A Drug Absorption Model Based on the Mucus Layer Producing Human Intestinal Goblet Cell Line HT29-H

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A new drug absorption model based on monolayers of the human intestinal goblet cell line HT29-H grown on permeable filters has been characterized. HT29-H cells have been shown (a) to form monolayers of mature goblet cells under standard cell culture conditions, (b) to secrete mucin molecules, (c) to produce a mucus layer that covers the apical cell surface, and (d) that this mucus layer is a significant barrier to the absorption of the lipophilic drug testosterone. This is the first demonstration of an intact human mucus layer with functional barrier properties produced in cell culture. The results indicate that monolayers of HT29-H cells provide a valuable complement to mucus-free drug absorption models based on absorptive cell lines such as Caco-2 cells.

KEY WORDS: drug absorption; mucus layer; HT29; Caco-2; epithelial; cell culture.

INTRODUCTION

Recently, monolayers of a number of human intestinal epithelial cell lines have been introduced as drug absorption models. The best characterized of these is the Caco-2 cell line (1). The differentiation of Caco-2 cells is characteristic of mature enterocytes, and their functional properties are specific for transporting epithelia (2). Thus, monolayers of Caco-2 cells act as rate-limiting cellular barriers to passive and active absorption of drugs (3–5). However, absorptive cells, including Caco-2 cells and other cell lines used as drug absorption models, do not produce a mucus layer (6). The production of mucin molecules (which are responsible for the gel properties of the mucus layer) is handled by another cell type of the intestinal epithelium: the goblet cells.

Several goblet cell clones have recently been established from the human intestinal epithelial cell line HT29 (7–11). The clones differentiate to monolayers with a large proportion of mature goblet cells (9,11). The goblet cell monolayers have been shown to secrete mucin molecules and to empty their mucin granules in response to various secretagouges (9). Whether the secreted mucin molecules form a mucus layer on the apical cell surface has not been established.

Thus cultured goblet cells would provide a drug absorption model incorporating the (extracellular) mucus barrier to drug absorption, thereby permitting detailed studies on the barrier properties of an intact human intestinal mucus layer. The significance of the mucus layer as a barrier to drug absorption is unclear. Results by Winne suggest that the mucus barrier is of limited importance since mucus decreased the diffusion coefficient of various nutrients and drugs by only approximately twofold (12). A recently established correlation between drug absorption in the mucus-free Caco-2 cell culture model and that in humans also suggests that the mucus barrier is of limited importance (13). Nevertheless, other studies suggest that the mucus layer can be a rate limiting barrier to drug absorption. For instance, Nimmerfall *et al.* showed that the absorption of a series of alkaloids correlated with their diffusion coefficients in a mucus layer (14).

In this paper, we characterize monolayers of a new goblet cell line, HT29-H, isolated from the human intestinal adenocarcinoma cell line HT29. The cell line forms confluent monolayers that contain mainly mature goblet cells. HT29-H cells secrete mucin molecules and produce a mucus gel layer that can be visualized on the apical surface on living cells. Further, preliminary results with testosterone suggest that the mucus layer may be a significant barrier to drug absorption. The results indicate that monolayers of HT29-H cells can be used as a drug absorption model for studies on the influence of the mucus layer on intestinal drug absorption.

MATERIALS AND METHODS

Cells

HT29-H cells was a generous gift from Dr. Daniel Louvard, Institut Pasteur, Paris (7). HT29-H cells were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, 1% nonessential amino acids (MEM), benzylpenicillin (100 U/mL), streptomycin (10 μg/mL), and human transferrin (5 μg/mL; Sigma, St Louis, MO), in an atmosphere of 90% air and 10% carbon dioxide, at 37°C (9). All tissue culture media were obtained from Gibco through Laboratorie design AB, Lidingö, Sweden. The medium was changed every second day.

Polycarbonate cell culture inserts (Transwell, 0.45-μm pore size; Costar, Cambridge, MA) and mixed cellulose ester cell culture inserts (Millicell-HA, 0.45-μm pore size; Millipore, Bedford, MA) were coated with 53 μg rat tail type I collagen/cm^2 (Sigma, St Louis, MO), dissolved in 1 part 1 *M* hydrochloric acid and 3 parts 60% ethanol. The collagen-coated filters were dried overnight in a laminar airflow hood and rinsed two times with phosphate-buffered saline, Dulbecco's, without calcium, magnesium, and sodium bicarbonate (PBS). HT29-H cells were seeded onto the collagen-coated cell culture inserts at a density of 8.5×10^5 cells/cm^2. Experiments were performed on 20- to 43-day-old monolayers. Cells of passage numbers 25–35 were used.

Caco-2 cells (15) were obtained from the American Type Culture Collection, Rockville, MD. The Caco-2 cells were cultivated under the same conditions as the HT29-H cells except that the cell culture medium did not contain human transferrin and that uncoated cell culture inserts were used (4). The Caco-2 cells were seeded onto the cell culture inserts at a density of 4.2×10^5 cells/cm^2. Experiments were

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performed on 7- to 28-day-old monolayers. Cells of passage numbers 87–95 were used.

Cell Density

The cell density of HT29-H cells was determined on 20- to 40-day-old cell monolayers. The cell monolayers were stained with the fluorescent probe Hoechst 33258. The stained cell nuclei were counted from photomicrographs taken in a fluorescence microscope, Axioskop, Zeiss, Oberkochen, Germany. Approximately 1000 cells were counted on each cell culture insert.

Permeability Studies

The integrity of the monolayers was studied by determining the transepithelial electrical resistance (TEER). Hanks' balanced salt solution (HBSS) was used as bathing solution, adding 3.0 and 4.0 mL to the apical and basolateral 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 programable current source (Keithley 224) 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 resistance of cell culture inserts without cells.

Absorption studies were performed with the marker molecule ^{14}C-mannitol, NEN Research Products, Boston, MA, at a concentration of 4 μ*M* as described by Artursson (4). Apparent permeability coefficients (P_{app}) were calculated according to

$$P_{app} = dQ/dt \times 1/A \times C_0$$

where dQ/dt is the permeability rate, A the surface area of the monolayer, and C_0 the initial concentration in the donor chamber.

Transmission Electron Microscopy

Cells were rinsed with PBS 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 uranylacetate and lead citrate. The specimens were studied in a Philips 420 electron microscope operated at 60 kV and photographs were taken.

Goblet Cell Density

The percentage of goblet cells in confluent HT29-H monolayers was determined in the transmission electron microscope by counting the number of cells with a large number of mucin granules gathered near the apical cell membrane.

Labeling and Identification of Secreted Mucin Molecules

HT29-H cells were cultured on cell culture inserts for 21 days. The radioactive precursors ^{3}H-glucosamine (20 μCi; sp act, 52.7 Ci/mmol) and ^{35}S-SO_4 (400 μCi; sp act, 1300 Ci/mmol; New England Nuclear Research Products, Boston, MA) were then added to each cell culture insert. After 24 hr the apical 2 mL and basolateral 2 mL of cell culture media and the mucus layer were analyzed for mucin. The apical and basolateral media were removed and the apical cell surface was washed with 2 mL of fresh medium to remove the mucus layer. The medium was removed and the apical cell surface was washed a second time. The samples were stored at −70°C until further analysis. Samples from two cell culture inserts were pooled (approx. 4 mL) and diisopropylphosphorofluoridate (50 μL of a 0.1 *M* solution in propanol) was added. Samples were then dialyzed in the cold against 5 vol of 6 *M* guanidinium chloride/5 m*M* *N*-ethylmaleimide/10 m*M* sodium phosphate buffer, pH 6.5, to remove excess radiolabeled precursor. After dialysis, CsCl and buffer were added to a final concentration of 4 *M* guanidinium chloride and an initial density of approx. 1.39 g/mL, and isopycnic density-gradient centrifugation was performed in a Beckman 70-Ti rotor (40,000 rpm, 15°C, 65 hr). The tubes were emptied from the bottom and fractions (approx. 0.75 mL) were collected with a fraction collector equipped with a drop counter (16). Aliquots (250 μL) were taken for radioactivity measurements in a liquid scintillator (Packard Instrument Co., Downers Grove, IL).

Measurement of the Mucus Gel Layer Thickness

The mucus layer was visualized by a modification of the procedures developed by Kerss *et al.* (17) and Sandzen *et al.* (18). Thin strips were cut from the viable cell monolayers grown on cell culture inserts (Millicell-HA) with a knife made of two razor blades mounted on each side of an objective glass. The cell strips were then stained with 0.25 or 1% toluidine blue in PBS for approx. 1 min. The strips were mounted transversely in silicone holders placed on an objective glass. The cells were maintained in their own cell culture medium during the measurements. The cell monolayer was stained dark blue and could therefore be distinguished from the more weakly stained mucus layer. The strips were viewed with an inverse phase-contrast microscope and the thickness of the mucus layer was recorded at intervals of approx. 100 μm with the aid of an eyepiece graticule. One hundred to three hundred serial measurements were made on each strip and two or three monolayers were used for each experiment. Three to five strips were obtained from each monolayer. The percentage surface coverage of the mucus layer was calculated according to

$$\% \text{ surface coverage} = \frac{\text{number of measurements of mucus covered areas}}{\text{total number of measurements}} \times 100$$

The thickness of the mucus layer was constant for at least 2 hr in the culture medium. Monolayers of mucus-free Caco-2 cells were used as controls.

Removal of the Mucus Gel Layer

Twenty-eight-day-old monolayers of HT29-H cells were washed by agitation on a Plate-shaker (Flow Laboratories, England, UK) at 135 rpm for 10 min with 2 mL fresh HBSS on the apical and basolateral side each. The HBSS was replaced with fresh HBSS and the agitation was repeated two times. Alternatively, monolayers were incubated with 10 mg/mL of the mucolytic agent, *N*-acetylcysteine, in HBSS for 1 hr. The reduction of the mucus gel layer thickness and surface coverage compared to untreated monolayers from the same batch was determined.

RESULTS

Characterization of HT29-H Monolayers

Measurements of transepithelial electrical resistance (TEER) and mannitol permeability indicated that HT29-H monolayers were more leaky than Caco-2 cell monolayers (Table I). Thus, 28-day-old monolayers of HT29-H cells had a transepithelial electrical resistance that was approximately four times lower than that of the Caco-2 monolayers. The permeability to the hydrophilic marker molecule, ^{14}C-mannitol, was approx. 50 times higher in the HT29-H monolayers than in the Caco-2 monolayers (Table I).

The distributions of the TEER and mannitol permeabilities were approximately the same for the two cell lines. However, as a result of the high mean permeability of the HT29-H monolayers, a fraction of the monolayers had very low TEER. Examination of these low-resistance monolayers by fluorescence microscopy revealed small areas on the porous filters that were not covered by cells (not shown). However, monolayers having a resistance of $\geq 50\ \Omega \times cm^2$ were continuous. Therefore, only monolayers having a TEER $\geq 50\ \Omega \times cm^2$ were used in subsequent studies.

Morphology of HT29-H Monolayers

Electron microscopy of confluent HT29-H cells grown on polycarbonate cell culture inserts showed monolayers of differentiated epithelial cells with tight junctions and apical brush borders. The monolayers consisted of cells with different phenotypes. The dominating cell type had a morphology similar to that of mature goblet cells. In these cells, clusters of large mucin granules were localized in the apical part of the cells (Fig. 1). The fraction of mature goblet cells was approx. 80%. Other cell populations included absorptive-like cells and immature goblet cells with few mucin granules (Fig. 1).

Often extracellular material could be observed above the apical mucin granules of the goblet cells. Examination of the apical part of the goblet cells at a higher magnification suggested that this material was secreted from mucin granules into the apical cell culture medium (Fig. 2).

Table I. Characteristics of HT29-H and Caco-2 Cell Monolayers[a]

Characteristic	HT29-H	Caco-2
Cell density (cells/cm^2 × 10^{-6})	1.02 ± 0.22 (11)	0.91 ± 0.04 (3)[b]
Transepithelial electrical resistance ($\Omega \times cm^2$)	100 ± 49 (64)	410 ± 67 (99)
Mannitol permeability, P_{app} (cm/sec × 10^6)	4.2 ± 2.7 (35)	0.08 ± 0.04 (25)

[a] The results are expressed as mean ± SD and the number of experiments is given in parentheses.
[b] From Ref. 4.

Identification of an Apical Mucus Layer

After cultivation of the HT29-H monolayers for 30 days, a gel layer could be visualized on the apical side of the monolayers using phase-contrast microscopy (Fig. 3). The gel layer was weakly stained by toluidine blue, indicating that it contained mucin molecules. No gel layer was seen in Caco-2 monolayers of the same age (not shown).

Mucin molecules were identified in the apical cell culture medium and in the gel layer after incubation of the monolayers with the metabolic precursors ^{3}H-glucosamine and ^{35}S-SO_4 (Fig. 4). Isopycnic density-gradient centrifugation in CsCl/4 *M* guanidinium chloride of the apical culture medium, showed a prominent ^{3}H-rich peak at a density of about 1.42 g/mL typical of mucus glycoproteins (Fig. 4a, arrow). Most of the radiolabel appeared at the top of the gradient as expected for proteins and lipids, and little material labeled with sulfate was found. The basolateral material, however, did not display a peak at 1.42 g/mL in the gradient, (Fig. 4b, arrow), and most of the ^{3}H-containing material was again present at the top of the gradient. A significant ^{35}S-labeled component at the high-density end of the gradient is presumably a connective tissue-type proteoglycan or glycosaminoglycan. A sharp peak at a density of 1.42 g/mL, corresponding to the putative mucin on the apical side of the cells, was the major component of the material removed by washing the cell monolayer (Fig. 4c, arrow). The result is consistent with the presence of an adherent mucus layer on the cells that is gradually dispersed and shed into the medium. Little of this material remained after washing with medium a second time, (Fig. 4d, arrow), suggesting that these molecules are loosely attached to the cell surface rather than firmly anchored to the cell membrane as expected for a mucus gel.

Thickness and Surface Coverage of the Mucus Layer

A gradual increase in thickness and surface coverage of the mucus layer was observed with time (Fig. 5). After approx. 2 weeks of culture, a thin and incomplete mucus layer could be seen. The thickness and surface coverage increased with time, attaining an average thickness of 40–60 μm and a surface coverage of >95% after approx. 4 weeks. The surface topography of the mucus layer was uneven and the thickness varied from 0 to >100 μm on a single strip (Fig. 6). Washing or incubation with *N*-acetylcysteine reduced the thickness and surface coverage of the mucus layer significantly. Thus, after washing the apical side of the monolayers, the thickness and surface coverage were reduced to 38

Fig. 1. Transmission electron micrograph showing a mature HT29-H goblet cell with apically clustered mucin granules, microvilli, and a basolaterally located cell nucleus. The cell to the left is an absorptive-like cell. The bar indicates 1 μm.

and 70%, respectively, compared to untreated monolayers from the same batch ($P < 0.0001$ and $P < 0.009$). The corresponding values for the *N*-acetylcysteine treatment were 22 and 43% ($P < 0.0001$ and $P < 0.002$). The Mann–Whitney *U* test was used for statistical analysis.

Effect of the Mucus Layer on the Absorption of Testosterone

The passive absorption of testosterone was investigated before and after washing the HT29-H and Caco-2 monolayers (Fig. 7). The washing procedure increased the absorption

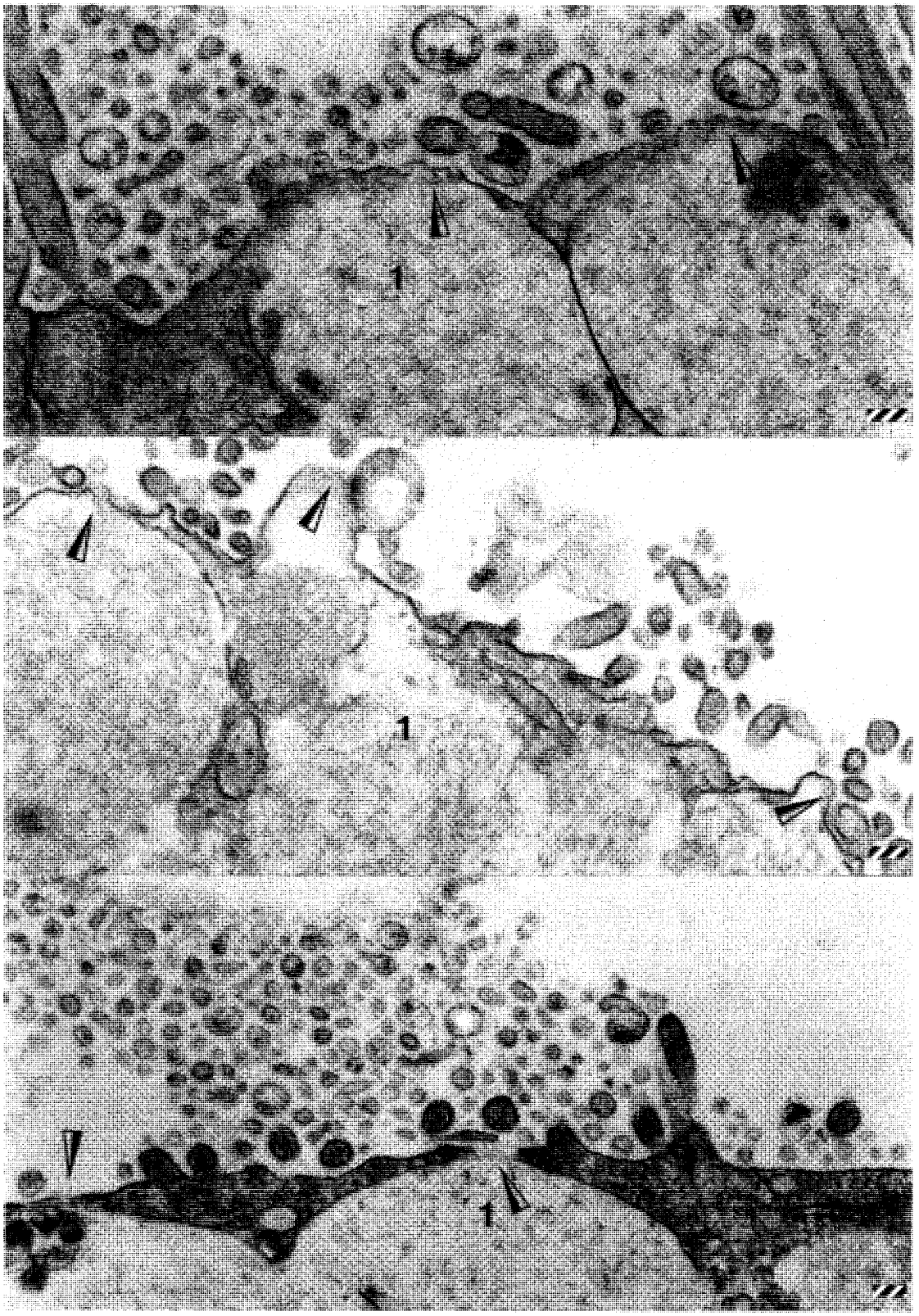

Fig. 2. Transmission electron micrograph showing mucin granules (1) that are fusing with the apical cell membrane (arrowheads) and releasing their content. The bars indicate 0.1 μm.

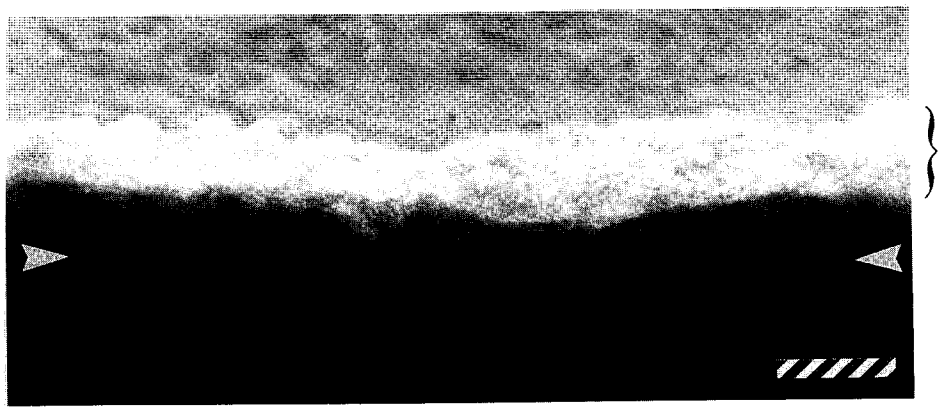

Fig. 3. Photograph on a strip of a viable HT29-H cell monolayer as it appears in the light microscope. The focus is on the mucus layer (bracket) and no individual cells are visible. The middle light area is the mucus gel layer and the lower black area is comprised of the cell monolayer and the porous filter support. The arrowheads indicate the border between the cell monolayer and the filter. The bar indicates 50 μm.

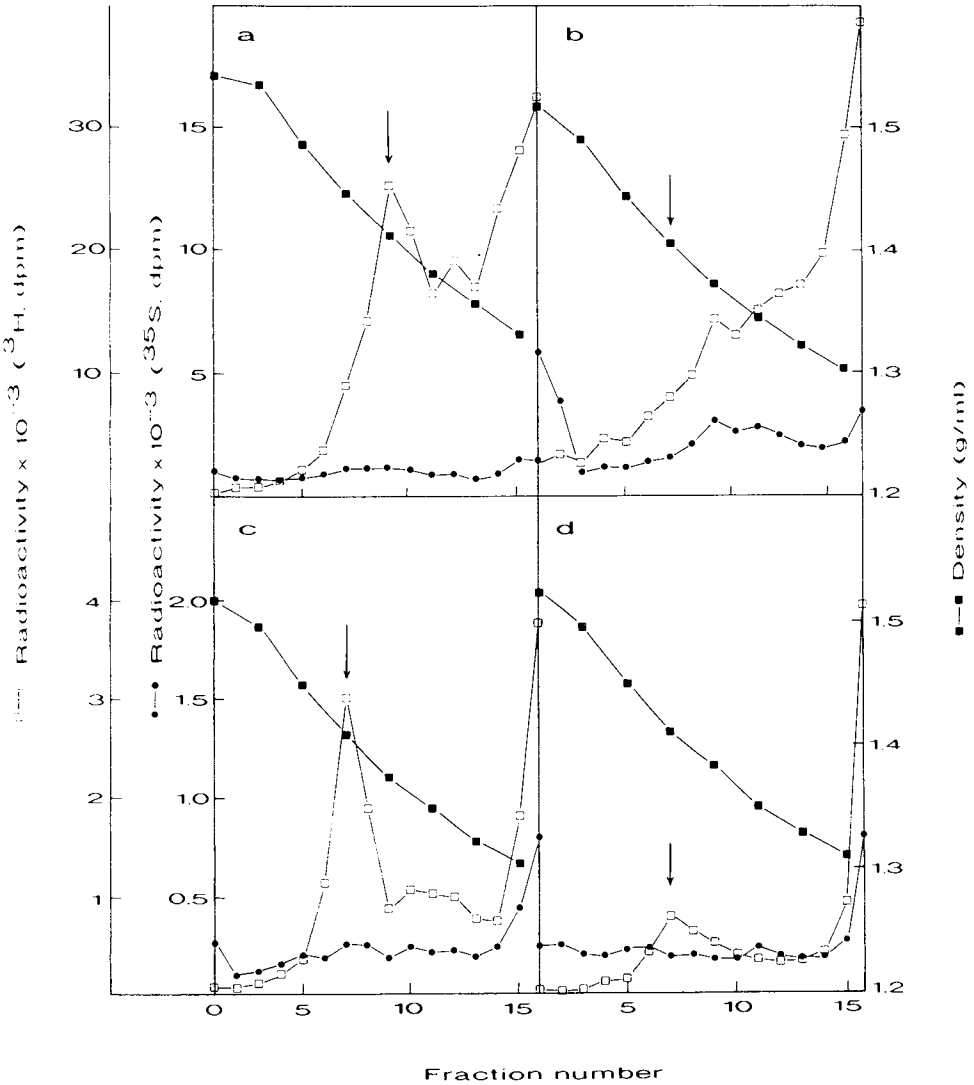

Fig. 4. Isopycnic density-gradient centrifugation in CsCl/4 *M* guanidinium chloride of (a) the apical culture medium, (b) the basolateral culture medium, (c) the adherent mucus-like material, and (d) "adherent material remaining after washing." The arrows indicate the density at which mucin molecules appear. Samples were adjusted to a density of 1.39 g/mL in 4 *M* guanidinium chloride and spun for 65 hr in a Beckman 70.1-Ti rotor at 40,000 rpm and 15°C. Fractions were collected from the bottom of the tubes and analyzed for ^{3}H (□——□), ^{35}S (●——●), and solution density (■——■). Note that the initial density in a was 1.41 g/mL instead of 1.39 g/mL; the mucin peak still appears at the same density.

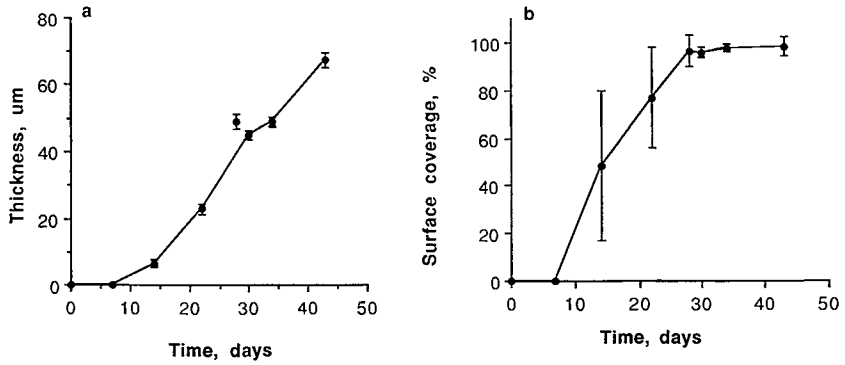

Fig. 5. (a) Mucus layer thickness as a function of time. Means ± SE. (b) Surface coverage of the mucus layer as a function of time. Means ± SD.

rate of testosterone across the HT29-H monolayers approximately twofold. The absorption rate in the Caco-2 cell monolayers remained unchanged. No difference in the TEER could be observed in the HT29-H and Caco-2 monolayers (not shown) after the washings. These results indicate that the washing procedure did not effect the epithelial integrity. However, the permeability of testosterone in the washed HT29-H monolayers was approximately four times lower than that in Caco-2 cell monolayers (Table II). Further, the absorption rate in the HT29-H monolayers decreased at later time points.

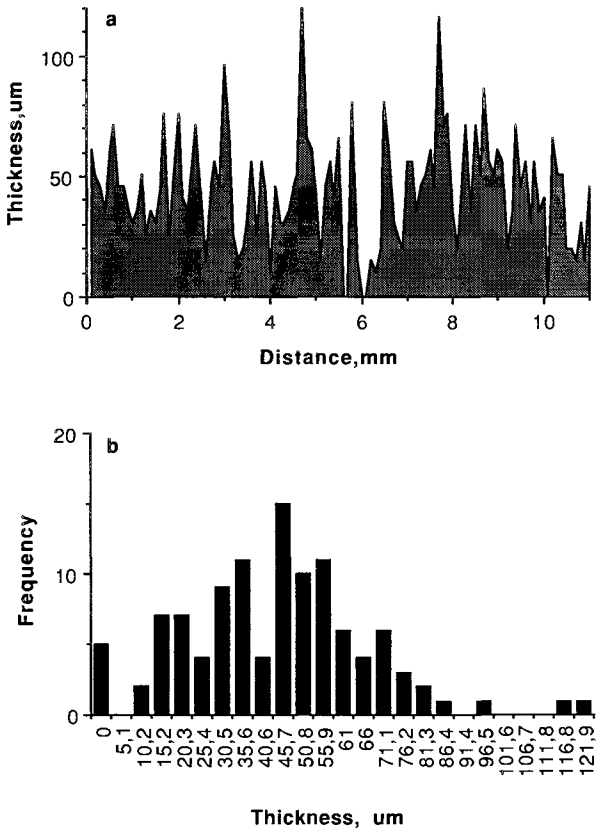

Fig. 6. (a) Variation in mucus thickness along a single strip from a viable monolayer of HT29-H cells. (b) Thickness distribution of the strip in a.

DISCUSSION

Extracellular mucus layers are normally partly or completely lost during routine fixation of mucosal tissues (19). Further, since mucus gels contain approx. 95% water, the dehydration steps cause a dramatic shrinkage of the mucus gel volume. Therefore, methods of measuring mucus layer thickness and surface coverage in viable tissues have been developed (17,18). However, none of these methods were directly applicable to the cell culture monolayers, and therefore a new method for determining mucus layer thickness and surface coverage was developed. Using this method, the formation of the mucus layer could be followed with time. The mucus layer was not visible until after approx. 2 weeks of culture, i.e., at a time when the HT29-H cells form almost confluent monolayers with a high proportion of mature goblet cells. Using another goblet cell clone, HT29-18N2, Phillips *et al.* observed that the goblet cells showed an increased degree of differentiation with time in culture (9). Four to eight days after confluence, the cell monolayers consisted of a mixture of predifferentiated, differentiated, and well-differentiated cells, while 8–15 days after confluence uniform monolayers of well-differentiated cells were obtained. Thus, the 2-week lag time before the appearance of the mucus layer represents the time required for the cells to develop into well differentiated goblet cells.

Studies with ^{3}H-glucosamine-labeled molecules in HT29-18N2 goblet cells indicated a turnover time of between 12 and 24 hr, a time roughly comparable to that found in explants of human large intestine (20). This contrasts with the apparently slow formation of the mucus layer in the HT29-H monolayers. However, the slow formation of the mucus layer is consistent with a rapid turnover of mucin molecules: The mucus layer was easily washed off the monolayers, suggesting that mucin molecules are continuously released from the apical side of the mucus layer, resulting in a rapid turnover of mucin molecules in the mucus layer. The release of metabolically radiolabeled putative mucin molecules into the apical medium was approximately eightfold larger than the radioactivity of the mucin fraction in the mucus layer (as calculated from the peak values of the mucin fractions in Figs. 4a and c), indicating that the turnover of mucin molecules is faster than, and does not reflect, the turnover of the mucus layer.

It should also be noted that the radiolabeled precursor is

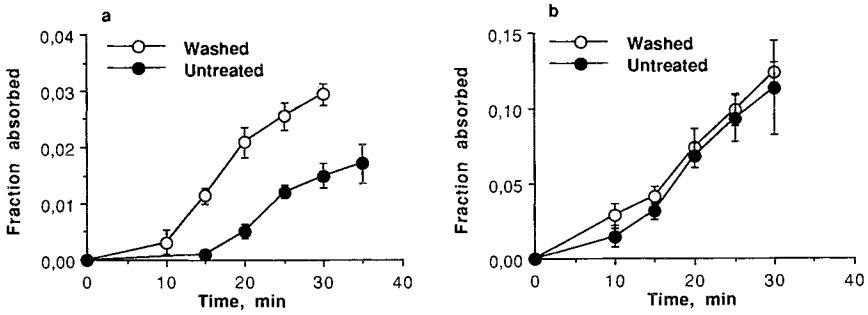

Fig. 7. (a) Absorption of ^{3}H-testosterone across washed and untreated monolayers of HT29-H cells. (b) Absorption of ^{3}H-testosterone across washed and untreated monolayers of Caco-2 cells.

incorporated not only into the mucin molecules, but also into other glycoproteins. The ^{3}H-glucosamine turnover (without the additional purification of the radiolabeled glycoproteins, e.g., an isopycnic density gradient) is therefore a rough assay of the turnover of a heterogeneous population of glycoproteins rather than mucin molecules (21).

The thickness of the mucus layer produced by HT29-H cells was less than the 80 μm reported for rat duodenum (22) and 180 μm reported for mouse colon (23). However, the variation in the thickness of the HT29-H mucus layer was large and comparable to the in vivo situation. A detailed analysis of the thickness of rat, frog, and human gastric mucus layers showed variations from 5 to 400 μm in a single mucosa (17).

The paracellular permeability of the HT29-H (goblet cell) monolayers was significantly higher than the Caco-2 (absorptive cell) monolayers. This is in agreement with the *in vivo* situation: it has been shown that tight junctions of goblet cells are more permeable to ions and have a more varied structure than those of absorptive cells in the rat small intestine (24). The heterogeneous structure of the tight junctions of goblet cells is presumably related to the large variations in the permeability of this cell population. Indeed, reports on TEER's of other HT29 goblet cell clones reveal significant differences between the clones (11,25).

The tight junction permeabilities of human intestinal cell lines that form monolayers of differentiated absorptive cells are often lower than those in whole tissues (4,26). As a result, drugs that are absorbed by the paracellular route are absorbed at very slow rates across these cells. One possible reason for the low permeability is the absence of a more leaky goblet cell population. The results in this study suggest that the permeability could be increased if cocultures between absorptive and goblet cell lines could be established. Not only would the permeability of those cocultures be more similar to the *in vivo* situation, but being composed of the two major cell types of the intestinal epithelium, they would therefore contain all the major cellular and extracellular barriers to drug absorption. Preliminary data from Allen *et al.* suggest that HT29-H cells and Caco-2 cells can be cocultivated (27).

Testosterone was chosen as a model drug for the absorption studies since it is lipophilic and therefore rapidly absorbed across the intestinal epithelium and since its membrane permeability in different intestinal epithelial cells is constant (26). We have shown previously that the absorption rate of rapidly absorbed drugs is unaffected by differences in paracellular permeability (28). Thus, the permeability of testosterone in HT29-H and Caco-2 monolayers could be compared (despite the clear difference in paracellular permeability of these two cell lines).

The twofold increase in absorption rate for testosterone after removal of the mucus layer clearly indicates that the drug and the mucus layer interact. In order not to saturate potential binding sites for testosterone in the mucus layer, only small quantities of the drug were used. The mucus layer, nevertheless, did not function as a complete barrier to testosterone absorption, indicating only weak interactions between the mucus layer and the drug. This is consistent with the postulate that mucus acts as a diffusion barrier for small molecules such as nutrients and ions while it is a more complete barrier for larger molecules and microorganisms (29). Previous studies with other drugs suggest that electrostatic as well as hydrophobic interactions may be of importance in drug–mucin interactions (14,30). In addition, hydrogen bonding to the hydroxyl groups of the carbohydrate residues is of importance (31). Since testosterone is an uncharged, lipophilic drug [$\log D_{(\text{oktanol/water; pH 7.4})}$ of 3.31 (32)] with one hydroxyl group, it is possible that hydrophobic interactions as well as hydrogen bindings were formed between the drug and the mucus layer. At present we cannot explain why the permeability of testosterone across the HT29-H monolayer is four to six times lower than across the Caco-2 monolayers or why the transport rate of testosterone becomes slower at later time points. Presumably an interaction between testosterone and the concentrated mucin molecules in the numerous mucin granules of the HT29-H cells is implicated.

Table II. Permeability of Testosterone Across HT29-H and Caco-2 Cell Monolayers[a]

	Permeability, P_{app} (cm/sec × 10^6)	
	HT29-H	Caco-2
Control	6.0 ± 0.6	35.3 ± 6.6
Washed	9.4 ± 1.2*	38.9 ± 2.6 (ns)[b]

[a] The results are expressed as mean ± SD; $n = 3$.
[b] Not significantly different from control monolayers.
* Significantly different from control monolayers ($P < 0.02$), two-tailed Student's t test.

A possible drawback of the present model is that the mucus layer produced by HT29-H cells could differ from a normal intestinal mucus layer. First, the continuous exposure to the serum-containing cell culture medium may influence the composition of the mucus layer. Additional factors that may modify the composition of the mucus layer in cell culture include the absence of luminal enzymes and microorganisms. Moreover, intestinal goblet cells produce multiple mucin species. The production of these species does not occur uniformly within the epithelium but is restricted to distinct subpopulations of goblet cells (29). It is therefore plausible that HT29-H, which is a clonal cell line, produces only a fraction of the mucin molecule species found in a mucus layer of the normal human intestine (8). However, although it is possible that the mucus layer produced by HT29-H cells is slightly different from a normal mucus layer, it is the first example of a human mucus layer with functional barrier properties produced in cell culture. Monolayers of HT29-H cells therefore provide a unique model for studying the effects of a native human mucus layer on drug and peptide absorption.

In conclusion, the results in this study show that the human goblet cell line HT29-H produce a mucus layer with significant barrier properties. This permits, for the first time, study of the influence of an intact human mucus layer on drug and peptide absorption under controlled cell culture conditions. Monolayers of HT29-H cells are therefore an interesting complement to mucus-free drug absorption models based on absorptive cell lines such as Caco-2 cells. An extension of this work would be the establishment of cocultures with Caco-2 cells in the proportions found *in vivo* as well as studies on mucoadhesion.

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