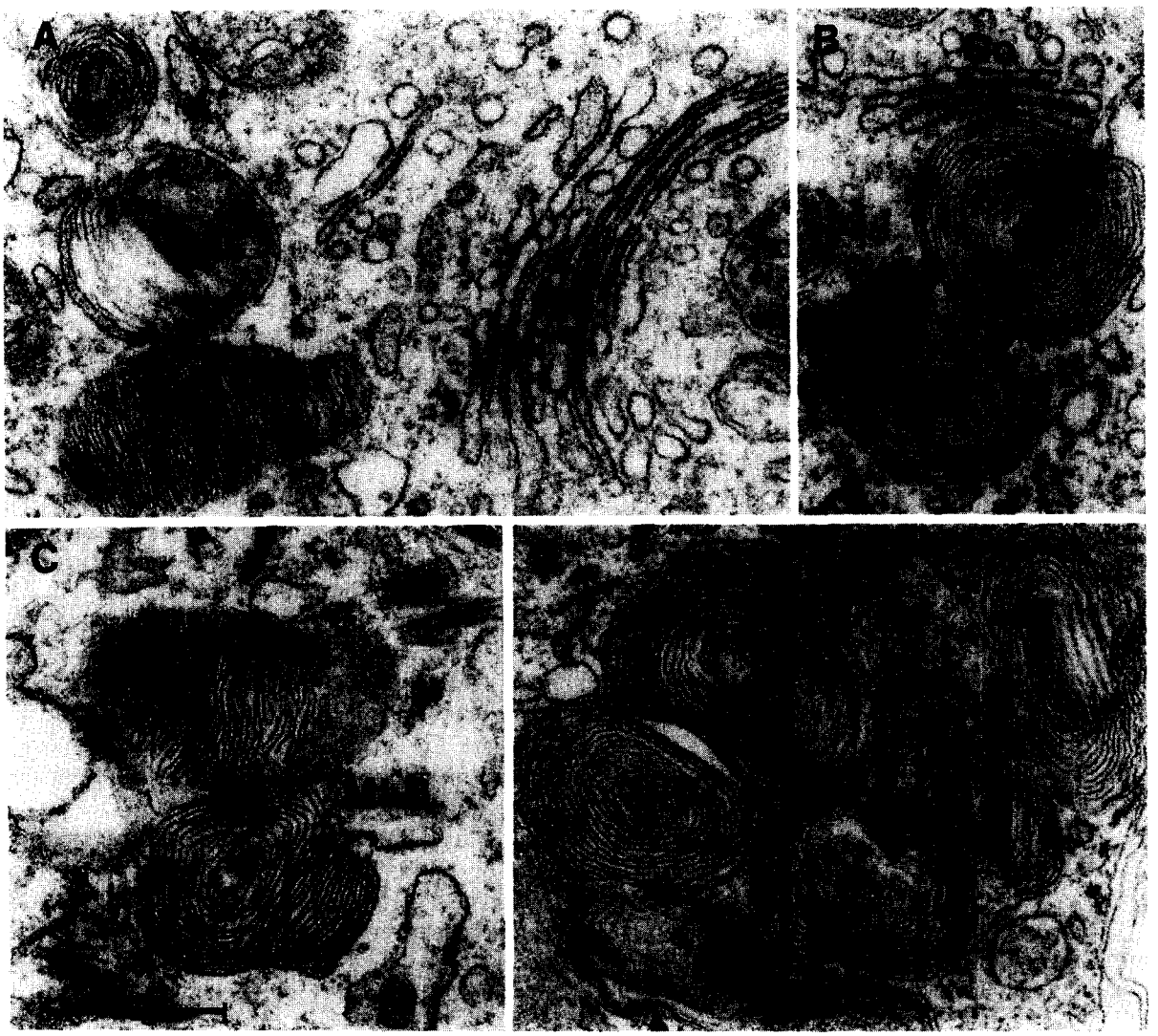

Fig. 6. TR146 cells grown for 30 days (C) or 50 days (A, B, and D) on filters. Note the many characteristic multilaminar bodies (MLB), often seen close to Golgi complexes (Go). Bar, 0.5 μm.

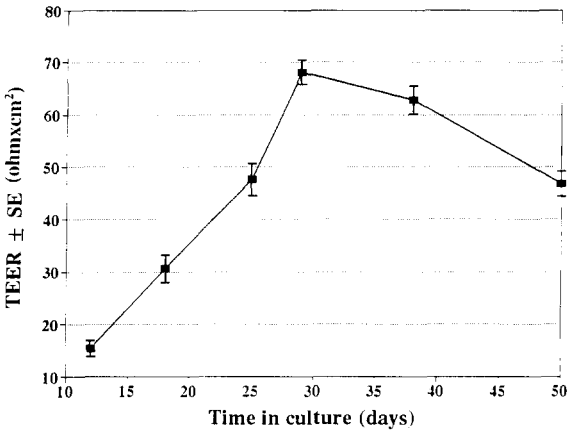

Fig. 7. Transepithelial electrical resistance (TEER) of confluent TR146 cell layers at various times in culture. 2.4×10^4 cells/cm^2 were seeded on day zero onto Falcon tissue culture inserts (pore size 0.45 μm). Resistance was measured with Endohm-24 and EVOM applying DMEM, in an 98% RH atmosphere of 5% CO_2/95% air at 37°C. Correction was made for the resistance of a cell-free insert containing DMEM (see Eq. 1 in section 2). Values are mean ± SE, $n = 3$–12.

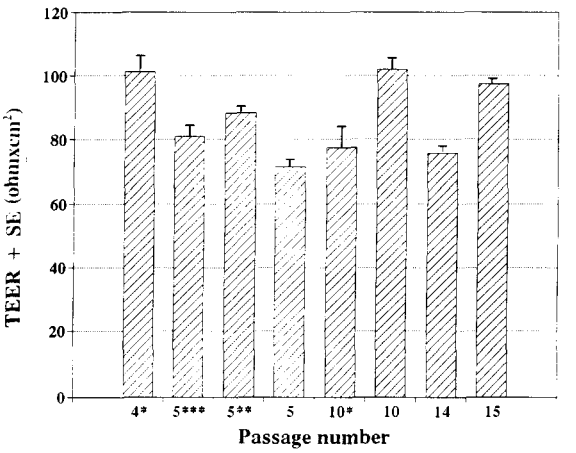

Fig. 9. Transepitelial electrical resistance (TEER) as a function of passage number. Outline of passage number: A stock cell suspension of passage number 3 was thawed several times and cultured giving rise to (1) passage 4*, (2) passage 4** → passage 5**, (3) passage 4*** → passage 5***, or passage 4 → passage 5 → → passage 10 → → passage 14 → passage 15. Passage 5 and passage 5*** are statistically significant differences at $p < 0.05$. Values are mean + SE ($n = 6$–27).

the actual experiment) was examined. The results indicated that P_{app} of [^{3}H]testosterone or [^{14}C]mannitol in the present studies were not related to the nutritional state of the cells (data not shown).

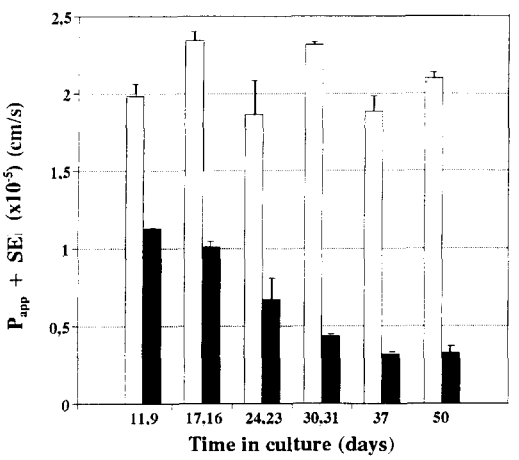

Fig. 8. Apparent permeability coefficient (P_{app}) across the cell layers of TR146 as a function of time in culture. (□) [^{3}H]Testosterone (final concentration 1×10^{-4} M); a single passage was used throughout the study. (■) [^{14}C]Mannitol; results on day 9–31 were derived from one passage and on day 37–50 from another passage. The receiver concentration remained below 90% of donor concentration, i.e., sink condition. Agitation rate, 163 rpm. Values are mean + SE ($n = 3$).

3.9. Unstirred water layer (UWL)

Adjacent to mucous epithelial membranes a UWL exists (Dainty and House, 1966) and P_{app} especially of lipophilic substances is influenced by the UWL (Karlsson and Artursson, 1991). In filter-grown cell cultures P_{app} is related to the permeability of the UWL, the cell layers, and the filter (see Eq. 6). To investigate the influence of UWL on P_{app} of lipophilic substances and to estimate h_{aq} and $h_{aq\ (app)}$ of the present model, the permeability of testosterone at different degrees of agitation was studied.

P_{app} at different agitation rates, estimated according to Eq. 3 (Table 4), was increased by a factor of 3 at the highest agitation rate 163 rpm compared to no agitation. h_{aq} was calculated according to Eq. 5 using P_c and P_f (Table 6) estimated from the slope of the curves in Fig. 12 (Eq. 7). The best fit of the curves in Fig. 12 was obtained with the exponent m of value 1 (Eq. 5, Table 3). A similar value of the exponent m has

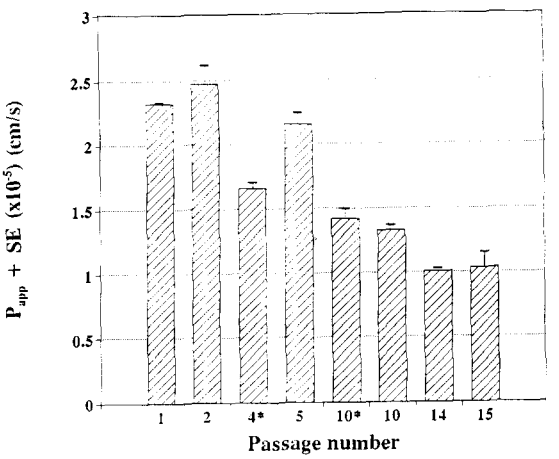

Fig. 10. Apparent permeability coefficients (P_{app}) of [^{3}H]testosterone at various passage numbers. [^{3}H]Testosterone (final concentration 10^{-4} M) transported across cell layers of TR146 cells grown for 30–31 days on filters. Agitation rate, 163 rpm. Outline of passage number: passage 1 gives rise to passage 2 and so on; passage 3 and 9 were both subcultivated to obtain passage 4 or 10, respectively, and stored at −80°C, later thawed to give rise to passage 4* or 10*. Passage 10 and passage 14 are statistically significant at $p < 0.05$. Values are mean + SE ($n = 3$).

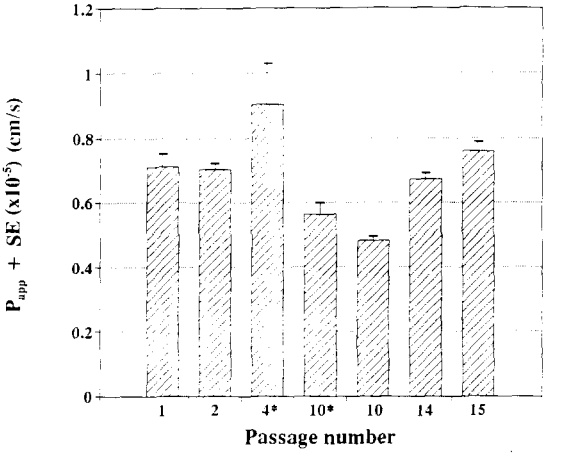

Fig. 11. Apparent permeability coefficients (P_{app}) of [^{14}C]mannitol at various passage numbers. [^{14}C]Mannitol transported across cell layers of TR146 grown for 30 days on filters. Agitation rate, 163 rpm. Outline of passage number: Passage 1 gives rise to passage 2 and so on; passage 3 and 9 were both subcultivated to obtain passage 4 or 10, respectively, and stored at −80°C, later thawed to give rise to passage 4* or 10*. Passage 10 and passage 14 are statistically significant at $p < 0.05$. Values are mean + SE ($n = 3$).

been reported for the Caco-2 cells (Karlsson and Artursson, 1991). h_{aq} was reduced by a factor of 6 at 163 rpm compared with no agitation (Fig. 13 and Table 4). $h_{aq\,(app)}$ was calculated according to Eq. 8. At 163 rpm $h_{aq\,(app)}$ and h_{aq} of filters with cells were approx. 3200 and 1450 μm, respectively (Fig. 13). The difference between the values shows that assuming $P_{app} \simeq P_{aq}$ in Eq. 8, e.g. the permeability of a lipophilic substance solely governed by the UWL, the magnitude of UWL will be overestimated ($h_{aq\,(app)} > h_{aq}$).

Ho and Higuchi (1971) proposed the dimensions of UWL in the human oral cavity to be 50 μm at the mucosal side, however, h_{aq} in vivo has not been measured.

Table 2
Percent recovery and percent adsorption at filters without or with TR146 cells grown for 30 days

Drug	% recovery + SE [a]		Adsorption ± SE [b]	
	Without TR146	With TR146	Without TR146	With TR146
Propranolol · HCl	107.3 ± 2.6	72.5 ± 4.9	0.2 ± 0.02	8.9 ± 3.6
Oxprenolol · HCl	99.7 ± 1.3	90.6 ± 10.5	n.m.	n.m.
Metoprolol tartrate	104.8 ± 0.9	106.4 ± 13.3	0.02 ± 0.002	4.1 ± 0.8
Atenolol	106.9 ± 0.8	87.7 ± 2.8	0.01 ± 0.005	0.7 ± 0.04
Testosterone	105.0 ± 1.8	93.9 ± 1.6	0.09 ± 0.02	1.9 ± 0.05
Mannitol	97.5 ± 1.7 ($n = 6$)	81.9 ± 2.0 ($n = 23$)	0.4 ± 0.1 ($n = 23$)	0.4 ± 0.2 ($n = 6$)

Agitation rate, 163 rpm. The transport study lasted 30 min, being conducted in the apical to basal direction. Number of replicates (n) was 3 if not stated otherwise. n.m., not measured.
[a] See section 2.
[b] Percent adsorbed radioactivity on the filter of initial amount of radioactivity in the donor chamber.

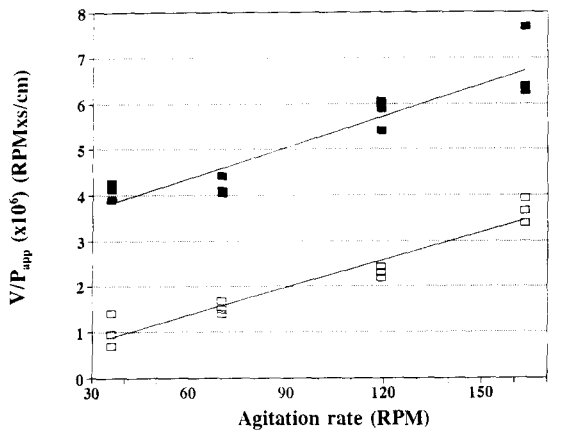

Fig. 12. Agitation rate (V)/apparent permeability coefficient (P_{app}) of [^{3}H]testosterone as a function of agitation rate. (■) TR146 cells grown for 30 days or (□) cell-free inserts. Each data point represents the result from a single filter.

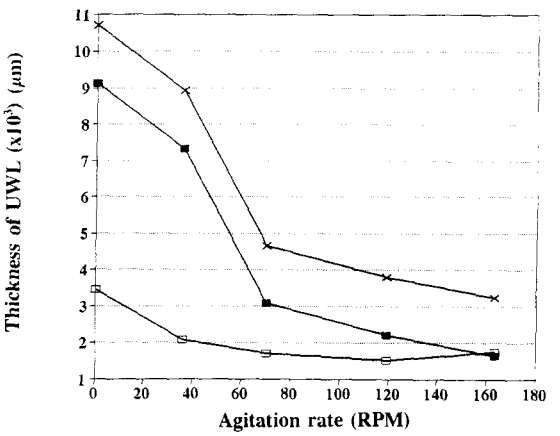

Fig. 13. Thickness (h_{aq}) of unstirred water layer (UWL) for [^{3}H]testosterone vs agitation rate: (■) shows h_{aq} adjacent to filters with TR146 cells grown for 30 days, (□) delineates h_{aq} adjacent to cell-free inserts, and (×) display apparent h_{aq} ($h_{aq\ (app)}$) adjacent to filters with TR146 cells grown for 30 days. Cells were cultured on Falcon tissue culture inserts (pore size 0.45 μm). h_{aq} was calculated via Eq. 5 through Eq. 6 and 7 and Table 3. $h_{aq\ (app)}$ was determined via Eq. 8.

3.10. Active or passive transport of mannitol and testosterone

Oral mucosa provides a barrier that protects both the deeper tissues of the oral cavity from entrance and insult of external substances, and prevents the exit of endogenous substances to the oral cavity. In defiance of this barrier function, simple diffusion as a mechanism of transfer across the oral mucosa has been proposed (reviewed by Rathbone and Hadgraft, 1991; and by Rathbone et al., 1994). However, carrier-mediated transport has been reported (reviewed by Rathbone and Hadgraft, 1991).

It was tested whether the layers of TR146 cells conduct passive or active transport of [^{3}H]testosterone and [^{14}C]mannitol. Four different initial concentrations (testosterone, 10^{-5}–10^{-4}M; mannitol, 10^{-6}–10^{-3} M) added apically showed no statistical significance between the obtained apparent permeability coefficients (P_{app}; cm/s), indicating a passive transfer of the substances in the applied concentrations.

The significance of transport direction, apical chamber to basal chamber and vice versa was examined (Table 5). P_{app} of the substances showed a statistically significant faster apical to basal transport rate than in the opposite direction. The degree of agitation of the apical chamber and the basolateral chamber, however, differed due to the employment of an orbital plate shaker. In order to investigate the influence of agitation, a transport study of [^{14}C]mannitol was conducted without agitation. The results showed that the transport of [^{14}C]mannitol apical to basolateral direction was still faster (Table 5) indicating active transport apical to basal direction. However, it is doubtful whether the surface area of the filter, denoted A in Eq. 3, is equal to the apical surface of the cell layers. Further investigations are needed to determine whether TR146 cells exhibit active transport of mannitol and testosterone in basal to apical direction.

Table 3
Estimation of the exponent m for Eq. 5 and 7

Exponent (m)	r [a]
0.1	0.9545
0.3	0.9656
0.5	0.9750
0.7	0.9827
0.9	0.9886
1	0.9910

[a] Correlation coefficient derived via linear regression of the curve (V'', P_{app}/V''). Data are listed in Table 4 and illustrated in Fig. 12.

Table 4
Apparent permeability coefficient (P_{app}) and thickness (h_{aq}) of unstirred water layer for [^{3}H]testosterone permeating TR146 cells, grown for 30 days on filters, employing different agitation rates (V)

V (rpm)	Filters with TR146 cells		Cell-free filters	
	P_{app} ($\times 10^5$) (cm/s) (mean ± SE, $n = 3$)	h_{aq} [a] (μm)	P_{app} ($\times 10^5$) (cm/s) (mean ± SE, $n = 3$)	h_{aq} [a] (μm)
0	0.73 ± 0.05	8922	2.27 ± 0.15	3457
36	0.87 ± 0.02	7138	3.80 ± 0.73	2063
70	1.68 ± 0.04	2894	4.64 ± 0.25	1690
119	2.06 ± 0.07	2021	5.18 ± 0.15	1514
163	2.42 ± 0.16	1448	4.47 ± 0.19	1754

[a] Calculated via Eq. 5 using exponent $m = 1$ according to Table 3 and D_{aq} of 7.84×10^{-6} cm^2/s (Karlsson and Artursson, 1991).

Table 5
Apparent permeability coefficent (P_{app}) of either transport direction for testosterone and mannitol permeating TR146 cells grown for 30 days on filters (direction of transport specified)

Drug	Agitation rate (rpm)	P_{app} ($\times 10^5$) (cm/s) (mean ± SE, $n = 3$)		Statistical significance level [a]
		Apical chamber to basal chamber	Basal chamber to apical chamber	
[^{3}H]Testosterone	163	2.15 ± 0.09	1.69 ± 0.1	0.047
[^{14}C]Mannitol	163	0.67 ± 0.02	0.58 ± 0.03	0.047
[^{14}C]Mannitol	0	0.52 ± 0.02	0.46 ± 0.04	0.027

[a] Differences between P_{app} of a substance at either transport direction were tested by Student's t-test and considered statistically significant if $p < 0.05$.

Table 6
Physiochemical characteristics and permeability constants (37°C, 163 rpm) of selected β-adrenoceptor antagonists, testosterone, and mannitol across filter-grown TR146

Drug	pK_a	M_w [a]	Log D [b]	P_{app} ($\times 10^5$) (cm/s) (mean ± SE, $n = 3$)	P_c ($\times 10^5$) (cm/s)	P_f ($\times 10^5$) (cm/s)	TEER pre-study (Ω cm^2) (mean ± SE, $n = 3$)	TEER post-study (Ω cm^2) (mean ± SE, $n = 3$)
Propranolol · HCl	9.45 [c]	259	1.540 [e]	1.54 ± 0.15	2.20	5.15	68 ± 5	28 ± 3
Oxprenolol · HCl	9.32 [d]	265	0.40 [f]	1.72 ± 0.071	n.c.	n.c.	95 ± 2	67 ± 6
Metoprolol tartrate	9.70 [c]	267	0.068 [e]	2.00 ± 0.044	0.17	3.68	84 ± 2	37 ± 7
Atenolol	9.55 [c]	266	−1.397 [e]	1.14 ± 1.33	1.46	4.03	87 ± 3	44 ± 4
Testosterone	–	288	3.31 [g]	2.42 ± 0.16	40.7	4.92	n.m.	n.m.
Mannitol	–	182	−3.10 [h]	0.39 ± 0.0099	0.41	4.39	n.m.	n.m.

n.c., not calculated; n.m., not measured.
[a] Molecular weight (M_w) of drug exclusive of the salt.
[b] Distribution coefficient (D).
[c] Temperature not specified (Tavakoli-Saberi and Audus, 1989b).
[d] 35°C (Schoenwald and Huang, 1983).
[e] D(octanol/PBSA-buffer pH 7.4) at 37°C (Tavakoli-Saberi and Audus, 1989b).
[f] D(octanol/phosphate-buffer pH 7.4) at 35°C calculated according to Eq. 2 in Schoenwald and Huang (1983).
[g] D(octanol/water), temperature and pH not specified in Komiya et al. (1980).
[h] Log partition coefficient (octanol/water), temperature and pH not specified in Grass and Sweetana (1988).

3.11. Functional application

In broad outline, two transport pathways exist, i.e., the paracellular and transcellular pathways of the buccal epithelium. In the former, drugs diffuse through the intercellular space occupied by an intercellular matrix, where discharge of the contents of membrane coating granules is proposed to contribute to formation of an intercellular permeability barrier (Squier, 1977; Harris and Robinson, 1992; Squier and Hill, 1994). Drugs transported by the transcellular route diffuse through the cells, i.e., passing through aqueous pores in the cell membranes or through the lipid bilayer of the cell membranes. Various factors have an effect on the extend and the rate of permeability across the buccal epithelium: (a) the physicochemical properties of the drug, e.g., molecular size, ionisation, lipophilicity, and bulkiness of the substances; and (b) the composition and the organisation of the epithelium (Rathbone and Tucker, 1993).

Permeability of the oral cavity of homologue series of acids and bases was reported with the more lipophilic compounds showing greater ability to penetrate into the oral mucosae (reviewed by Rathbone and Tucker, 1993).

The permeability of filter-grown TR146 cells apical to basal direction was studied using a series of β-adrenoceptor antagonists of similar pK_a and molecular weight, differing approx. 2-log fold range in lipophilicity (see Table 6). The curve displaying the quantity of β-adrenoceptor antagonist permeated basolaterally as a function of time was rectilinear above lag-time. Light microscopy of TR146 cells before and after performing a permeability study of propranolol, oxprenolol, metoprolol, and atenolol showed slight increased intercellular spaces after the permeability study. These microscopic observations were supported by a decrease in the TEER values regardless of whether the study was performed at a high agitation rate (163 rpm) (Table 6) or a low agitation rate (36 rpm) (data not shown). Hence, the drugs affected the integrity of the cells, but obviously did not affect the transport properties of the cell layers during the time of study. Permeability studies of mannitol and testosterone indicated no change of TEER pre-to post-study (data not shown). The four β-adrenoceptor antagonists and testosterone showed almost similar P_{app} values (see Table 6) indicating, as expected, that the transport of the more lipophilic compounds was unstirred water layer controlled. P_c values showed a positive correlation with the lipophilicity (expressed as distribution coefficient) of the compounds except for metoprolol. Available data from experimental animals and the buccal absorption test indicate a higher permeability to the more lipophilic β-adrenoceptor antagonists: A sigmoid relationship was reported between lipophilicity (expressed as logarithmic distribution coefficient) and logarithmic P_{app} values derived from cultured hamster pouch buccal epithelium (Tavakoli-Saberi and Audus, 1989b). TR146 cells showed higher permeabilities, a factor of approx. 2–6 compared to results obtained with porcine buccal epithelium (Le Brun et al., 1989) or porcine buccal mucosa (Bland et al., 1991) in diffusion chamber and a factor of 18–65 lower than data from cultured hamster pouch buccal epithelium (Tavakoli-Saberi and Audus, 1989b). The structure and composition of porcine buccal epithelium are considered to be more equivalent to human buccal mucosa as opposed to hamster pouch epithelium (De Vries et al., 1991; Harris and Robinson, 1992). A buccal absorption test carried out in one subject indicated that about 42% propranolol partitioned into oral mucosa in opposition to less than 2% atenolol in 5 min independently of the initial concentration (Schürmann and Turner, 1978).

In summary, this paper reports the morphology, growth characteristics, active and/or passive transport of mannitol and testosterone, inter-passage variation, TEER, and the thickness of UWL for filter-grown TR146 cells. The functional application (P_{app} and P_c) of a series of β-adrenoceptor antagonists of different lipophilicity has been examined. The findings of this study indicate that filter-grown TR146 cells have the potential to model human buccal epithelial permeation of drugs, although further characterization is required to establish an in vivo-in vitro correlation.

Acknowledgements

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