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The mucus layer as a barrier to drug absorption in monolayers of human intestinal epithelial HT29-H goblet cells

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Summary

The efficacy of the intestinal mucus layer as a barrier to drug absorption was investigated in monolayers of a mucus-producing human goblet cell line (HT29-H) using testosterone as a model drug. The investigation included comparison of the permeability of the HT29-H monolayers to testosterone before and after removal of the mucus layer, and comparison of the permeability of HT29-H and mucus-free Caco-2 cell monolayers. The apparent permeability coefficients of testosterone in HT29-H monolayers were $6.8 \pm 1.0 \times 10^{-6}$ cm/s before and $21.5 \pm 5.9 \times 10^{-6}$ cm/s after removal of the mucus layer ($p < 0.02$). The corresponding permeability coefficients in Caco-2 monolayers were $31.5 \pm 5.3 \times 10^{-6}$ and $32.1 \pm 9.3 \times 10^{-6}$ cm/s ($p > 0.05$) or approx. 50% higher than that of 'mucus-free' HT29-H monolayers. A comparison of the relative importance of the mucus layer, the unstirred water layer and the goblet cell monolayer as barriers to testosterone absorption showed that the mucus layer contributes 78% of the total resistance to absorption. We conclude that the intestinal mucus layer produced by HT29-H goblet cells is a significant barrier to testosterone absorption and that HT29-H monolayers can be used to study the barrier functions of an intact human intestinal mucus layer in cell culture.

Introduction

The major barriers to drug and peptide absorption in the intestine are the epithelial cell layer and the mucus layer (reviewed by Wilson et al., 1989). While the importance of the epithelial barrier is undisputed, the significance of the mucus layer as a barrier to drug absorption is unclear. It has been suggested that the mucus layer is only a minor barrier to drug absorption (e.g., Winne and Verheyen, 1990), but others suggest that the mucus layer functions as a rate limiting barrier (Nimmerfall and Rosenthaler, 1980; Smithson, 1981). Comparisons between studies are complicated since different models of the mucus layer have been used. Some studies have been performed in everted rat intestinal sacs (e.g., Kearney and Marriott, 1987b) or in samples of fresh mucus from experimental animals (Nimmerfall and Rosenthaler, 1980; Smith et al., 1986). In others, partially purified mucin in soluble or insoluble form has been used (Niibuchi et al., 1985; Kearney and Marriott, 1987a; Hughes, 1988). However, there are several disadvantages associated with these models: the properties of

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the mucus may vary from one preparation to another; isolation of the mucus layer or purification of mucin molecules is relatively time consuming; and the mucus is not of human origin.

Recently, several drug absorption models based on the cultivation of monolayers of various intestinal epithelial cell lines on permeable supports have been characterized (reviewed by Artursson, 1991). The most commonly used model is based on the human intestinal epithelial Caco-2 cell line (Hidalgo et al., 1989; Wilson et al., 1990). This cell line forms confluent monolayers of enterocyte-like cells with the functional properties of transporting epithelia (Pinto et al., 1983; reviewed by Neutra and Louvard, 1989). These properties make it suitable for studies of both passive and active drug transport (Artursson, 1990; Hilgers et al., 1990; Hu and Borchardt, 1990; Lundin and Artursson, 1990; Artursson and Karlsson, 1991; Cogburn et al., 1991, Inui et al., 1992; Ranaldi et al., 1992). However, Caco-2 cell monolayers do not form a mucus layer.

We therefore developed a new drug absorption model based on monolayers of the mucin producing human intestinal goblet cell line HT29-H grown in permeable cell culture inserts (Wikman et al., 1993). The goblet cell monolayers produce mucin molecules and form a native human mucus layer that covers the apical side of the cells. In this paper, we investigate the barrier properties of the mucus layer produced by HT29-H goblet cells.

Materials and Methods

Materials

HT29-H cells, a mucus secreting subclone of the HT29 human colon carcinoma cell line, were a gift from Dr Daniel Louvard, Institute Pasteur, Paris (Huet et al., 1987). The Caco-2 cell line was obtained from American Type Culture Collection, Rockville, MD, U.S.A. (Fogh et al., 1977). Dulbecco's Modified Eagle Medium (DMEM), fetal calf serum (FCS), non-essential amino acids (NEAA), penicillin-streptomycin solution, (10000 IU/ml and 10 mg/ml, respectively) and Hanks' Balanced Salt Solution were obtained from Gibco Laboratories through Laboratorie Design AB, Lidingö, Sweden. Rat tail collagen (type I), human transferrin, Hepes and testosterone were from Sigma Chemical Co, St. Louis, MO, U.S.A. Transwell™ cell culture chambers, polycarbonate membrane, 24.5 mm diameter and 0.4 μm pore size were purchased from Costar, Badhoevedorp, The Netherlands. Millicell-HA filter inserts, mixed cellulose esters membrane, 30 mm diameter 0.45 μm pore size were obtained from Millipore, Bedford, MA, U.S.A. [^{3}H]Testosterone (spec. act. 141.1 Ci/mmol) and [^{14}C]mannitol (spec. act. 52.0 mCi/mmol) were from New England Nuclear, Boston, MA, U.S.A.

Cell culture

The culture of HT29-H and Caco-2 cells has been described and characterized elsewhere (Artursson, 1990; Wikman et al., 1993). HT29-H cells of passage numbers 25–35 and Caco-2 cells of passage numbers 90–100 were used. HT29-H cells were seeded at a density of 8×10^5 cells/cm^2 on Transwell™or Millicell-HA inserts, which had been coated with a thin layer of collagen. Caco-2 cells were seeded at a density of 4×10^5 cells/cm^2 on uncoated Transwell™ inserts. The cells were grown in culture medium consisting of DMEM supplemented with 10% heat-inactivated FCS, 1% NEAA and 1% penicillin-streptomycin solution. Human transferrin, 5 μg/ml, was also added to the HT29-H cell culture medium. The cells were maintained at 37°C in a humidified atmosphere of 10% CO_2 in air. They were fed every second day with 2.5 ml culture medium in both the apical and basal chambers. Monolayers of HT29-H were used in experiments 28–35 days after seeding. Monolayers of Caco-2 were used in experiments 20–30 days after seeding.

Transmission electron microscopy

Cells grown on permeable inserts were rinsed with PBS (phosphate buffered saline) and fixed in 1.5% glutaraldehyde in phosphate buffer. Specimens were treated consecutively with 1% osmium tetroxide and 1% uranyl acetate. They were then dehydrated and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate. The specimens were studied in a Philips

420 electron microscope (Philips, Eindhoven, The Netherlands) operated at 60 kV.

Thickness of the mucus layer

The thickness of the mucus layer on the apical side of the HT29-H monolayers was measured as described in detail previously (Wikman et al., 1993). Briefly, 1-mm thick strips were cut from the Millicell-HA filter-grown monolayers. The strips were stained and viewed using an inverse phase contrast microscope and the thickness of the mucus layer was determined using an eyepiece graticule. Mean thickness was computed from measurements of three monolayers. Three strips were cut per monolayer and 13–36 measurements of the thickness were made along each strip.

A washing procedure was used to decrease the thickness of the mucus layer. The HT29-H monolayers were washed by agitation on a plate-shaker at 135 rpm for 10 min with 2.0 ml fresh HBSS on both the apical and basolateral sides. The HBSS on the apical side was replaced with fresh HBSS and the agitation was repeated twice more (Wikman et al., 1993).

Transport studies

All transport experiments were performed at 37°C in Hanks' Balanced Salt Solution containing 25 mM Hepes (HBSS). The culture medium of Transwell filter-grown Caco-2 or HT29-H monolayers was replaced with 1.0 ml of HBSS in the insert (apical chamber) and 2.0 ml of HBSS in the basal chamber. Subsequently, the monolayers were equilibrated at 37°C for 30 min. To initiate the experiment, 1.0 ml of prewarmed HBSS containing [^{3}H]testosterone and/or [^{14}C]mannitol was added to the apical chamber. At selected times, a 100 μl sample was collected from the basal chamber and replaced with an equal volume of fresh HBSS to maintain constant volume. The initial donor concentration in the apical chamber, C_0, was determined from the mean value of at least three samples (100 μl) taken from the radiolabelled drug solutions. The radioactivity of the samples was determined in a Tricarb 1900 CA liquid scintillation counter (Canberra Packard Instruments, Downers Grove, IL, U.S.A.).

The passive transport of testosterone and mannitol was studied before and after washing the HT29 and Caco-2 monolayers. These experiments were performed without stirring of the monolayers, in order to preserve the mucus layer.

Calculations

Apparent permeability coefficients (P_{app}) P_{app} was calculated by using the following equation:

$$P_{app} = (dQ/dt)(1/AC_0)\ (cm/s) \qquad (1)$$

where dQ/dt is the linear rate of appearance of the compound on the basolateral side of the monolayer (mol/s), under 'sink' conditions, A denotes the surface area of the monolayer (4.71 cm^2), and C_0 is the initial concentration on the apical side of the monolayer (mol/ml).

The reciprocal of P_{app} ($1/P_{app}$) is equal to the apparent resistance to drug absorption (R_{app}) of the barriers in the cell culture model. The barriers can be interpreted as resistances in series (Flynn et al., 1974; Komiya et al., 1980; Winne, 1984). Thus, the Caco-2 and HT29-H models can be defined in terms of permeability coefficients by:

$$\text{Caco-2: } R_{app} = 1/P_{app} = 1/P_f + 1/P_c + 1/P_{aq} \qquad (2)$$

$$\text{HT29-H: } R_{app} = 1/P_{app} = 1/P_f + 1/P_c + 1/P_{aq} + 1/P_{muc} \qquad (3)$$

where P_f is the permeability coefficient of the porous polycarbonate filter, P_c denotes the permeability coefficient of the cell monolayer, P_{aq} is the combined permeability coefficients of the unstirred water layers (UWL) present on each side of the cell monolayer, and P_{muc} represents the permeability coefficient of the mucus layer on the HT29-H monolayers.

Permeability coefficient of the polycarbonate filter (P_f) The permeability of testosterone

through the polycarbonate filter was calculated as follows (King, 1980):

$$P_f = \frac{D_{aq}\epsilon}{h_f} = \frac{7.84 \times 10^{-6} \times 0.1256}{1 \times 10^{-3}}$$

$$= 985 \times 10^{-6} \text{ cm/s} \quad (4)$$

where D_{aq} is the aqueous diffusion coefficient of testosterone (cm^2/s), ϵ denotes the porosity and h_f the thickness (cm) of the polycarbonate filter. D_{aq} of testosterone was calculated from the expression $D_{aq} \cdot MW^{1/3} = \text{constant}$, using mannitol (MW = 182; $D_{aq} = 9.14 \times 10^{-6}$ cm^2/s) as the reference solute. ϵ was calculated from data provided by the manufacturer.

Permeability coefficients of the cell monolayers (P_c) We have previously determined P_c of testosterone in the mucus-free Caco-2 model by using the following linear relationship between stirring rate and P_{app} (Karlsson and Artursson, 1991, 1992):

$$V/P_{app} = 1/K + (1/P_c + 1/P_f) \cdot V \quad (5)$$

where V is the stirring rate and K is a lumped empirical constant. A plot of V/P_{app} against V gives a straight line with a slope of $(1/P_c + 1/P_f)$. $1/P_c$ is then obtained by subtracting $1/P_f$ from the slope value.

P_{app} of testosterone was measured at one low (135 rpm) and one high (1090 rpm) stirring rate on a plate shaker with adjustable speed (Titertec, Flow Laboratories Ltd, U.K.) and P_c was calculated by using Eqns 4 and 5. Washed monolayers were used to determine P_c of the HT29-H cells.

Permeability coefficient of the unstirred water layer (P_{aq}) P_{aq} in the Caco-2 model can be calculated at any stirring rate according to Eqn 2, when P_c and P_f are known:

$$P_{aq} = 1/(1/P_{app} - 1/P_c - 1/P_f) \quad (6)$$

Permeability coefficient of the mucus layer (P_{muc})

P_{muc} cannot be separated from P_{aq} in the HT29-H model. However, if the assumption is made that P_{aq} is equal in the HT29-H and Caco-2 models, then by inserting P_{aq} (Caco-2) into Eqn 3, P_{muc} is given by:

$$P_{muc} = 1/(1/P_{app} - 1/P_c - 1/P_f - 1/P_{aq}) \quad (7)$$

Transepithelial electrical resistance measurements

The transepithelial electrical resistance (TEER) of the monolayers was measured to investigate the effect of the washing procedure and stirring during transport experiments. 3.0 and 4.0 ml of HBSS were added to the apical and basal chambers, respectively. The monolayers were allowed to equilibrate for 15 min at 25°C prior to measurements. Each chamber was connected via salt bridges (polyethylene tubing, 2 M KCl and 2% agar), to voltage-sensitive calomel electrodes and to Ag/AgCl current-passing electrodes. Current pulses of 100 μA were generated across the monolayers using a programmable current source (Keithley 224, Keithley Instruments, Cleveland, OH, U.S.A.) and the voltage changes were detected on a microvolt multimeter (Keithley 197). Apparent electrical resistance values were obtained from Ohm's law. The actual TEER values were calculated by subtracting the electrical resistance of cell culture inserts without cells.

Statistics

Unpaired Student's t-test (two-tailed) was used to test the significance of the difference between two mean values. When comparisons of more than two mean values were made, the data were analyzed with one-way ANOVA. The Mann-Whitney U-test was used as a non-parametric test for testing the significance of differences in mucus layer thickness.

Results and Discussion

The significance of the mucus layer as a barrier to drug absorption was investigated in monolayers of mucus-producing human intestinal HT29-H goblet cells using testosterone as a model drug. Testosterone was chosen as a model drug for several reasons: We have previously used

testosterone in the Caco-2 model to develop a method of quantitatively determining the extracellular and cellular drug permeability coefficients (Karlsson and Artursson, 1991, 1992). By using the same marker in the present study, the method could be extended to incorporate the quantitative determination of the permeability of the extracellular mucus layer. Moreover, when Hughes investigated the diffusion of various drugs in purified gastric mucus, the diffusion of testosterone was reduced more than 4-fold as compared to a 2-fold reduction for most other drugs, suggesting an unusually strong interaction between testosterone and mucus (Hughes, 1988). Further, [^{3}H]testosterone has a very high specific activity which makes it possible to use small amounts of the drug. This reduces the risk of saturation of the binding sites in the mucus layer in the small scale cell culture model. Finally, since testosterone is very lipophilic and therefore very rapidly absorbed across the intestinal epithelium, its permeability in the cell monolayer is only dependent on the permeability of the cell membranes and independent of the permeability of the tight junctions (Artursson and Magnusson, 1990; Karlsson and Artursson, 1991). This makes it possible to compare testosterone permeability in HT29-H and Caco-2 cell monolayers, despite quite different tight junction permeabilities (Wikman et al., 1993).

The barrier properties of the mucus layer were investigated by comparing the permeability of the HT29-H monolayers before and after removal of the mucus layer and by comparing the permeabilities of HT29-H monolayers and mucus-free Caco-2 monolayers. A mild washing procedure was used to remove the mucus layer from the HT29-H monolayers. After washing, the mean thickness of the mucus layer was reduced from 25 ± 1 to 9 ± 1 μm and the surface covered with mucus from 94 ± 3 to 65 ± 10% (Table 1). Thus, the washing procedure did not remove the mucus layer completely. The effects of the washing procedure on the integrity of the cell monolayers were investigated using two methods: transepithelial electrical resistance and permeability to

TABLE 2

Effect of washing on the apparent permeability coefficient (P_{app}) of mannitol and the transepithelial electrical resistance (TEER) of the cell monolayers

Monolayers	P_{app} [a] ($\times 10^6$) (cm/s)	TEER [a] (Ω cm^2)
HT29-H control	1.8 ±1.0 (9)	159±10 (3)
HT29-H washed	2.2 ±1.3 (9)	118±42 (3)
p	n.s.	n.s.
Caco-2 control	0.071±0.01 (4)	401±18 (4)
Caco-2 washed	0.085±0.02 (4)	415± 9 (4)
p	n.s.	n.s.

[a] Values are means ± S.D. for the number of determinations indicated within parentheses. Tests of the significance (*p*) of differences between mean values of washed and control monolayers were made by using a two-tailed Student's *t*-test for unpaired data. n.s., not significant ($p > 0.05$).

TABLE 1

Effect of washing on the thickness of the mucus layer on HT29-H cell monolayers

Monolayers [a]	Mean thickness of the mucus layer [b] (μm)	Surface covered with mucus [c] (%)	Distribution of measurements of mucus thickness (%)				
			0 μm	1–10 μm	11–20 μm	21–30 μm	31–66 μm
Control	25 ± 1	94 ± 3	5	20	26	18	31
Washed	9 ± 1 [d]	64 ± 10 [e]	36	35	21	7	1

[a] Three strips were cut from each of three monolayers and 13–36 measurements were made along each strip.
[b] Values are means ± S.E.; calculated from 166 and 206 measurements on control and washed monolayers, respectively. Values of zero thickness of the mucus layer were included in the calculation of mean thickness of the mucus layer.
[c] Values are means ± S.D.; calculated from values of three monolayers.
[d] Significantly different from control, $p < 0.0001$, as determined by Mann-Whitney *U*-test.
[e] Significantly different from control, $p < 0.001$, as determined by Mann-Whitney *U*-test.

the hydrophilic paracellular marker molecule [^{14}C]mannitol (Table 2). No significant differences in the integrity of HT29-H and Caco-2 monolayers were observed. This indicates that the mucus layer can be partly removed from the HT29-H monolayers without negative effects on epithelial integrity and that the permeability to [^{14}C]mannitol and small ions across HT29-H monolayers is independent of the mucus layer. (The transepithelial electrical resistance is an inverse measure of epithelial ion permeability.)

In contrast, the permeability to testosterone in HT29-H monolayers was significantly influenced by the mucus layer (Fig. 1). After washing, the apparent permeability coefficient of testosterone in HT29-H monolayers increased 3-fold from $6.8 \pm 1.0 \times 10^{-6}$ to $21.5 \pm 5.9 \times 10^{-6}$ cm/s ($p < 0.02$), while no change in apparent permeability

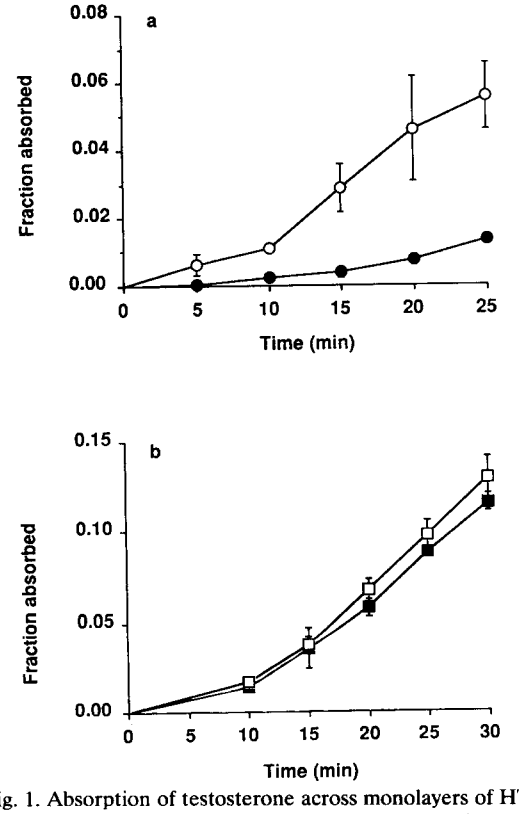

Fig. 1. Absorption of testosterone across monolayers of HT29-H and Caco-2 cells. (a) Control (●) and washed (○) HT29-H monolayers. (b) Control (■) and washed (□) Caco-2 monolayers. Data points are means ± S.D.; $n = 3$.

TABLE 3

Effect of washing on the apparent permeability coefficient (P_{app}) of testosterone in HT29-H and Caco-2 cell monolayers

Monolayers	P_{app} ($\times 10^6$) (cm/s) [a]	
	HT29-H	Caco-2
Control	6.8 ± 1.0	31.5 ± 5.3
Washed	21.5 ± 5.9	32.1 ± 9.3
p	< 0.02	n.s.

[a] Values are means ± S.D.; $n = 3$ (HT29-H) and $n = 6$ (Caco-2). To avoid removal of the mucus layer the apparent permeability coefficients were determined in unstirred monolayers (see Materials and Methods). Tests of the significance (p) of differences between mean values of washed and control monolayers were made by using a two-tailed Student's t-test for unpaired data. n.s., not significant ($p > 0.05$).

of the mucus-free Caco-2 monolayers was observed (Table 3). Interestingly, the apparent permeability coefficients of testosterone in Caco-2 monolayers, before and after washing, were approx. 50% higher than those obtained in washed HT29-H monolayers ($p < 0.05$ and not significantly different, respectively). The most likely explanation for this difference was that the residual mucus layer (after washing) still formed a significant barrier to testosterone absorption. To test this hypothesis, the apparent permeability coefficients of testosterone were determined at two different stirring rates and permeability coefficients of the cell monolayers were calculated as described previously (Karlsson and Artursson, 1991, 1992) (Table 4). Clear increases in the apparent permeability coefficients of testosterone were observed under the stirred conditions. In addition most of the difference between the apparent permeability coefficients in HT29-H and Caco-2 monolayers disappeared (Table 4). This was not a result of any effect on epithelial integrity since no changes in transepithelial electrical resistance or mannitol permeability were observed even at the highest stirring rate (Table 5). This indicates that most of the 50% difference in the apparent permeability coefficients of washed HT29-H and Caco-2 monolayers (Table 3) was a result of the barrier properties of the remaining mucus.

TABLE 4

Cellular permeability coefficients (P_c) of testosterone in HT29-H and Caco-2 monolayers

Monolayers	Stirring rate (rpm)	Permeability coefficients ($\times 10^6$) (cm/s) [a]	
		P_{app}	P_c
HT29-H	135	70.0 ± 3.4 (3)	124.7 ± 2.7 (6)
washed	1090	103.3 ± 3.1 (3)	
Caco-2	135	61.6 ± 10.4 (6)	139.6 ± 8.5 (12)
	767	104.2 ± 9.3 (6)	$p < 0.001$

[a] Values are means ± S.D. for the number of determinations indicated within parentheses. The significance (p) of the difference between the mean values of P_c in the Caco-2 and HT29-H cell monolayers was tested by using the two-tailed Student's t-test for unpaired data.

TABLE 5

Effect of stirring on the apparent permeability coefficient (P_{app}) of mannitol and the transepithelial electrical resistance (TEER) of washed HT29-H cell monolayers

Stirring (rpm)	P_{app} [a] ($\times 10^6$) (cm/s)	TEER [a] (Ω cm^2)
0	2.2 ± 1.3 (9)	118 ± 42 (3)
135	2.9 ± 1.3 (3)	171 ± 29 (3)
1090	3.4 ± 0.4 (3)	146 ± 16 (3)
p	n.s.	n.s.

[a] Values are means ± S.D. for the number of determinations indicated within parentheses. Tests of the significance (p) of differences between mean values at 0, 135, and 1090 rpm were made by using a one-factor analysis of variance (ANOVA). n.s., not significant ($p > 0.05$).

However, a small (approx. 10%) but significant difference in the permeability of the cell monolayer remained, ($p < 0.001$) (Table 4). The remaining difference is probably associated with differences in the epithelial barrier properties. At least two possible explanations of the difference can be found after a morphological examination of the two different cell monolayers (Fig. 2). Firstly, Caco-2 cells, being the more differentiated phenotype, express a higher density of microvilli on their apical membrane and should therefore have a larger absorptive surface area than HT29-H goblet cells. Secondly, more than 80% of the cells in HT29-H monolayers express the morphology of mature goblet cells defined by clusters of large mucin granules localised in the apical part of the cells (Wikman et al., 1993) (Fig. 2). Thus, it is fully possible that testosterone interacted not only with extracellular mucus, but also with intracellular mucin molecules.

The results show that the extracellular mucus produced by HT29-H goblet cell monolayers is a significant barrier to the passive absorption of the lipophilic and uncharged drug testosterone. The

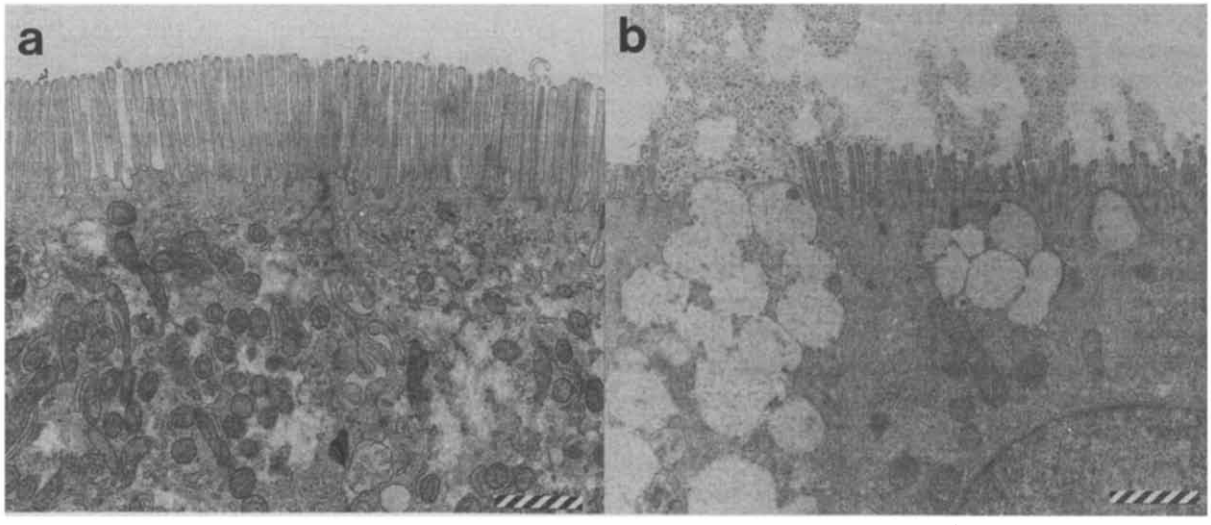

Fig. 2. Transmission electron micrographs showing the apical membranes of (a) Caco-2 absorptive cells and (b) HT29-H goblet cells. Note the difference in the length and number of microvilli. The bar indicates 1 μm.

resistances to absorption of the different barriers in the HT29-H model, i.e., the epithelial cells, the unstirred water layer and the mucus layer, are shown in Fig. 3. In the unstirred situation, the cell monolayer was the smallest absorption barrier. However, it should be noted that in the well-stirred in vivo situation, the unstirred water layer is minimal (Levitt et al., 1990, 1992; Strocchi and Levitt, 1991) and the absorption barrier of the cell monolayer becomes more important (Karlsson and Artursson, 1992). It is clear from Fig. 3 that the mucus layer is the largest barrier to absorption of testosterone. Thus, in mucus-covered HT29-H monolayers, the relative resistance to absorption of the mucus layer is 78% while the corresponding values for the unstirred water layer, the cell monolayer and the filter are 16, 5 and 1%, respectively. The results are summarized in Fig. 4 where the apparent permeability coefficients and the resistances to absorption of the mucus layer are represented as a function of the thickness of the mucus layer.

The applicability of HT29-H monolayers as a model for the intestinal mucus barrier will depend on several factors including

(1) The results of further characterization of the mucin molecules produced by this and similar cell lines. It is well established that tumour cell lines such as HT29-H produce mucins that are structurally different from those produced by normal goblet cells and this may influence the properties of the mucus gel (Maoret et al., 1989; Capon et al., 1992).

(2) The reproducibility of the cultivation of HT29-H monolayers. We have observed that HT29-H monolayers do not always form confluent monolayers and a demanding sorting procedure based on examination in the light microscope must be used in order to eliminate subconfluent monolayers (Wikman et al., 1993).

(3) The binding capacity of the mucus layer. The amount of mucus in the present model is relatively low and this limits the binding capacity of the mucus layer. While this may not be a problem when radioactively labelled drugs are studied it may be a limitation in studies with unlabelled drugs, i.e., when higher amounts of drugs are needed.

In conclusion, our results clearly indicate that HT29-H monolayers can be used to study the barrier functions of an intact human intestinal mucus layer in cell culture. To the best of our knowledge, this is the first report of the quantitative measurement of a human mucus layer as a barrier to drug absorption.

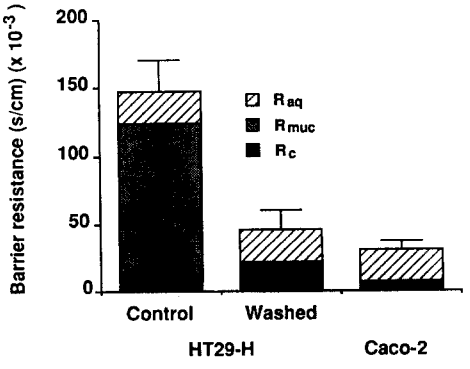

Fig. 3. Resistance to absorption of testosterone across the different barriers in the cell models: the cell monolayer (R_c) (filled bars), the unstirred water layer (R_{aq}) (hatched bars) and the mucus layer (R_{muc}) (stippled bars). Height of bars indicates total mean resistance ± S.D.; $n = 3$.

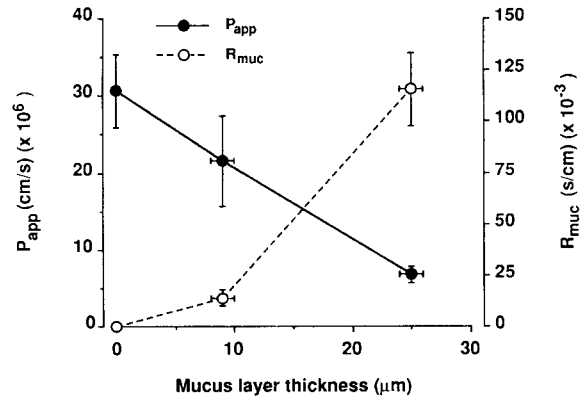

Fig. 4. Absorption of testosterone in the HT29-H model. Apparent permeability coefficient (●) and resistance of the mucus barrier (○) as a function of the measured thickness of the mucus layer. The apparent permeability coefficient of mucus-free monolayers ($P_{muc} = 0$) was calculated according to Eqn 7. Values are means ± S.D. (± S.E. for the mean thickness of the mucus layer); $n = 3$.

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