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Permeable support type influences the transport of compounds across Caco-2 cells

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Summary

The influence of aluminium oxide and nitrocellulose inserts on the uptake and transport of taurocholic acid in Caco-2 cells has been determined. Caco-2 cells grown on nitrocellulose inserts displayed a higher rate of taurocholic acid transport than those grown on aluminium oxide inserts; 59.3 ± 4.1 ng 4 h^{-1} $insert^{-1}$ compared to 29.7 ± 4.1 ng 4 h^{-1} $insert^{-1}$ at day 14. In addition, Caco-2 cells grown on aluminium oxide and nitrocellulose inserts were not comparable with respect to adsorption potential, cell morphology, cell numbers and transepithelial electrical resistance. The low adsorption potential of aluminium oxide inserts, particularly for high molecular weight or lipophilic ligands such as propranolol and testosterone, allowed basolateral uptake events to be studied with a precision not possible with nitrocellulose inserts. In addition, the translucent nature of aluminium oxide inserts offered significant advantages over the nitrocellulose insert in terms of visualising the cells by light microscopy. Caco-2 cells grown on either type of insert appear fully differentiated when observed by light and electron microscopy. Occasional dome-like structures were present when Caco-2 monolayers were grown on aluminium oxide but not nitrocellulose inserts. Cell numbers were significantly lower on aluminium oxide inserts than on nitrocellulose inserts. Moreover, Caco-2 cells grown on aluminium oxide inserts displayed a higher transepithelial electrical resistance than those grown on nitrocellulose inserts; 871 ± 149 compared to 513 ± 32 at day 14. The reasons for these differences and the respective merits of each type of insert are considered.

Introduction

Current knowledge of the mechanisms available for the absorption of drugs and nutrients from the gastrointestinal tract (GI tract) has been developed using a variety of models. These include luminal perfusion, isolated loops, everted sacs, intestinal rings, brush-border membrane vesicles and isolated enterocytes (Csáky, 1984; Osiescka et al., 1985). Each of these systems lacks the versatility and viability for detailed study of transport across the GI epithelium which is the major cellular barrier to drug absorption. The development of primary intestinal cell culture has been limited by poor retention of anatomical and biochemical features found in vivo (Moyer, 1983). Recently, the enterocyte-like Caco-2 cell line, de-

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rived from a colon adenocarcinoma (Fogh et al., 1977; Pinto et al., 1983), has been proposed as a useful in vitro transport model for the distal ileal epithelium (Hidalgo et al., 1989; Artursson, 1990; Artursson and Magnusson, 1990; Wilson et al., 1990). When Caco-2 cells are grown on permeable supports the cells form a confluent monolayer. Moreover, they display low permeability to macromolecules whilst expressing many transport systems for low molecular weight molecules, such as amino acids (Hidalgo and Borchardt, 1990a), bile acids (Hidalgo and Borchardt, 1990b; Wilson et al., 1990; Woodcock et al., 1991) and vitamin B_{12} (Dix et al., 1990) in a specific and polar fashion. In addition, passive transport of drug molecules across Caco-2 monolayers correlates well with their oral bioavailability in vivo (Artursson and Karlsson, 1991; Cogburn et al., 1991).

We have previously reported that the Caco-2 cell line is a unique in vitro model with respect to the polar transport of taurocholic acid (Wilson et al., 1990). This process is specific and saturable with an apparent K_m for the transcellular transport of [^{14}C]taurocholic acid of 42.5 ± 2.8 μM. This apparent K_m is consistent with the reported value of 37 ± 7 μM (Barnard and Ghishan, 1987). We have recently isolated clones from the Caco-2 cell line that showed marked differences in the amounts of taurocholic acid actively transported (Woodcock et al., 1991). Whilst some clones did not actively transport taurocholic acid, others transported much higher amounts than the parental population. As part of a detailed study to analyse the factors affecting the drug transport in GI epithelial cells we have investigated the influence of the type of permeable support used.

Ideally, a permeable support for the Caco-2 model should promote enterocyte-like differentiation but should not influence uptake and transport kinetics of the monolayer. For example, when Caco-2 cells are grown on nitrocellulose inserts, which are polymeric microporous membranes manufactured from mixed esters of cellulose, they display enterocyte-like behaviour and this combination has been used successfully to study transport and secretion phenomena (Rindler and Traber, 1988; Dix et al., 1990; Wilson et al., 1990). However, these inserts are thick (150 μm; pore size 0.45 μm) and it has been proposed that significant molecular adsorption may limit their application when investigating the uptake and transport properties of some compounds (Wilson et al., 1990). Recently, the aluminium oxide culture insert has been introduced which has a thin (45 μm), rigid, membrane. Its high-porosity, controlled pore size (0.2 μm at the upper surface and 0.02 μm at the lower surface or 0.2 μm throughout in the case of a specially prepared batch) and low adsorption characteristics offer potential advantages over other inserts as permeable cell culture supports for uptake and transport studies. We have compared the adsorption, morphological and functional properties of Caco-2 cells cultured on aluminium oxide and nitrocellulose inserts and assessed their relative merits with respect to taurocholic acid uptake and transport.

Materials and Methods

Cell culture

The Caco-2 cell line was obtained from Professor Colin Hopkins, Imperial College, University of London. Cells were cultured in 150 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum, 1% non-essential amino acids and 1% glutamine (maintenance medium). All medium components were obtained from Gibco (Paisley, U.K.). Flasks were incubated at 37°C in a humidified atmosphere of 10% CO_2 and 90% air. The culture medium was renewed on alternate days. Stock cultures were passaged (1:3) weekly by trypsinisation with 0.25% trypsin and 0.2% Na_2EDTA in phosphate-buffered saline (PBS; Oxoid, U.K.). Cells of passage number 94–108 were used.

For permeable support cultures, 2–3 day post-confluent monolayers were trypsinised and resuspended in maintenance medium supplemented with 1% penicillin (10000 IU ml^{-1}) and streptomycin (10 mg ml^{-1}) (permeable support medium). The apical chambers of aluminium oxide (Anocell, Anotec separations, Banbury, U.K.) and nitrocellulose (Millicell HA, Millipore, Bedford, MA, U.S.A.) inserts were seeded with 4 ml

(2.0×10^6 cells per insert) and 3 ml of permeable support medium added to the basolateral chamber. The inserts were not disturbed for 72 h, thereafter, the permeable support medium was replaced on alternate days.

Permeable support adsorption studies

Aluminium oxide and nitrocellulose inserts were conditioned for 2 h in permeable support medium. They were washed with PBS before incubation (1 ml apical, 1 ml basolateral) with [^{3}H]leucine, [^{14}C]mannitol, [^{35}S]methionine, [^{14}C]polyethylene glycol 4000 (PEG 4000), [^{3}H]-phenylalanine, [^{3}H]proline, [^{14}C]taurocholic acid and ^{125}I-human calcitonin (all Amersham, U.K.), [^{3}H]thyrotrophin releasing hormone and [^{3}H]-testosterone (Dupont, Germany). After 4 h at 37°C the inserts were washed three times with ice-cold PBS containing sodium azide (0.05% w/v). The residual insert radioactivity of ^{3}H, ^{14}C and ^{35}S ligands was measured using a Beckman LS1801 scintillation counter following removal of each insert into a vial containing Lumagel (Lumac, The Netherlands; 12 ml). Inserts incubated with ^{125}I-ligands were analysed using an LKB 1260 Multigamma-II gamma spectrometer. The insert-associated radioactivity was expressed as a percentage of the total radioactivity in the incubation chamber.

Light microscopy and cell counting

Caco-2 monolayers on aluminium oxide inserts were photographed during culture (Leica Diavert, Wild MPS 51S and Photoautomat 45). For cell counting, the monolayers grown on both types of insert were photographed after fixing and staining at days 5, 10, 14, 20 and 25. Briefly, the cells were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde for 1 h then rinsed with PBS. The monolayers were stained with Harris' haematoxylin (BDH, U.K.) for 5 min, washed with water that was slightly alkaline after the addition of a few drops of 35% ammonia and finally destained with acidified methanol. The inserts were dehydrated with an ascending series of ethanol (50, 70, 90, 95 and 100%) and washed in xylene before mounting onto glass slides with DPX (BDH, U.K.). The monolayers were photographed (Leitz Labovert, Wild MPS 51S and Photoautomat 45). Six photographs were taken randomly from each sample, the nuclei counted and used to calculate the number of cells per insert.

Transmission electron microscopy (TEM)

Cells cultured on aluminium oxide and nitrocellulose inserts at 5, 10, 14, 20 and 25 days post-seeding were washed with fresh maintenance medium and fixed with a solution of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3 for 2 h at 25°C, then washed with 0.1 M cacodylate buffer. Aluminium oxide inserts were processed in situ, whereas nitrocellulose inserts were removed from their housings and cut into squares for subsequent processing. The cells were post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer before dehydration through an ascending series of ethanol (70, 90 and 100%). Inserts were embedded in Epon resin and incubated at 60°C for 48 h. The aluminium oxide inserts were embedded in their housings and the nitrocellulose squares were embedded in flat embedding moulds. Cells were sectioned on a Reichert OM4 microtome, stained with uranyl acetate and lead citrate, visualised and were photographed with a Philips CM10 electron microscope.

Scanning electron microscopy (SEM)

Cells were fixed and dehydrated using the procedures for TEM. At the final 100% ethanol wash, the samples were processed in an Edwards freeze-drier (Cambridge, U.K.). Inserts were glued onto an SEM stub, and a fine gold film evaporated onto the surface in an Edwards sputter coater. The cells were viewed in a Cambridge Stereoscan 200 electron microscope.

Transepithelial electrical resistance (TER)

Minimum essential medium (MEM), supplemented with 0.1% bovine serum albumin (BSA) was added to the apical (5 ml) and basolateral (4 ml) chambers and allowed to equilibrate to 25°C for 15 min. The TER of each monolayer was measured using an epithelial voltmeter (EVOM, World Precision Instruments, U.S.A.). The total resistance (cell monolayer + insert) was sub-

tracted from the intrinsic resistance of the system (insert alone) to give monolayer resistance. The resistance was corrected for surface area (aluminium oxide, 4.15 cm^2; nitrocellulose, 4.2 cm^2) and expressed as Ω cm^2.

Taurocholic acid transport

Caco-2 monolayers were washed with PBS before 0.2 μCi of [^{14}C]taurocholic acid (Amersham, U.K.; 56 mCi $mmol^{-1}$) in 2 ml of permeable support medium was added to the apical or basolateral chamber. They were incubated at 37°C in a humidified atmosphere of 10% CO_2 and 90% air. After 4 h, the inserts were transferred to a cold table (0–4°C), the apical and basolateral transport media collected and the monolayers washed with ice-cold PBS-sodium azide. The ^{14}C content of each sample was measured using a Beckman LS1801 scintillation analyser with

TABLE 1

Percentage ligand adsorption to aluminium oxide and nitrocellulose permeable supports

Ligand	Percentage absorbed to insert	
	Nitrocellulose	Aluminium oxide
^{125}I-Human calcitonin	29.70	0.38
[^{3}H]Leucine	0.19	< 0.02
[^{35}S]Methionine	0.54	< 0.02
[^{14}C]Mannitol	0.10	< 0.02
[^{14}C]PEG 4000	36.50	0.11
[^{3}H]Phenylalanine	0.40	< 0.02
[^{3}H]Proline	0.10	0.04
[^{3}H]Propranolol	25.40	0.05
[^{14}C]Taurocholic acid	0.40	< 0.02
[^{3}H]Testosterone	72.70	0.01
[^{3}H]Thyrotropin releasing hormone	0.30	0.03

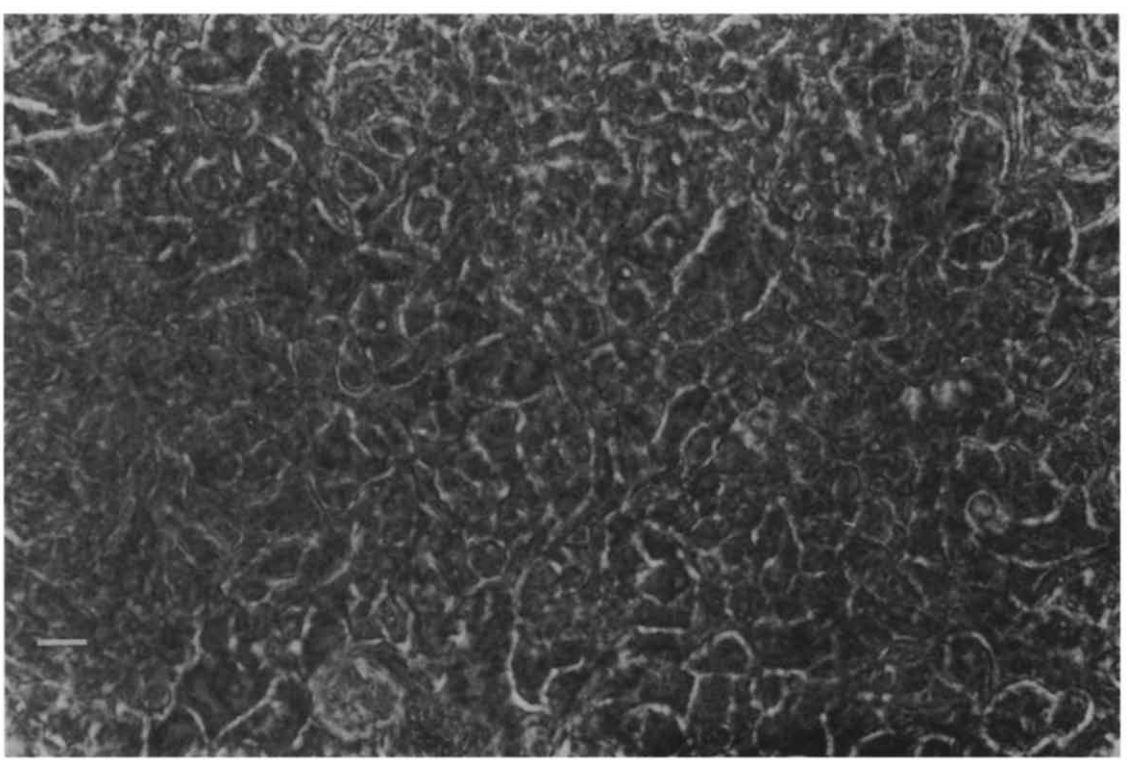

Fig. 1. Phase-contrast light micrograph of day 19 Caco-2 cells grown on an aluminium oxide insert. Bar scale represents 50 μm.

Lumagel as the scintillant. The appearance of ^{14}C at the opposite side of the monolayer gave total (specific + non-specific) transport. The non-specific transport component was determined by measuring transport in the presence of a 1000-fold molar excess of taurocholic acid. Specific transport was calculated by subtraction of the total and non-specific transport components. Similarly, [^{14}C]taurocholic acid uptake was measured by removing the insert from its housing using a specialised cutting tool (aluminium oxide) or scalpel (nitrocellulose) and its ^{14}C content determined as described above.

Proline transport

Amino acid-free buffer used for proline transport experiments consisted of 1% BSA, 3.2 mM calcium chloride, 1.2 mM magnesium chloride, 4 mM potassium chloride, 150 mM sodium chloride and 5 mM glucose in double-distilled water, buffered to pH 7.4 with Hepes. Caco-2 monolayers were washed once with PBS and finally with amino acid-free buffer before 2.0 μCi of [^{3}H]proline (Amersham, U.K.; 26 Ci mmol^{-1}) in 2.0 ml (44 nM) of amino acid-free buffer was added to the apical chamber. After 30 min at 37°C the inserts were transferred to a cold table (0–4°C) and the apical and basolateral transport media collected. The monolayers were washed three times with ice-cold PBS-sodium azide. The ^{3}H content of each sample was measured using a Packard Tricarb 2000CA liquid scintillation analyser with OptiPhase HiSafe 3 (LKB) as the scintillant. The appearance of ^{3}H at the basolateral side of the monolayer gave total (specific + non-specific) transport. The non-specific transport component was estimated by measuring transport in the presence of a 1.0×10^{6} molar excess of proline. Specific transport was calculated by subtraction of the total and non-specific transport components.

Results

Ligand adsorption

The amount of ligand adsorption to aluminium oxide and nitrocellulose inserts is listed in Table 1. There was much greater adsorption to nitrocellulose than to aluminium oxide inserts in all cases, but especially for the high molecular weight and lipophilic ligands, [^{14}C]polyethylene glycol 4000, ^{125}I-human calcitonin, [^{3}H]testosterone and [^{3}H]propranolol. For example, 36.5% of the applied dose of [^{14}C]polyethylene glycol 4000 adsorbed to the nitrocellulose insert whilst only 0.11% adsorbed to the aluminium oxide insert. In the case of [^{3}H]propranolol, 25.4% adsorbed to the nitrocellulose insert whereas 0.05% adsorbed to the aluminium oxide inserts. Significant adsorption of [^{3}H]propranolol to nitrocellulose inserts has been reported previously and polycarbonate inserts were used in preference to reduce adsorption of this and other β-blocker drugs during transport studies (Wilson et al., 1990). Testosterone proved a most striking example with 72.7% adsorbed to the nitrocellulose insert whereas only 0.01% adsorbed to the aluminium oxide insert. This represents a differential in binding of over 5000-fold. [^{14}C]Taurocholic acid bound more

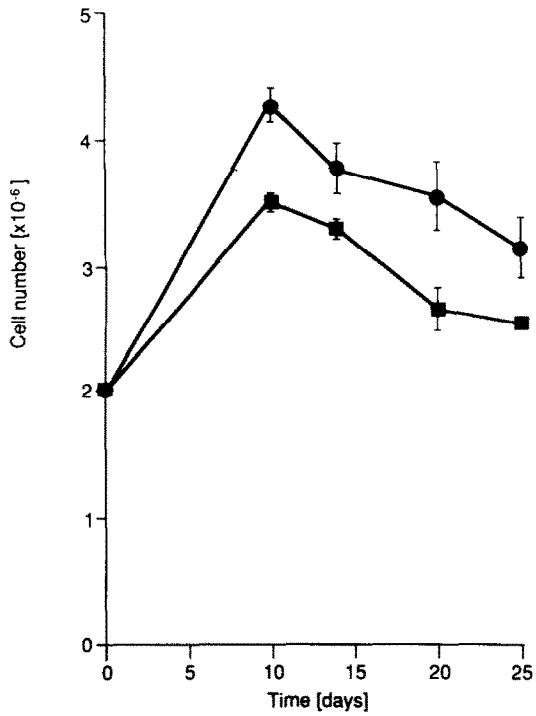

Fig. 2. Growth of Caco-2 cells on aluminium oxide inserts (■) and nitrocellulose inserts (●). Results + SE for six samples.

avidly to nitrocellulose inserts with 0.4% adsorption compared to less than 0.02% with the aluminium oxide inserts.

Light microscopy

Direct visualisation of Caco-2 monolayers on nitrocellulose inserts during culture was not possible due to the opacity of this support. In contrast, Caco-2 cells can be observed by phase contrast light microscopy when grown on aluminium oxide inserts allowing visual inspection of the monolayer before transport experiments. Fig. 1 shows a phase contrast light micrograph of Caco-2 cells grown on aluminium oxide inserts for 19 days. When visualised, individual Caco-2 cells become more discrete with time in culture, possibly reflecting greater monolayer organisation. After fixing and staining, a lower cell density on aluminium oxide compared with nitrocellulose inserts at each time interval was apparent (see next section). Monolayers cultured on aluminium oxide exhibited occasional blister-like domes similar to those formed by Caco-2 cells cultured on plastic (Pinto et al., 1983). This phenomenon was not observed when Caco-2 cells were grown on nitrocellulose inserts.

Cell counting

The observed lower cell density on aluminium oxide inserts was confirmed by cell counting (Fig. 2). After seeding with 2.0×10^6 cells, numbers increased initially to $3.5 \pm 0.1 \times 10^6$ in the case of aluminium oxide inserts and $4.3 \pm 0.1 \times 10^6$ with nitrocellulose inserts at day 10. Cell numbers decreased after 10 days to $2.5 \pm 0.1 \times 10^6$ and $3.1 \pm 0.2 \times 10^6$ at day 25 on aluminium oxide and nitrocellulose inserts, respectively. The decline in cell numbers after 10 days reflects cells sloughing off the insert as the monolayer becomes more organised. The optimal time for transport studies has been reported as 14–25 days post-seeding (Wilson et al., 1990). Over this period the cell

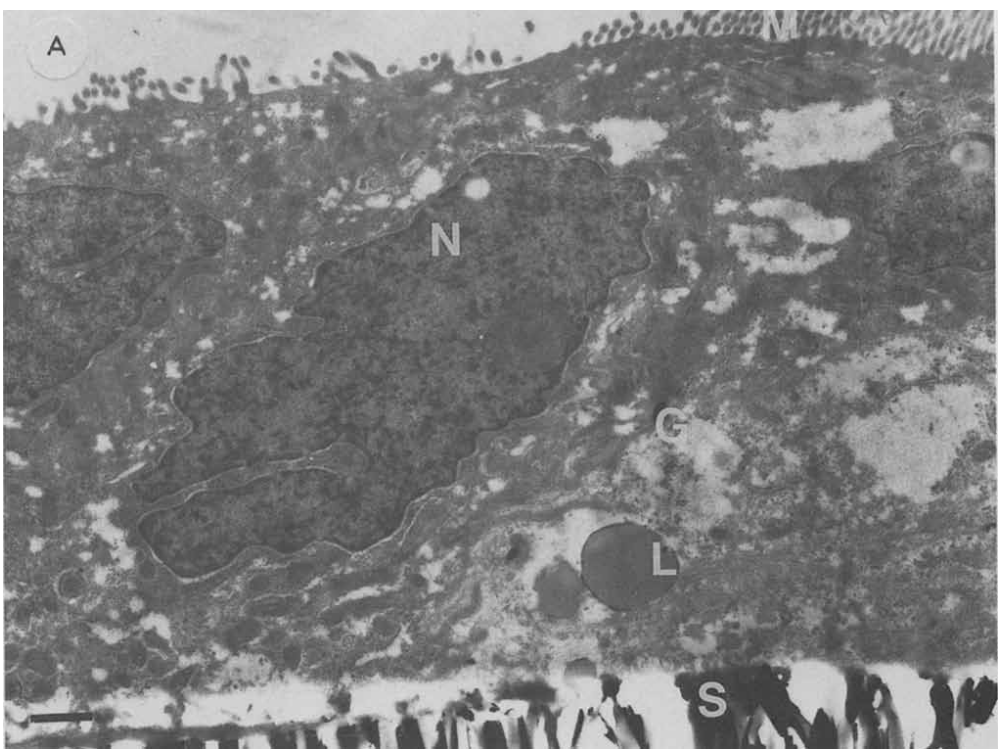

Fig. 3. TEM of day 20 Caco-2 cells cultured on (A) an aluminium oxide insert and (B) a nitrocellulose insert, showing the insert (S), glycogen (G), lipid vesicles (L), microvilli (M) and nuclei (N). Bar scale represents 1 μm.

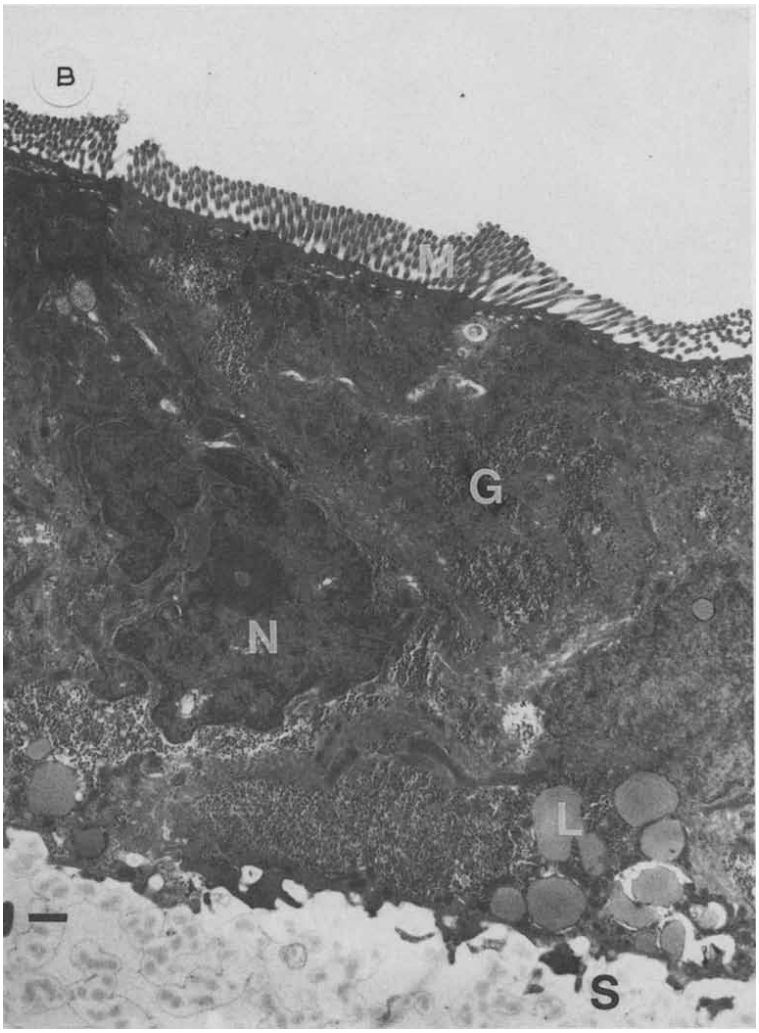

Fig. 3 (continued).

density on aluminium oxide inserts is significantly lower than on the nitrocellulose inserts.

Electron microscopy

Cells grown on either insert formed monolayers. Fig. 3A and B shows TEM of Caco-2 cells grown on aluminium oxide and nitrocellulose for 20 days, respectively. On nitrocellulose inserts, Caco-2 cells displayed the highly polarised morphology with good intracellular organisation described previously (Wilson et al., 1990). In contrast, cells grown on aluminium oxide inserts appeared less polar having irregular membrane processes and a dense disorganised cytoplasm with large areas of glycogen accumulation. Throughout the culture period the basolateral membrane of cells on the nitrocellulose insert had grown into the insert spaces whereas cells grown on the aluminium oxide insert did not penetrate the insert mesh. Microvilli were present on the apical surface of either aluminium oxide or nitrocellulose grown cultures. On nitrocellulose, apical microvilli were present as dense 'carpet-like' and clustered orientations whereas cells on aluminium oxide displayed sparse irregular clusters.

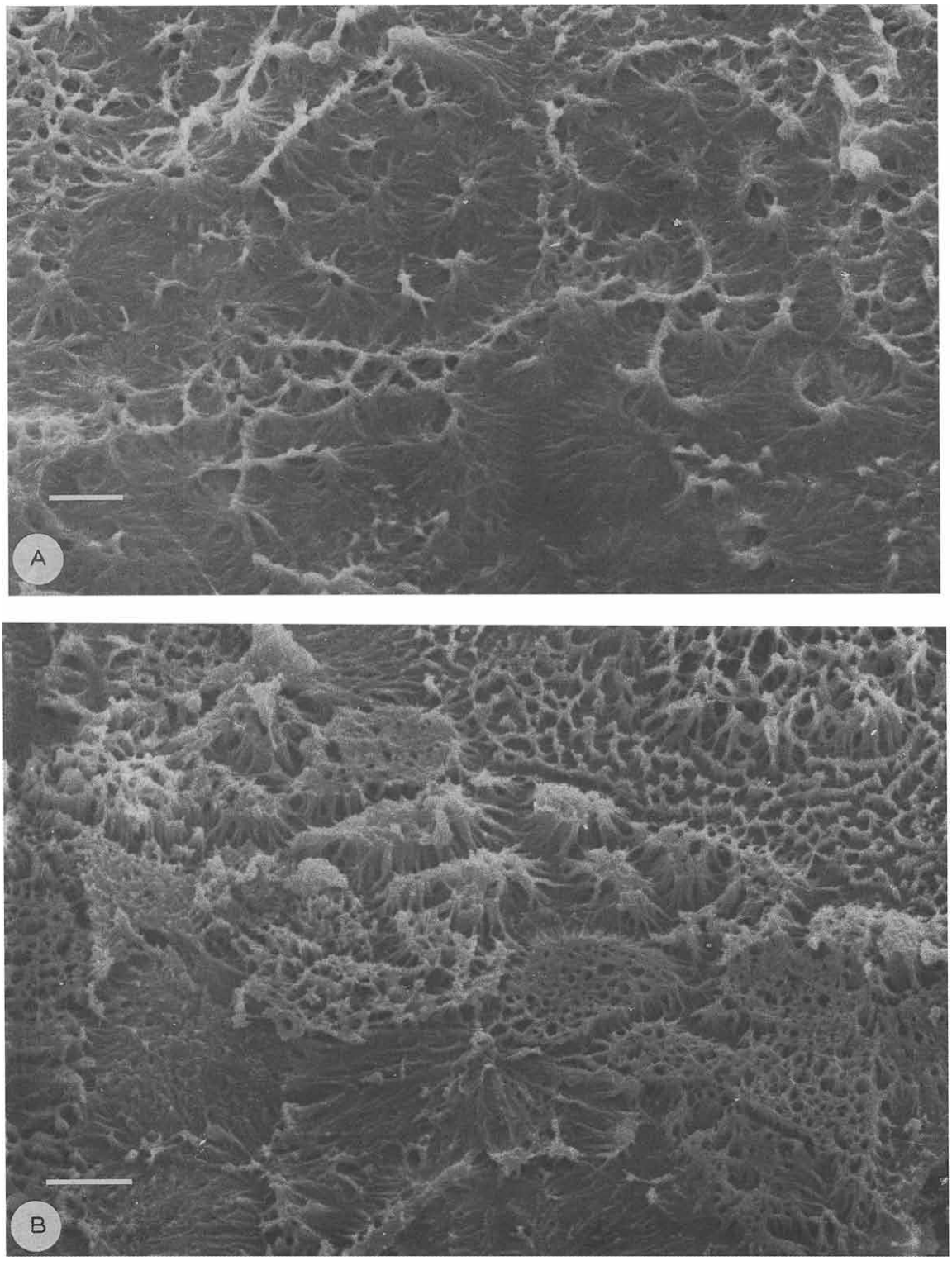

Fig. 4. SEM of day 14 Caco-2 cells cultured on (A) an aluminium oxide insert and (B) a nitrocellulose insert. Bar scale represents 1 μm.

These differences are demonstrated by SEM at day 14 (Fig. 4A and B).

The height of Caco-2 cells grown on aluminium oxide and nitrocellulose inserts is shown in Fig. 5A. The height of aluminium oxide grown cells remained constant throughout the culture period, 10.0 ± 0.7 μm at day 5 to 8.5 ± 1.0 μm at day 20. In contrast, cells grown on nitrocellulose inserts increased in height over the time period from 7.7 ± 0.8 μm at day 5 to 17.7 ± 0.6 μm at day 20. Fig. 5B shows the height of microvilli of Caco-2 cells grown aluminium oxide and nitrocellulose inserts. The height of microvilli remained constant for aluminium oxide grown cells, 1.4 ± 0.2 μm at day 5 to 1.3 ± 0.1 μm at day 20. The height of microvilli of nitrocellulose grown cells

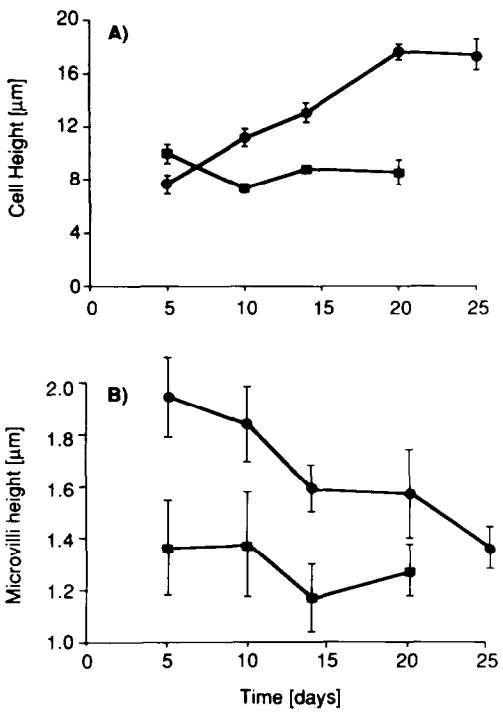

Fig. 5. (A) Cell height (μm) of Caco-2 cells with time on aluminium oxide inserts (■) and nitrocellulose inserts (●). (B) Microvilli height (μm) of Caco-2 cells with time on aluminium oxide inserts (■) and nitrocellulose inserts (●). The morphological measurements made on micrographs from at least six randomly chosen areas in the case of cell height and at least 11 randomly chosen areas in the case of microvilli height. Results ± SE.

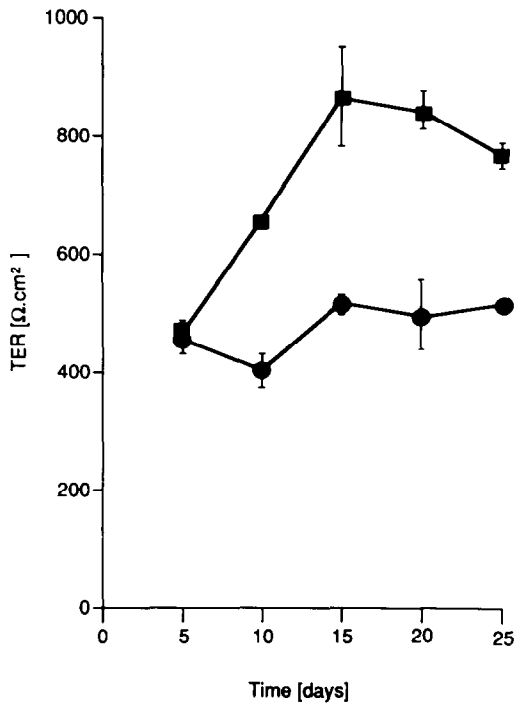

Fig. 6. TER of Caco-2 cells grown on aluminium oxide inserts (■) and nitrocellulose inserts (●) with time. Results ± SE for three samples.

was significantly higher at day 5, 1.9 ± 0.2 μm, although the height decreased over the time period to 1.6 ± 0.2 μm at day 20 which was not significantly different from the microvilli height of aluminium oxide grown cells at day 20.

TER

At 5 days after high density seeding (2.0×10^6 cells insert^{-1}), Caco-2 monolayers on aluminium oxide and nitrocellulose inserts have similar TER values, 460.0 ± 26.7 and 454.5 ± 53.4 Ω cm^2, respectively (Fig. 6). Therefore, electrically tight monolayers were rapidly formed on both inserts. However, as culture progressed Caco-2 cells grown on the two types of inserts developed disparate electrical characteristics. The TER of monolayers on nitrocellulose inserts increased only slightly through to day 25 of culture, remaining between 454.5 ± 53.4 and 524.1 ± 16.0 Ω cm^2. This is in good agreement with values achieved in previous studies from this laboratory (Dix et al.,

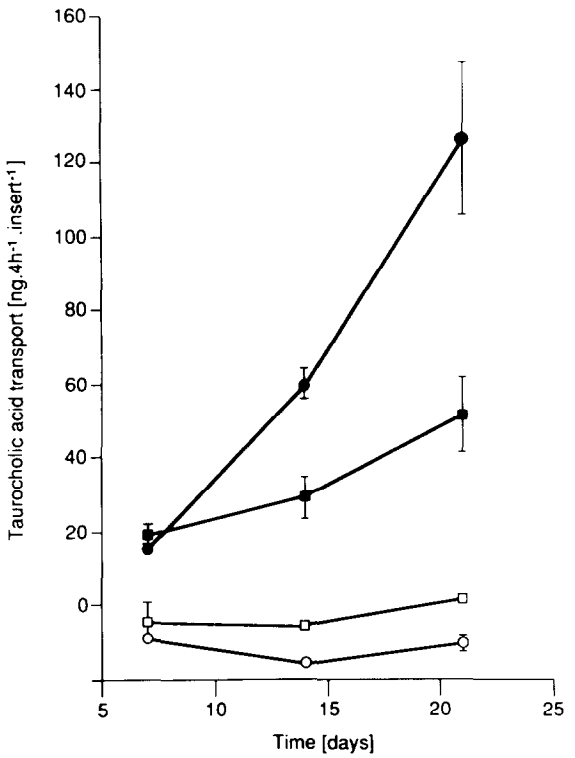

Fig. 7. Specific transport of [^{14}C]taurocholic acid across Caco-2 cells grown on aluminium oxide inserts (■, □) and nitrocellulose inserts (●, ○) in the apical to basolateral direction (■, ●) and in the basolateral to apical direction (□, ○). Results ± SE for three samples.

1990; Wilson et al., 1990). In contrast, the TER on aluminium oxide inserts increased up to day 14 (871.2 ± 149.7 Ω cm^2) and remained constant through to day 25 (780.7 ± 42.8 Ω cm^2).

Taurocholic acid uptake and transport

The ability of Caco-2 monolayers grown on aluminium oxide and nitrocellulose inserts to specifically transport taurocholic acid after 7, 14 and 21 days is illustrated in Fig. 7. On both types of insert, transport was highly polar, essentially only occurring in the apical to basolateral direction. However, higher rates of apical to basolateral transport were observed when Caco-2 cells were grown on nitrocellulose inserts (14.4 ± 0.8 ng 4 h^{-1} insert^{-1} at day 7 to 126.9 ± 13.2 ng 4 h^{-1} insert^{-1} at day 21) compared to aluminium oxide grown cells (13.2 ± 3.0 ng 4 h^{-1} insert^{-1} at day 7 to 51.5 ± 10.3 ng 4 h^{-1} insert^{-1} at day 21). Table 2 shows that the difference in the rate of transport was not due to the smaller pore size of the aluminium oxide inserts. The rate of taurocholic acid transport was not significantly different on normal pore size aluminium oxide inserts and a specially prepared batch where the pore size was 0.2 μm throughout.

Fig. 8A shows that the apical uptake of taurocholic acid into Caco-2 cells increased concomitantly with transport when cultured on nitrocellulose inserts, 1.53 ± 0.1 ng 4 h^{-1} insert^{-1} at day 7 to 3.2 ± 0.2 ng 4 h^{-1} insert^{-1} at day 21, but remained relatively constant with time on aluminium oxide inserts, 0.9 ± 0.4 ng 4 h^{-1} insert^{-1} at day 7 to 0.7 ± 0.1 ng 4 h^{-1} insert^{-1} at day 21.

Specific basolateral uptake of [^{14}C]taurocholic acid by Caco-2 monolayers on aluminium oxide and nitrocellulose inserts is compared in Fig. 8B. In the case of nitrocellulose insert grown cells the apparent large and positive basolateral uptake probably results from insert adsorption rather than genuine cellular uptake (see Table 1). However, on aluminium oxide inserts, basolateral uptake can be determined due to the low adsorption of [^{14}C]taurocholic acid by aluminium oxide inserts (see Table 1). In this case the lack of specific uptake is consistent with the lack of basolateral to apical transport (see Fig. 7).

Proline transport

Fig. 9 depicts the transport of proline across aluminium oxide and nitrocellulose inserts. The results demonstrated that there was no significant difference in the rate of proline transport on either insert at day 14 with total transport mea-

TABLE 2

The transport of taurocholic acid across Caco-2 cells grown on aluminium oxide inserts, normal pore size (0.2 μm upper surface to 0.02 μm lower surface) and special pore size (0.2 μm throughout)

Transport (ng^{-1} h^{-1} insert^{-1})	Normal insert	Special insert
Total	9.12 ± 0.32	9.92 ± 0.67
Non-specific	3.57 ± 0.74	4.48 ± 0.25
Specific	5.40 ± 0.63	5.41 ± 0.85

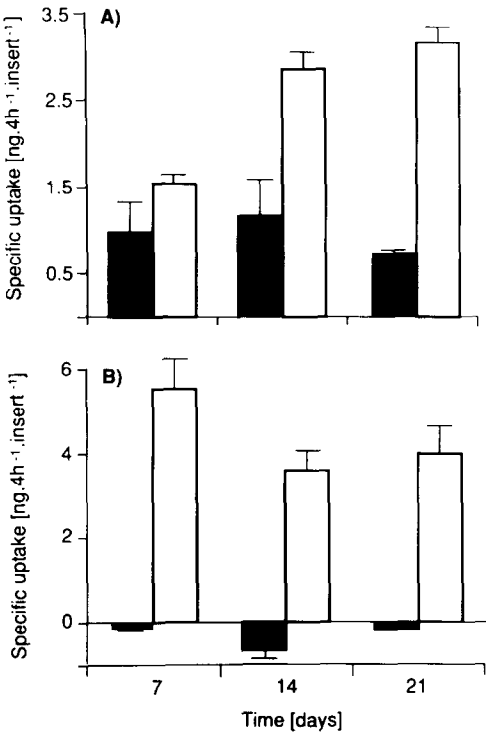

Fig. 8. (A) Apical uptake and (B) basolateral uptake of [^{14}C]taurocholic acid into Caco-2 cells grown on aluminium oxide inserts (filled bars) and nitrocellulose inserts (open bars). Results ± SE for three samples are shown.

sured at 1153.4 ± 201.0 fmol 30 min^{-1} insert^{-1} on aluminium oxide inserts and 1118.9 ± 120.5 fmol 30 min^{-1} insert^{-1} on nitrocellulose inserts.

Discussion

The ideal permeable support insert would not influence transport kinetics so that appearance in the apical or basolateral chamber accurately reflects permeation of the cellular barrier. This criterion can only be met by an insert with low adsorption properties. In this respect, the aluminium oxide insert is superior to the nitrocellulose insert. Therefore, use of aluminium oxide inserts will significantly enhance the Caco-2 transport model, notably for high molecular weight and/or lipophilic ligands. Previous studies have shown the development of Caco-2 cells to be dependent on the nature of their basement membrane (Stratford et al., 1988; Wilson et al., 1990). For example, Stratford and co-workers (1988) showed that 0.3% fluorescein isothiocyanate dextran (FITC-dextran) was transported across Caco-2 cells grown on Matrigel inserts (a solubilised tissue membrane preparation, consisting predominantly of laminin, type IV collagen, heparan sulphate proteoglycans and nidogen) compared with 0.04% transported across collagen coated nitrocellulose inserts.

Between days 14 and 25, the TER was approximately 350 Ω cm^2 higher when Caco-2 cells were grown on aluminium oxide inserts compared to those grown on nitrocellulose inserts. The TER across monolayers of Caco-2 cells cultured on 'permeable supports' predominately reflects the resistivity across tight junctions rather than the

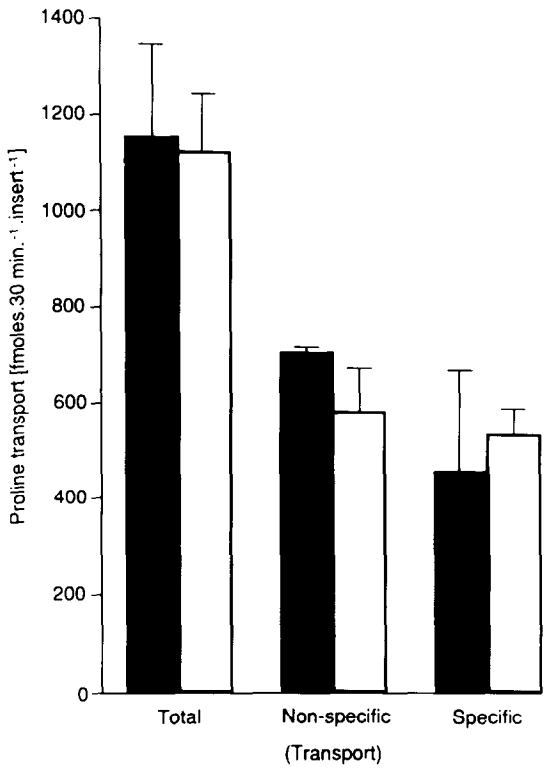

Fig. 9. Transport of [^{3}H]proline across Caco-2 cells grown on aluminium oxide inserts (filled bars) and nitrocellulose insert (open bars) in the apical to basolateral direction. Results ± SE for three samples.

cell membranes (Madara, 1983; Grassett et al., 1984). The lower cell density on aluminium oxide inserts results in a smaller number of intercellular contacts per unit area of monolayer, thereby increasing resistance to ion flux. This could partially explain the higher TER of Caco-2 monolayers on aluminium oxide inserts. However, the likeliest reason for the different electrical properties is due to the selection of different sub-populations of Caco-2 cells on the respective types of insert. We have previously isolated a number of clones which displayed a wide range of TER profiles (Woodcock et al., 1991). For example, at day 15 the TER of clones ranged from 100 to 1500 Ω cm^2. These clones were stable with respect to a number of electrophysiological, transport and morphological characteristics showing that the parental Caco-2 cell population consists of a number of sub-populations.

Caco-2 monolayers actively transport taurocholic acid in a polar fashion when grown on nitrocellulose (Wilson et al., 1990) or collagen-coated polycarbonate inserts (Hidalgo and Borchardt, 1990b). We have shown that this is also true for Caco-2 cells grown on aluminium oxide inserts. However, the rate of taurocholic acid transport differs depending on the type of insert. The difference in rate of transport can be partially explained in terms of cell numbers. However, after correction for this variant, taurocholic acid transport occurs almost twice as rapidly across Caco-2 cells cultured on nitrocellulose (35.7 ± 11.2 ng 4 h^{-1} 10^6 cells^{-1}) compared to aluminium oxide inserts (19.5 ± 8.3 ng 4 h^{-1} 10^6 cells^{-1}), suggesting that a functional difference exists between monolayers grown on different permeable supports. This difference can also be explained in terms of selection of a sub-population of Caco-2 cells. We have shown that the specific transport of taurocholic acid also varied with different clones grown on nitrocellulose inserts ranging from negligible amounts to approx. 150 ng 4 h^{-1} insert^{-1} at day 14 (Woodcock et al., 1991). The difference in rate of transport could not be explained by the difference in pore size between the insert types strengthening the claim that the difference is related to selection of a sub-population of the parental cell line.

The correlation between basolateral uptake and basolateral to apical transport of taurocholic acid is better defined when Caco-2 cells are grown on aluminium oxide inserts. This is due to the lack of adsorption to the aluminium oxide insert. Conversely, in the case of nitrocellulose inserts a relatively high adsorption to the insert conceals the true amount of basolateral uptake. Interestingly, specific apical to basolateral proline transport across Caco-2 cells was similar irrespective of the insert used for growth.

A number of conclusions can be drawn from this study. Firstly, the low adsorption potential of aluminium oxide is a significant advantage particularly for determining transport of lipophilic compounds. In addition, basolateral uptake is more accurately measured using aluminium oxide inserts. Secondly, the physical characteristics of aluminium oxide inserts make them better suited for light microscopy. Thirdly, it is evident that different sub-populations of Caco-2 cells grow on the respective inserts. This manifests itself in different TER profiles and different taurocholic acid transport characteristics. The benefits of using permeable supports for cell studies are obvious. However, the work presented here shows that consideration must be given to the type of insert used depending on the study to be undertaken.

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