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# Development of the $Na^+$-dependent hexose carrier in LLC-$PK_1$ cells is dependent on microtubules

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**The $Na^+$-dependent hexose carrier, an endogenous apical marker, develops during differentiation of LLC-$PK_1$, an established cell line with characteristics of the proximal tubule. This development was inhibited by the microtubule-disrupting drugs, colchicine and nocodazole, while it was insensitive to lumicolchicine. This strongly suggests that microtubules are involved in the plasma membrane expression of the $Na^+$-dependent hexose carrier. We also analyzed the increase in activity of endogenous apical and basolateral membrane proteins during the polarization process. The development of three apical ($Na^+$-dependent hexose carrier, γ-glutamyltransferase and alkaline phosphatase) and one basolateral membrane protein ($Na^+/K^+$-ATPase) was studied during the reorganization of LLC-$PK_1$ cells into a polarized epithelium. Colchicine inhibited the rapid, transient increase in the expression of the $Na^+$-dependent hexose carrier during this polarization process. A similar result was observed for the development of the other apical proteins, while the development of $Na^+/K^+$-ATPase seemed to be largely insensitive to colchicine. Our results are in agreement with the model that the vesicles containing the apical membrane proteins use microtubules as tracks to reach the plasma membrane. The transport of vesicles containing basolateral membrane proteins clearly occurs by a different pathway which is independent on an intact microtubular network. Since the inhibition by the microtubule-disrupting drugs was complete, it can be concluded that after disruption of microtubules, the apical vesicles do not use the basolateral pathway by default.**

## Introduction

Cells of transporting epithelia exhibit both structural and functional polarity. This is reflected in the asymmetric distribution of enzymes and transport activities between the apical and basolateral domain of the plasma membrane [1–3]. These two domains are separated by the tight junctions. The mechanisms by which epithelial cells generate and maintain the different composition of both membrane domains are largely unknown. Specific sorting pathways from the *trans* Golgi network to the different membrane domains have been postulated [3].

Using the established cell line, LLC-$PK_1$, we wanted to obtain an insight into a possible role of microtubules in the development of apical and basolateral proteins in renal epithelial cells. LLC-$PK_1$ is derived from the pars recta of the proximal tubule of a pig kidney and expresses a number of characteristic enzymes, e.g., γ-glutamyltransferase and alkaline phosphatase [4]. The apical membrane also contains transport systems in which $Na^+$ is cotransported with hexoses [5,6], amino acids [7] and phosphate [8] or in exchange with hydrogen ions [9]. The development and polarization of γ-glutamyltransferase and alkaline phosphatase [4], of the $Na^+/H^+$ antiport [10] and of the $Na^+$-dependent hexose carrier [11] have been studied during reorganization of LLC-$PK_1$ cells into a polarized epithelium. Using the non-metabolizable D-glucose analog, AMG, we focused on the development of the $Na^+$-dependent hexose carrier. This carrier shows a 2:1, $Na^+$:hexose stoichiometry [12] and is competitively inhibited by phlorizin [6]. An overview of the kinetic characteristics [13], as well as some aspects of the development of the carrier [14,15] were reported earlier.

Using colchicine and nocodazole, two microtubule-disrupting drugs [16,17], we present evidence that an intact microtubular network is essential for the delivery of the $Na^+$-dependent hexose carrier to the plasma membrane during differentiation as well as during

Abbreviations: AMG, α-methyl-D-glucoside; EMEM, Eagle's minimal essential medium; HBSS, Hanks' balanced salt solution; SDS, sodium dodecyl sulfate.

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polarization. The development of other apical membrane proteins (γ-glutamyltransferase and alkaline phosphatase) was also sensitive to colchicine, while for the development of the activity of a basolateral protein ($Na^+/K^+$-ATPase), an intact microtubular network seemed not to be a prerequisite. These findings for LLC-$PK_1$, a cell line with characteristics of the renal proximal tubule, confirm recent studies on cultured intestinal epithelial cells (Caco-2) [18], on proximal tubules [19] and on intestinal epithelium [20]. However, we have found in LLC-$PK_1$ cells that the $Na^+$-dependent hexose carrier can not use the basolateral pathway to reach the plasma membrane. This was also found in renal proximal tubules [19] but seems to be different in intestinal epithelial cells [18,20].

## Material and Methods

LLC-$PK_1$ cells (American Type Culture Collection CRL 1392/ CL 101) were obtained through Flow Laboratories Ltd. (Irvine, Ayrshire, Scotland) and were used between passages 187 and 202. Cells were cultured at 37°C in a humidified 5% $CO_2$ atmosphere using EMEM with 3.5 mM L-glutamine, 0.9% (v/v) non-essential amino acids, 87 μg/ml streptomycin, 87 IU/ml penicillin and 10% fetal calf serum.

To study the development of AMG uptake and of the activity of different enzymes during reorganization of a monolayer into a polarized epithelium, confluent and quiescent LLC-$PK_1$ cells (seeded at 20 000 cells/$cm^2$, 11 days old) were rinsed twice with EDTA (5 mM) (Flow Laboratories Ltd.) and harvested in a 0.05% (w/v) trypsin/ 5 mM EDTA mixture. After centrifugation (15 min, $320 \times g_{max}$), cells were resuspended in EMEM and seeded in 12-well clusters of 3.8 $cm^2$ or 6-well clusters of 9.5 $cm^2$ (Costar Europe, Badhoevedorp, The Netherlands) at a superconfluent density ($(6–8) \cdot 10^5$ cells/$cm^2$). Thymidine (final concentration: 10 mM) was added to the medium to arrest cell division. About 2 h 30 min after plating, the medium containing the nonattached cells was removed and new medium supplemented with the respective additives and thymidine (10 mM) was added. The delay of 2 h 30 min after seeding was introduced in order to avoid a possible effect of the different agents on the attachment of the cells.

The uptake procedure for radioactive labeled AMG was essentially the same as previously described [13]. After an uptake period of 15 min at 37°C, the uptake medium (final AMG concentration: 0.1 mM) was aspirated and the monolayers grown in 12-well clusters were rinsed twice with 2.5 ml HBSS buffered to pH 7.4 with 10 mM Hepes. The cells were solubilized in 2% SDS and radioactivity was measured using Insta-Gel II (Packard Instruments International, Zürich, Switzerland) as scintillation liquid. A correction was made for the phlorizin-insensitive AMG uptake by subtracting from the total uptake the value found in the presence of 40 μM phlorizin. The phlorizin-insensitive AMG uptake was in all cases less than 3% of the phlorizin-sensitive uptake.

Ouabain binding was measured as described by Simmons [21]. Cells were cultured on nitrocellulose filters (HATF, surface area 4.2 $cm^2$; Millipore Corporation, Bedford, MA) and [$^3$H]ouabain (0.2 μM; 1 μCi/ml) binding was determined after a 20-min incubation at 37°C in $K^+$-free or 15 mM $K^+$-containing HBSS. To obtain the specific binding of [$^3$H]ouabain, the value in the presence of 15 mM $K^+$ was subtracted from that in the absence of $K^+$.

The rate of protein synthesis was determined by incubating the cells in serum-free EMEM without L-methionine (NV Gibco Europe SA, Gent, Belgium) and adding L-[$^{35}$S]methionine (≈ 1000 μCi/mmol) at a radioactive concentration of 5 μCi/ml. Thereafter, the cells were incubated for 30 min at 37°C in a 5% $CO_2$ atmosphere. The L-[$^{35}$S]methionine incorporation was terminated by aspirating the medium, washing and chasing for 30 min in a buffer containing: NaCl 140 mM, L-methionine 2 mM, Tris-HCl 5 mM (pH 7.4). The cells were solubilized with 2% SDS and radioactivity was measured using Insta-Gel II (Packard Instruments International).

For the determination of enzyme activities, monolayers cultured in 6-well clusters were washed twice with HBSS. Cells were scraped in phosphate-buffered saline and homogenized by sonication (3 × 5 s) using a sonicator (M.S.E.) equipped with an exponential probe (20 kHz, 15 μm amplitude). This treatment did not affect the activities of the enzymes [22]. γ-Glutamyltransferase (EC 2.3.2.2) and alkaline phosphatase (EC 3.1.3.1) were determined using the optimized kinetic tests from Merck (Darmstadt, F.R.G.; Merckotest 14302 and 3344). $Na^+/K^+$-ATPase (EC 3.6.1.3) was measured as described elsewhere [23]. Enzyme units (U) are micromoles of substrate hydrolyzed per minute. Protein concentration was determined by the method of Lowry et al. [24].

The transepithelial electrical resistance was measured using the Millicell-ERS Epithelial Voltohmmeter (Millipore Corporation) on monolayers grown on culture plate inserts from the Millipore Corporation (Millicell HA, ø 12 mm).

[$^{14}$C]AMG, [$^3$H]ouabain and L-[$^{35}$S]methionine (in vivo cell labeling grade) were from Amersham International plc (Amersham, U.K.). Nonradioactive AMG, ouabain, methionine, colchicine, lumicolchicine and the anti-α-tubulin monoclonals (DM 1A) were from Sigma Chemical Company (St. Louis, MO). Nocodazole was obtained from Janssen Chimica (Beerse, Belgium). The stocks of colchicine and lumicolchicine were made in ethanol, while the stock solution of nocodazole was

made in dimethyl sulfoxide. In all cases the final concentration of ethanol or dimethyl sulfoxide was less than 0.5%. It was verified that these concentrations had no effect on the phenomena observed.

## Results

*Effect of colchicine, lumicolchicine, nocodazole and cycloheximide on the AMG uptake during differentiation*

In order to evaluate the role of microtubules during the development of the $Na^+$-dependent hexose carrier, colchicine was added to a polarized epithelium and the AMG uptake was measured as a function of time (Fig. 1). The experiment was initiated on the moment that the AMG uptake started to develop, that is between one and two days after the monolayer became confluent. Thymidine was added in order to avoid a possible effect via an influence on the cell proliferation. As pointed out by Lasheras et al. [11], thymidine inhibited in a very specific way the DNA synthesis in LLC-$PK_1$ cells. Thymidine had no effect on the development of the $Na^+$-dependent hexose carrier. This was shown by the fact that confluent cells cultured for several days in the absence or presence of 10 mM thymidine, showed a similar development of the AMG uptake (own observation, not shown and [11]).

Colchicine affected the development of the AMG uptake very drastically. The increase was already completely inhibited 2 h 30 min after starting the treatment. We have verified immunocytochemically, by using monoclonal antibodies against $\alpha$-tubulin, that a 4 h treatment with colchicine completely disrupted the microtubular network present in the control condition (not shown). In order to demonstrate that the observed effect of colchicine was due to microtubule disruption, lumicolchicine was added. Lumicolchicine, an isomer of colchicine, shares most of the properties of colchicine, but it does not bind to tubulin and it does not depolymerize microtubules [25]. Lumicolchicine had no effect on the development of the AMG uptake (Table I). Nocodazole, a different microtubule-disrupting drug, had a similar effect as colchicine on the development of the AMG uptake (Fig. 1).

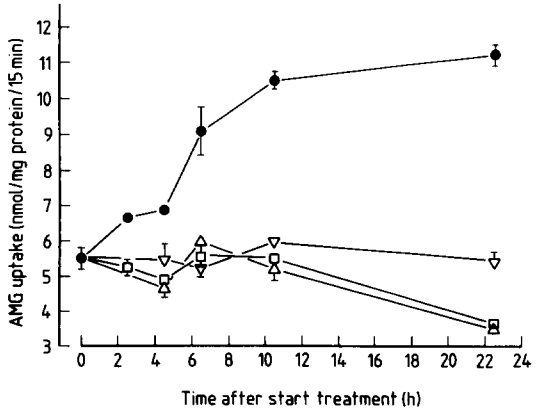


Fig. 1. Influence of different substances on the development of the AMG uptake during differentiation. LLC-$PK_1$ cells were seeded at day 0 at a concentration of 20000 cells/$cm^2$. At day 5, different conditions were created by adding EMEM (●), EMEM containing colchicine (20 $\mu$M, □), nocodazole (10 $\mu$g/ml, △) or cycloheximide (1 $\mu$g/ml, ▽). In all conditions, thymidine was added to a final concentration of 10 mM. The initial AMG uptake (15 min, final AMG concentration: 0.1 mM) at 37°C was measured as a function of time after starting the treatment. Each value is an average ± S.E. of three measurements.

TABLE I

*Influence of colchicine and lumicolchicine on the AMG uptake during differentiation*

LLC-$PK_1$ cells were seeded at day 0 at a concentration of 20000 cells/$cm^2$. At day 4, colchicine (20 $\mu$M) or lumicolchicine (20 $\mu$M) together with thymidine (10 mM) were added. On that moment ( = time 0), the AMG uptake amounted to 3.43 nmol/mg protein per 15 min. After the indicated time periods, the AMG uptake was determined in the different condition. Each value is an average ± S.E. of four determinations.

| | AMG uptake (nmol/mg protein per 15 min) | |
|---|---|---|
| | 6 h 30 min | 22 h 30 min |
| Control | 3.91 ± 0.14 | 5.54 ± 0.12 |
| + colchicine (20 $\mu$M) | 2.80 ± 0.08 | 2.22 ± 0.05 |
| + lumicolchicine (20 $\mu$M) | 3.85 ± 0.25 | 5.42 ± 0.12 |

Cycloheximide inhibited the increase of the AMG uptake (Fig. 1) indicating that synthesis of new molecules was necessary to get the normal development of the $Na^+$-dependent hexose carrier. In our experimental conditions, cycloheximide inhibited 80% of the [$^{35}$S]methionine incorporation, a measure for protein synthesis (Table III).

These observations indicate that the development of the AMG uptake during differentiation is dependent on an intact microtubular network and on protein synthesis.

*Influence of colchicine on the AMG uptake during polarization*

Differentiation of LLC-$PK_1$ cells as a function of the time in culture is a complex process influenced by many parameters. Therefore, we also evaluated the effect of colchicine on the development of the $Na^+$-dependent hexose carrier during polarization, an early stage of differentiation. This process can be isolated by studying the reorganization of already differentiated LLC-$PK_1$ cells into a polarized epithelium after the destruction of polarity by harvesting the cells using trypsin. Treatment of the cells for about 20 min with trypsin, decreased the AMG uptake by about 20%. This was concluded from a comparison of the AMG uptake in cells detached using trypsin (13.7 nmol/mg protein per 15 min) and cells still attached (16.5 nmol/mg protein per 15 min). The

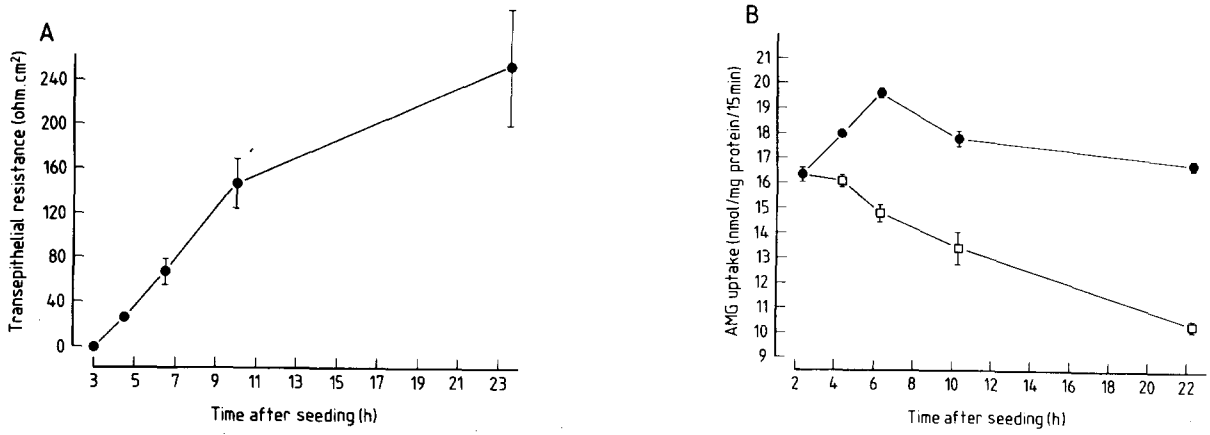


Fig. 2. Development of the transepithelial electrical resistance (A) and of the AMG uptake (B) during reorganization of LLC-PK$_1$ cells into a polarized monolayer. Cells from a confluent, quiescent monolayer (11 days old) were harvested using trypsin and seeded at time 0 at a superconfluent density in EMEM. Medium was aspirated and EMEM (●) or EMEM containing 20 μM colchicine (□) was added 2 h 30 min after seeding. Thymidine (10 mM) was always present. The transepithelial electrical resistance was determined using the Millicell-ERS Epithelial Voltohmmeter, while the AMG uptake was measured as described in Fig. 1. Each value is an average ± S.E. of three determinations.

cells were replated at superconfluent density and thymidine was included in order to inhibit cell proliferation.

The polarization process, which is initiated subsequent to replating, can be monitored by measuring the formation of the tight junctions. This occurred progressively during the experiment as can be observed from the increase in transepithelial resistance (Fig. 2A). We have followed the development of phlorizin-sensitive AMG uptake during this polarization process. In the control condition, a transient overshoot was observed with a maximum value at 6 h 30 min after seeding (Fig. 2B). This overshoot was completely inhibited by colchicine. This result was not critically dependent on the choice of the matrix since a similar inhibition was found when cells were cultured on filters (Table II). In con-

TABLE II

*Influence of colchicine, lumicolchicine and of the substrate on the AMG uptake during polarization*

Quiescent LLC-PK$_1$ cells were harvested using trypsin and seeded at a superconfluent density in 6-well clusters containing culture plate inserts ( = filter) or in 12-well clusters ( = plastic). After 2 h 30 min the different agents were added. Another 4 h later, the phlorizin-sensitive AMG uptake was measured. Thymidine (10 mM) was always present during the experiment. Each value is an average ± S.E. of three determinations.

| | AMG uptake (nmol/mg protein per 15 min) | |
|---|---|---|
| | filter | plastic |
| Control | 16.3 ± 1.3 | 16.6 ± 0.2 |
| + colchicine (20 μM) | 12.9 ± 0.7 | 13.7 ± 0.5 |
| + lumicolchicine (20 μM) | | 16.1 ± 0.3 |

TABLE III

*Influence of colchicine and cycloheximide on methionine incorporation, $Na^+/K^+$-ATPase activity and ouabain binding*

Quiescent LLC-PK$_1$ cells were seeded at a superconfluent density in EMEM containing 10 mM thymidine. After 2 h 30 min, medium was aspirated and new medium containing 10 mM thymidine and the respective agents was added. After a treatment of 4 h ( = 6 h 30 min after seeding) the methionine incorporation and the ouabain binding and after a treatment of 20 h ( = 22 h 30 min after seeding) the $Na^+/K^+$-ATPase activity and again the ouabain binding were measured. The methionine incorporation was measured at 37°C and an incorporation period of 30 min was taken. During the incorporation process the additives remained present. $Na^+/K^+$-ATPase was measured in homogenates obtained after scraping and sonicating the cells. To measure ouabain binding, cells grown on nitrocellulose filters were incubated for 20 min at 37°C in the presence of [$^3$H]ouabain (0.2 μM). Each value is an average ± S.E. of three determinations.

| | Methionine incorporation ($10^6$ cpm/mg protein) | $Na^+/K^+$-ATPase activity (mU/mg protein) | Ouabain binding (pmol/mg protein) | |
|---|---|---|---|---|
| | | | 4 h | 20 h |
| Control | 1.66 ± 0.10 | 37.4 ± 0.7 | 2.03 ± 0.03 | 3.67 ± 0.21 |
| + colchicine (20 μM) | 2.06 ± 0.07 | 33.8 ± 1.7 | 1.97 ± 0.03 | 3.27 ± 0.27 |
| + cycloheximide (1 μg/ml) | 0.33 ± 0.02 | 11.1 ± 1.3 | 1.58 ± 0.04 | 2.01 ± 0.15 |

trast to colchicine, lumicolchicine had no effect at all on the overshoot (Table II).

The increase in the AMG uptake during the overshoot is interpreted as resulting from the synthesis of new sugar transporters plus the recruitment or recycling of transporters originally present in the monolayer [11]. This suggests that one of these processes is critically dependent on an intact microtubular network. Colchicine had no effect on protein synthesis (Table III). On the other hand, experiments with microtubule-disrupting drugs have shown that microtubules may be important for vesicular transport [26]. Therefore, it is tempting to speculate that the influence of colchicine on the overshoot is owing to an interference with some form of vesicular transport, e.g., from the Golgi towards the plasma membrane.

*Influence of colchicine on the activity of apical and basolateral enzymes during polarization*

An overshoot during reorganization of LLC-$PK_1$ cells into a polarized epithelium was also observed for the activity of two apical enzymes. Figs. 3A and 3B show the results obtained for alkaline phosphatase and γ-glutamyltransferase, respectively. In both cases, the maximum was observed 6 h 30 min after seeding. In contrast to this, the development of $Na^+/K^+$-ATPase, a basolateral protein, didn't show the over-response (Fig. 3C). The $Na^+/K^+$-ATPase activity increased steadily over the time period of the experiment.

The effect of colchicine on the overshoot of the apical enzymes was similar as on the development of the $Na^+$-dependent hexose carrier. As is shown in Figs. 3A and 3B, the overshoot for alkaline phosphatase as well as for γ-glutamyltransferase was inhibited. After a colchicine treatment of 10 h or longer, no difference in the activity of these two apical enzymes was observed compared to the control condition. This is in contrast to the colchicine effect on the AMG uptake which remained large and continued to grow.

Colchicine had no significant effect on the development of the total $Na^+/K^+$-ATPase activity (Fig. 3C). Table III shows that a treatment of 20 h with cycloheximide largely inhibited the development of this activity. This indicates that the increase of the $Na^+/K^+$-ATPase activity observed in Fig. 3C is dependent on the synthesis of new molecules.

We also measured ouabain binding to the $Na^+/K^+$-ATPase in order to get information on the fraction of molecules localized in the plasma membrane. After a 4 h treatment, the ouabain binding was not affected at all by colchicine (Table III). After a treatment of 20 h, colchicine also didn't decrease the number of specific binding sites to the same extent as cycloheximide (Table III). This means that newly synthesized $Na^+/K^+$-ATPase molecules could still reach the plasma membrane after the microtubular network was depolymerized by colchicine.

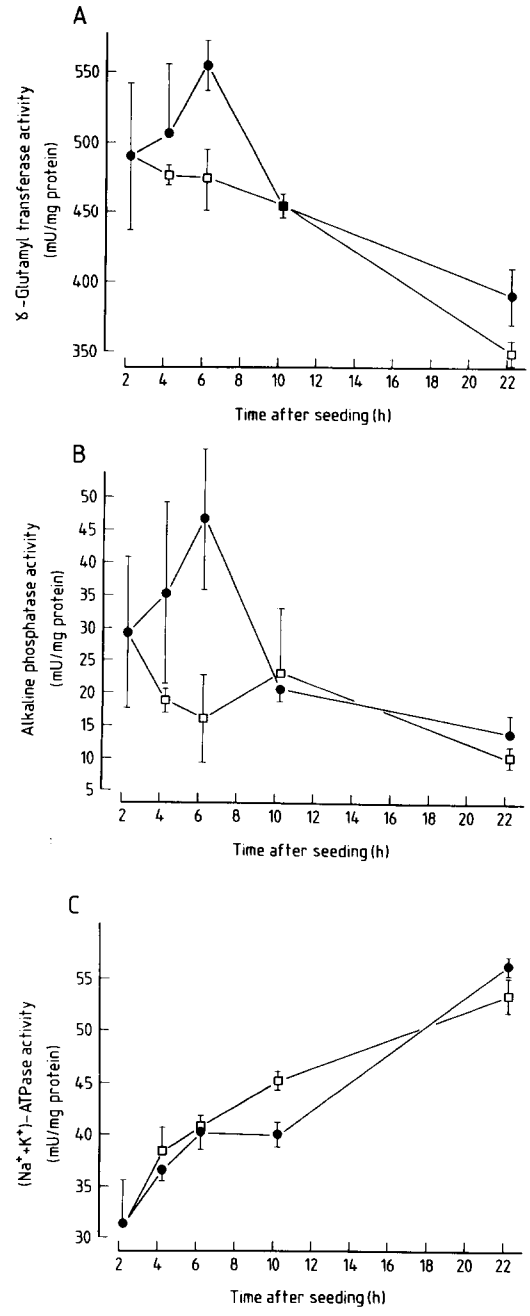


Fig. 3. Influence of colchicine on the development of γ-glutamyltransferase (A), alkaline phosphatase (B) and $Na^+/K^+$-ATPase (C). Seeding conditions were as described in Fig. 2. Medium was aspirated and new EMEM (●) or EMEM containing colchicine (20 μM, □) were added, 2 h 30 min after seeding. Thymidine (final concentration: 10 mM) was always present. At different time points, batches of cells were scraped, sonicated and the enzyme activities were measured in the homogenates. Each value is an average ± S.E. of three determinations.

## Discussion

From experiments with microtubule-disrupting agents, it has been concluded that in intestinal epithelial

cells, microtubules are important for the transport of apical membrane proteins from the Golgi apparatus towards the apical membrane [20, 27–29]. This has been confirmed using cultured intestinal epithelial cells [18]. The question arises whether this is a mechanism typical for intestinal epithelial cells or a general characteristic of epithelia. In a study on proximal tubules, an effect of colchicine was found on the transport of an apical glycoprotein (gp 330). However, controversial results were obtained when the effect of microtubule-disrupting agents was studied on the polarized delivery of virus proteins in the renal cell line, MDCK [30,31]. A problem in the use of infected MDCK cells may arise from the cytopathic effect of virus infection. The degree to which this affects the normal sorting pathways is difficult to predict. We used endogenous proteins expressed in LLC-$PK_1$ cells to evaluate the effect of microtubule-disrupting agents. The $Na^+$-dependent hexose carrier is particularly suited for this purpose since it is exclusively located at the apical cell pole and its expression is entirely dependent on the development of the apical membrane.

The development of the $Na^+$-dependent hexose carrier during differentiation of LLC-$PK_1$ cells seemed to be critically dependent on an intact microtubular network. There was also an effect of colchicine on the development of the $Na^+$-dependent hexose carrier during the polarization process. To study the latter process, we used essentially the same approach as Lasheras et al. [11]. By seeding cells at a superconfluent density, cell-substratum and cell-cell contact could start to develop from the beginning of the experiment. As a consequence, the process of polarization could also start very rapidly. When cells were seeded from previously quiescent LLC-$PK_1$ cells, an overshoot in the activity of the $Na^+$-dependent hexose carrier [11] and of the $Na^+/H^+$ antiport system [10] was observed. For both apical transporters it was found that the increase in transport activity represented synthesis and/or sorting of transporters rather than changes in the affinity or changes in the driving force [10,11]. Another argument for this conclusion is our observation that also the development of the apical enzymes, alkaline phosphatase and $\gamma$-glutamyltransferase, showed this increase. The subsequent decrease in the activity of all these apical markers may be attributed to a reduction in the supply of molecules and/or an accelerated breakdown. The overshoot was only observed when the experiment was done with cells dispersed from a differentiated monolayer. When cells from cultures in active growth were taken, no overshoot for the $Na^+$-dependent hexose carrier [11] and for the $Na^+/H^+$ antiporter [10] was observed. This indicates that the overshoot phenomenon is owing to the translation of mRNA and/or the transport and incorporation of proteins that were already present before seeding the cells. Experiments with cycloheximide indicated that at least part of the overshoot was related to de novo synthesis of proteins [11]. Although the exact nature of the overshoot phenomenon is not fully clear, it offers an interesting experimental advantage. Since the appropriate conditions for the functional incorporation of proteins can be restored within a few hours, this greatly facilitates the study of factors which interfere with this process.

Intact microtubules may be necessary at different stages to get the expression of the $Na^+$-dependent hexose carrier. First of all, by displacement and partial fragmentation of the Golgi apparatus, the sorting pathway may be blocked [32]. However, for Chinese hamster ovary cells, it has been shown that movement of newly synthesized glycoproteins through the Golgi apparatus was not altered by a fragmentation of the Golgi due to a disassembly of microtubules [33]. Secondly, intact microtubules may serve as tracks for apical membrane vesicles. It has been described for several cell systems that vesicle and organelle movement is mediated by microtubules [26,34].

In intact intestinal [20] as well as in cultured intestinal epithelial cells (Caco-2)[18], it was found that microtubule-disrupting drugs caused a partial missorting of apical proteins. On the other hand, the delivery of basolateral proteins was not affected. Gutman et al. [19] focused their attention on the sorting of an apical membrane glycoprotein (gp 330) in proximal tubules. Using colchicine they observed a redistribution of the vesicles containing gp 330 as well as reduced labeling of the brush-border membrane by anti-gp 330 antibodies. However, there was no significant insertion of gp 330 into the basolateral membrane. Using cultured proximal tubule cells (LLC-$PK_1$), we have also found a complete inhibition of the expression of new carrier molecules after a colchicine treatment. Our uptake values can be considered as a good measure for the total number of carriers present in the plasma membrane. It is very improbable that insertion of carrier molecules in either the basolateral or the apical membrane would have been undetected by our assay, since the same results were obtained with monolayers grown on filters. As a consequence, we conclude that, after disruption of the microtubular network, the vesicles containing the carriers did not follow the basolateral pathway by default to reach the plasma membrane. This seems to be a difference between intestinal and proximal tubule cells. In intestinal epithelial cells only the correct sorting to the apical plasma membrane domain was affected by microtubule-disrupting drugs [18,20]. On the other hand, in renal proximal tubule cells, both the traffic to and the incorporation of the apical vesicles in the plasma membrane seem to be inhibited.

In contrast to the AMG uptake as a measure of the $Na^+$-dependent hexose carrier, the activity of the apical enzymes in total cell homogenates is not necessarily a

good measure of their functional incorporation in the plasma membrane. Nevertheless, we observed an effect of colchicine on the overshoot of two apical enzymes. Although we don't know in this case which additional mechanism is responsible for the effect of colchicine, a number of possibilities can be proposed. It is possible that the enzyme activities are not fully active until they reach the plasma membrane. Furthermore, the colchicine effect may also be attributed to an intracellular breakdown of enzymes after the depolymerization of the microtubular network. This may be due to the fact that enzymes present in internal membranes are more susceptible to degradation than those of the plasma membrane. Moreover, the accumulation of the enzymes in intracellular compartments following the colchicine treatment may also be responsible for a decrease of their synthesis.

After a longer colchicine treatment, a remarkable difference was observed between the apical enzymes and the $Na^+$-dependent hexose carrier. For the enzyme activities, the values after 10 – 22 h were not much lower than for control, while this difference remained large and continued to grow for the AMG uptake. This is most likely owing to the fact that total activities were measured in the former, while only the protein fraction present in the plasma membrane was measured in the latter case. These results indicate that the loss of apical proteins from the cell surface is relatively greater then their total decrease. As was proposed by Gutmann et al. [19], the removal of apical proteins from the plasma membrane after colchicine treatment may be due to endocytotic recycling from the cell surface into the cytoplasm, while the transport back to the plasma membrane is blocked.

We found no effect of colchicine on the expression of $Na^+/K^+$-ATPase. For renal epithelial cells, the pathway for apical and basolateral proteins was found to be the same until the proteins reach the *trans* Golgi network [35]. In this part of the Golgi apparatus, different classes of proteins are sorted into different vesicle populations. As a consequence, from the observation that basolateral proteins can be transported to the membrane after the disruption of the microtubular network it can be concluded that the Golgi apparatus still functions properly. A second conclusion is that microtubules are apparently not critically involved in the transport of basolateral proteins to the plasma membrane.

In conclusion, our data confirm the different sensitivity to microtubule-disrupting agents of the transport pathways taken by the apical and by the basolateral proteins. In addition, it seems that the apical proteins could not reach the plasma membrane by another pathway after disruption of the microtubular network. This is in accordance with the following model. Microtubules are used as tracks by the apical vesicles to reach the zone just beneath the apical membrane. As the concentration of apical vesicles becomes high, the chance for fusion of these vesicles with the apical membrane increases. This process may involve recognition of a protein exposed on the cytoplasmic side of the vesicle with a specific 'docking receptor' on the apical plasma membrane [36].

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