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USE OF CULTURED EPITHELIA TO STUDY TRANSPORT AND ITS REGULATION

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SUMMARY

Epithelial cells from a variety of species and organs form polarized epithelia in culture. When epithelia are grown on a porous surface, such as a millipore filter, transport can be studied using adaptations of standard techniques. In the few years in which cultured epithelia have been studied by transport physiologists, most work has been focused on identification and description of the differentiated transport exhibited by cultured epithelia. Epithelia formed by a continuous line of cells derived from pig kidney ($LLC\text{-}PK_1$) exhibit sodium-coupled glucose transport similar to that of the proximal tubule and have vasopressin-sensitive adenylate cyclase that has been studied in great detail. Also of interest are epithelia formed by continuous lines of cells derived from amphibian kidney (A6) and from amphibian urinary bladder (TBM). Each line forms epithelia that have high electrical resistance and amiloride-sensitive sodium transport. Transport is stimulated by aldosterone and by cAMP or hormones that raise cell cAMP levels. In $LLC\text{-}PK_1$ and in A6 epithelia, transport and the response to hormones can be manipulated by manipulating the culture conditions. Cultured epithelia have also been used to explore the cell biology of epithelia. Most interesting in this regard are studies of the development and maintenance of epithelial cell polarity. This approach should be especially valuable.

INTRODUCTION

Half a century after Chambers & Kempton (1933) used chick embryo mesonephros in organ culture (to establish secretion as a function of kidney tubules) transport physiologists are again studying cultured epithelia. Although major revelations about transport have not yet emerged, it is apparent from work over the past 7 years that cultured epithelia offer unique advantages for the study of transport and its control. I will briefly summarize some studies of primary cultures and then, in more detail, investigations using cell lines. Finally, I will review studies that do not focus on transport, but approach other facets of epithelial biology. Eventually, the latter may prove to be of special value in understanding epithelial function and its regulation.

Uniform cellular polarity, which is essential for the transport function, occurs in cultured epithelia. Regardless of the species or tissue of origin, epithelia in standard

Key words: Epithelial polarity, hormones, differentiation.

culture conditions, orientate with their basal plasma membrane attached to the supporting surface (e.g., the Petri dish), and apical plasma membrane facing the medium. The polarity of epithelia formed in culture has been demonstrated by autoradiography of binding sites for ^{3}H-ouabain (Mills, MacKnight, Dayer & Ausiello, 1979), immunofluorescent localization of specific apical and basolateral membrane antigens (Louvard, 1980), the inhibition of sodium transport by amiloride only when the drug is added to the solution bathing the apical surface of the amiloride-sensitive epithelium (Handler, Perkins & Johnson, 1981*a*), and in many studies by morphological evidence and the direction of net transport (apical to basal) by epithelia formed by cells derived from absorptive epithelia. The signal that initiates cell polarization has not been identified.

EPITHELIAL TRANSPORT

The first indication of transport under standard culture conditions is a consequence of polarity. Many cultured epithelia form domes or hemicysts when grown on a solid surface such as a standard plastic or glass Petri dish. The domes are usually small cyst-like areas of a confluent epithelium that are elevated from the attachment surface by the accumulation of solution. Leighton, Brada, Estes & Justh (1969) realized that domes might be the result of apical to basolateral transepithelial transport of solution. They proposed that the transported solution is trapped below the cells by tight junctions and lifts the epithelium off the Petri dish. Subsequently they supported the idea by demonstrating that ouabain inhibits dome formation (Abaza, Leighton & Schultz, 1974). There is no reason to question the proposed mechanism of dome formation, or quarrel with the use of dome formation to identify transporting epithelia in culture. Unfortunately, because domes are easy to identify, their number or area has been relied on as a quantitative assay for transport. However, these are unreliable measures (even for testing the effect of an experimental manipulation on the rate of transport), since dome formation depends not only on transport, but also on the ability of tight junctions to seal in the transported fluid and the degree of adhesion of the basal plasma membrane to the surface of the dish. Currently, Misfeldt, Tanner & Frambach (1983) are studying the dynamics of dome formation by measuring the pressure and composition of dome fluid and the rate of expansion of domes. Even though it may become possible to estimate the rate of fluid transport into domes, the information will be of limited value because of limitations in the preparation. The solution at the basal surface of the epithelium cannot be varied to suit experimental requirements. Furthermore, in a variety of epithelia cultured on plastic Petri dishes, differentiation is limited when compared to that on other surfaces (*vide infra*).

To study transport, epithelia should be grown on porous supports so that the solution on both surfaces of the epithelium can be sampled and manipulated, and transepithelial electrical measurements can be performed. Indeed, the demonstration of such preparations (Misfeldt, Hamamoto & Pitelka, 1976; Cereijido *et al.* 1978) led to the current interest in cultured epithelia. When grown on millipore or other filters, or on a variety of preparations of collagen, the cultures can be treated like naturally occurring epithelia such as frog skin, and placed in chambers for standard measurements of flux, short-circuit current, and electrical resistance. R. Steele, J. Johnso

Preston & J. Handler (manuscript in preparation) have gone further and developed techniques to follow the electrical characteristics of the same cultured epithelium intermittently over a period of months.

Many primary cultures of epithelial cells and many continuous epithelial cell lines form domes and in that sense form transporting epithelia. Not all of these are of physiological interest. A number of continuous cell lines form domes but are without measurable potential difference or resistance when grown on a porous surface. I will not speculate on the reason for this. It may be intrinsic to the epithelium or related to the culture conditions.

PRIMARY CULTURES

Primary cultures are those which are initially formed when cells are taken from their natural site. Continuous cell lines are repeatedly sub-cultured from dish to dish (typically over 100 times). Each preparation offers special advantages. Primary cultures are likely to resemble the epithelium of origin. In Table 1 are listed some primary cultures that have been reported to maintain a transepithelial potential difference, or provide evidence of a special epithelial transporter, and have at least one other interesting differentiated property. I have not included cultures in which transport has been identified solely by dome formation.

CONTINUOUS CELL LINES

Continuous cell lines are easy to grow, can yield enormous amounts of material and can be stored frozen for later study. Continuous epithelial cell lines have been most

Table 1. *Primary cultures*

Organ	Species	Characteristic	Reference
Kidney	rabbit	apical surface positive PD sensitive to furosemide	(a)
Kidney	rabbit	sodium-coupled alpha-methylglucoside uptake inhibited by phlorizin; PTH-sensitive cAMP production	(b)
Stomach	dog	apical surface negative PD stimulated by histamine, inhibited by amiloride	(c)
Stomach	rabbit foetus	apical surface begative PD inhibited by amiloride	(d)
Mammary gland	mouse	apical surface negative PD stimulated by prolactin, inhibited by amiloride	(e)
Thyroid	pig	follicles with apical surface negative PD, secrete thyroglobulin in response to thyrotropin	(f)
Lung	rat	apical surface negative PD inhibited by amiloride	(g)

(a) Burg *et al.* (1982).
(b) Chung *et al.* (1982).
(c) Ayalon *et al.* (1982).
(d) Logdson, Bisbee, Rutten & Machen (1982).
(e) Bisbee, Machen & Bern (1979).
(f) Chambard, Verrier, Gabrion & Mauchamp (1983).
(g) Mason *et al.* (1982).

extensively studied. Almost all of these were derived from mixed cell populati[illegible] After many subcultures, epithelial cells became the sole cell type (Hull, Cherry & Weaver, 1976; Madin & Darby, 1958; Rafferty, 1969). With two exceptions (Ambesi-Impiombato, Parks & Coon, 1980; Handler *et al.* 1979), attempts to establish continuous cell lines from selected populations of normal epithelial cells have not led to the development of continuous epithelial cell lines with differentiated properties.

Two differentiated apical membrane transporters have been identified and studied extensively in epithelia formed by continuous cell lines. Sodium-coupled glucose transport, like that of the pars recta of the renal proximal tubule, is expressed by LLC-PK_1 epithelia (Rabito & Ausiello, 1980; Mullin, Weibel, Diamond & Kleinzeller, 1980) and amiloride-sensitive sodium transport, like that of the toad urinary bladder, is expressed by A6 (Perkins & Handler, 1981) and by TBM (Handler *et al.* 1979) epithelia. The cultures are particularly interesting in that both transporters are subject to regulation. Another continuous cell line, derived from dog kidney and designated MDCK, has been studied in several laboratories. The following is a summary of the characteristics of these cultured epithelia, as well as some new information about their function.

LLC-PK_1 epithelia

The continuous line, designated LLC-PK_1, was derived in 1958 from an unknown site in the kidney of a Hampshire pig to serve as host for the growth of viruses (Hull *et al.* 1976). The cell line attracted the attention of physiologists when shown to have adenylate cyclase that is sensitive to vasopressin and calcitonin (Goldring, Dayer, Ausiello & Krane, 1978). Subsequently it was found that LLC-PK_1 epithelia express sodium-coupled hexose transport like that of the proximal tubule. Many techniques used to investigate hexose transport by natural epithelia have been used to study sodium-coupled hexose transport by LLC-PK_1 epithelia. Rabito & Ausiello (1980) and Mullin *et al.* (1980) measured hexose uptake in cells cultured on collagen-coated filters. Like slices of kidney cortex, the cultures concentrate the non-metabolizeable hexose analogue α-methyl-D-glucoside (AMG). Hexose transport by LLC-PK_1 epithelia has been characterized further using similar techniques, by measurement of transport into apical membrane vesicles (Lever, 1982; Moran, Handler & Turner, 1982), and by direct examination of transepithelial transport of glucose and sodium by epithelia grown on collagen-coated millipore filters (Misfeldt & Sanders, 1981). Almost all net sodium transport is coupled to hexose transport (Misfeldt & Sanders, 1982). There is agreement that the apical membrane transporter has a selective affinity for hexose analogues like that of the proximal tubule, that hexose transport is coupled to that of sodium (with a ratio of two sodium ions to one hexose) and is inhibited by phlorizin and not by phloretin. The coupling ratio is similar to that in brush border membrane vesicles from the pars recta of rabbit kidney (Turner & Moran, 1982), but the transporters differ in that the latter is not sensitive to inhibition by phlorizin. As in the proximal tubule, there is little or no metabolism of transported glucose (Misfeldt & Sanders, 1981). This is difficult to reconcile with the observation that, in contrast to the proximal tubule, glycolysis is an essential energy source for maximal transport by LLC-PK_1 epithelia (Sanders, Simon & Misfeldt, 1983).

LLC-PK_1 epithelia also concentrate D-aspartate across the apical plasma membr[illegible]

sodium dependent fashion (Rabito & Karish, 1983). The uptake of some other amino acids is sodium-dependent (Sepulveda & Pearson, 1982; Cook, Amsler, Weiss & Shaffer, 1982; Rabito & Karish, 1982), but appears to involve basolateral transporters. LLC-PK_1 epithelia may also transport phosphate actively (Rabito, 1980). Although hormones that stimulate adenylate cyclase also activate cAMP-dependent protein kinase in LLC-PK_1 epithelia (Ausiello, Hall & Dayer, 1980), there is no evidence that transport is affected by the hormones.

For this extensive characterization to be of value it must provide insight into the mechanism of transport or its regulation. A number of studies indicate that the expression of the transporter in LLC-PK_1 epithelia is not constant and can be manipulated. The ability of LLC-PK_1 epithelia to concentrate AMG increases with the confluency or age (days after seeding) of the culture (Mullin *et al.* 1980; Cook *et al.* 1982). The rate of AMG transport by apical membrane vesicles increases in a parallel fashion as the culture matures (Lever, 1982; Moran, Handler & Turner, 1982). The expression of the transporter can also be manipulated by chemical agents. Amsler & Cook (1982) found that agents that affect differentiation of Friend erythroleukaemia cells in culture have a similar effect on the expression of the sodium coupled hexose transporter in LLC-PK_1 epithelia. Hexamethylene bisacetamide, which accelerates differentiation in Friend cells, speeds up the development of AMG transport in LLC-PK_1 epithelia. As in Friend cells, the effect of hexamethylene bisacetamide is blocked by the tumour promoter 12-*o*-tetradecanoylphorbol-13-acetate. Because of reports that the transition of cells from a growth to a differentiating phase is accompanied by a rise in cell cAMP, they measured the cAMP content of LLC-PK_1 epithelia and manipulated the cAMP content by adding 1-methyl-3-isobutylxanthine (MIX), a cAMP phosphodiesterase inhibitor, to the growth medium. In many experiments there was a correlation between slow growth rate, cell cAMP levels, and AMG uptake. For example, hexamethylene bisacetamide inhibited growth, raised cAMP levels and AMG uptake. Cultures treated with hexamethylene bisacetamide plus 12-*o*-tetradecanoylphorbol-13-acetate grew faster and had lower cAMP levels and AMG uptake. MIX raised cell cAMP and accelerated the development of AMG uptake. Amsler & Cook (1982) noted that the correlations were not strong. There were differences in the time course of changes in cAMP content and AMG uptake and under certain conditions cultures had high cAMP content but low AMG uptake. Amsler & Cook tentatively proposed that elevated cAMP content may be a necessary step in the early development of Na-dependent hexose transport, but is not a sufficient condition. The effects of the age of the culture, of tumour promoters, and of cAMP content or MIX treatment on differentiation have been seen previously in non-epithelial cells. Using a different approach, Moran, Turner & Handler (1983) have found that the expression of the transporter is regulated by the concentration of glucose in the growth medium. Cultures grown in medium containing 25 mM glucose have lower AMG uptake and fewer phlorizin binding sites (assumed to be a quantitative assay for the number of transporters) than cultures grown in 5 mM glucose. The regulation appears to be specific for the hexose transporter in that there is no change in the activity of an apical membrane marker enzyme, alkaline phosphatase, or in the activity of marker enzymes of other cellular components. The concentration of glucose in the growth medium has been shown to have a similar effect on the expression of a different hexose

transporter in fibroblasts (Kalckar & Ullrey, 1973). The mechanism by which concentration of glucose in the growth medium affects the expression of hexose transporters is unknown.

The homogeneity of cells in culture and the ability to manipulate growth conditions make cultured epithelia ideal for studying hormone-receptor interactions. On the basis of their studies on LLC-PK$_1$ epithelial cells Roy & Ausiello (1981) and Roy, Hall, Karish & Ausiello (1981) have extended earlier models for vasopressin-receptor binding and activation of adenylate cyclase. In brief, they demonstrated vasopressin-induced desensitization of adenylate cyclase associated with a transition to lower receptor affinity for the hormone. Based on kinetic considerations, they proposed a model in which vasopressin receptors are dimeric units, each one being able to bind hormone and activate adenylate cyclase molecules in its interaction field. Binding of the hormone to one receptor subunit results in activation of adenylate cyclase and transition of the second subunit to a low affinity state. The model is compatible with their data for vasopressin binding ($K_d = 10$ nM) and other studies with naturally-occurring kidney tissue. It describes a system that would lead to rapid, sensitive and reversible control of antidiuretic action at the low concentrations (10 pM) of hormone normally found *in situ*. In addition to the above effect of vasopressin on its receptors, other factors affect receptor number in a strain of LLC-PK$_1$ cells that grows in a defined medium without serum or hormones. The strain has only 5 % of the receptors for vasopressin of the parent strain grown in medium with serum. When serum or insulin are added to the growth medium, receptor number increases without a change in affinity for vasopressin. There is a parallel increase in maximal adenylate cyclase activity that is stimulated by vasopressin, indicating that the number of receptors limits the adenylate cyclase response to the hormone (Roy, Preston & Handler, 1980). Calcium-calmodulin modulates vasopressin-sensitive adenylate cyclase in LLC-PK$_1$ cells (Ausiello & Hall, 1981).

LLC-PK$_1$ cells also express some regulatory functions of the proximal tubule in vitamin D metabolism. *In situ*, conversion of 25-(OH)D$_3$ to the active metabolite 1,25-(OH)$_2$D$_3$ or the inactive metabolite 24,25-(OH)$_2$D$_3$ is regulated in part by the circulating level of 1,25-(OH)$_2$D$_3$. High levels of 1,25-(OH)$_2$D$_3$ stimulate conversion of 25-(OH)D$_3$ to 24,25-(OH)$_2$D$_3$. LLC-PK$_1$ epithelia have receptors for 1,25-(OH)$_2$D$_3$ with an apparent K_d of 0·12 nM. Binding of 1,25-(OH)$_2$D$_3$ to the receptor appears to induce 24-hydroxylase activity and formation of the inactive metabolite (Colston & Feldman, 1982).

A6 epithelia and TBM epithelia

Two continuous amphibian cell lines, one derived from an unknown site in the kidney of *Xenopus laevis*, A6 (Rafferty, 1969), and the other from the urinary bladder of *Bufo marinus*, TBM (Handler *et al.* 1979), form epithelia with high electrical resistance and amiloride-sensitive sodium transport as in toad urinary bladder. When grown on collagen-coated nucleopore filters, both lines form epithelia with trans-epithelial potential differences of 10 mV (apical surface negative), resistances of 5000 Ωcm^2, and short-circuit currents of 2 $\mu A\,cm^{-2}$, which are equivalent to their net sodium transport (Perkins & Handler, 1981; Handler *et al.* 1979). Sodium transport in both epithelia is rapidly and reversibly inhibited by the addition of amiloride to

[redacted]cal solution. In A6 epithelia, amiloride inhibits the apical sodium entry pathway competitively with $K_i = 50$ nM (Sariban-Sohraby, Burg & Turner, 1983). In both, epithelial sodium transport is stimulated by cAMP and by adrenal steroid hormones. The time course of the response to each agent is like that in naturally-occurring epithelia in that the response to cAMP is evident in minutes, while the response to aldosterone develops over hours. In A6 epithelia, insulin stimulates sodium transport over a period of 1 h (Fidelman, May, Biber & Watlington, 1982).

Studies of the two cultured amphibian epithelia have focused on the receptors for and on the enzymes induced by aldosterone, and on the effect of the hormone on sodium uptake across the apical membrane. They have yielded new information about the action of aldosterone on sodium transport. Watlington, Perkins, Munson & Handler (1982) studied the specific binding of adrenal steroid hormones by A6 epithelia and correlated it with stimulation of sodium transport by the steroids. The epithelia have two classes of steroid receptors, one with a high affinity for aldosterone (apparent $K_d = 85$ pM) and a more numerous population with a lower affinity (apparent $K_d = 16$ nM). The low affinity receptor appears to be involved in stimulation of sodium transport, for occupancy of the low affinity receptor correlates linearly with stimulation of sodium transport. The linear relationship between binding and stimulation of sodium transport in A6 epithelia differs from that reported for toad urinary bladder epithelia *in situ* (Farman, Kusch & Edelman, 1978). The latter is non-linear and there is no stimulation of sodium transport when fewer than 50 % of receptors are occupied. The significance of the difference between aldosterone binding and stimulation of sodium transport in A6 epithelia as compared to toad urinary bladder epithelia is unknown. The receptors also differ in their affinity for other adrenal steroid hormones. Receptors have been examined for a role in another interesting aspect of the response to aldosterone. As in the intact toad urinary bladder (Rossier *et al.* 1979), the stimulation of sodium transport by aldosterone is blocked by prior incubation of TBM epithelia with thyroxine. The block does not involve a change in the number of receptors or their affinity for aldosterone (Pratt & Johnson, 1983). Thyroxine does not block the response of A6 epithelia to aldosterone.

Aldosterone increases the activity of a number of enzymes in epithelia, and at least two, citrate synthase and Na,K-ATPase, may have an important role in the stimulation of sodium transport. Aldosterone induces citrate synthase in the outer medulla of rat kidney (Law & Edelman, 1978) and increases the activity of the enzyme in toad urinary bladder epithelia *in situ* (Kirsten, Kirsten, Leaf & Sharp, 1968) and in rabbit cortical collecting tubule *in situ* (Marver & Schwartz, 1980); all are epithelia in which aldosterone stimulates sodium transport. Since citrate synthase plays a key role in metabolism, its induction might be an essential step in stimulation of sodium transport. This was ruled out by studies of A6 and TBM epithelia. Aldosterone stimulates sodium transport in both cultured epithelia without affecting citrate synthase activity (Johnson & Green, 1981). After prolonged exposure to aldosterone, a number of epithelia develop increased Na,K-ATPase activity. To distinguish whether the effect on Na,K-ATPase is direct or secondary to the stimulation of sodium transport, the enzyme was assayed (using a ^{3}H-ouabain binding assay on membranes from broken cells) in A6 epithelia that had been incubated with aldosterone, or with aldosterone [redacted]s amiloride. Amiloride was added to block sodium transport. Incubation with

aldosterone results in increased Na,K-ATPase. Amiloride, which alone has no eff[illegible] on Na,K-ATPase, blocks the effect of aldosterone on the enzyme (Handler *et al.* 1981*b*). A similar pattern has been seen in rabbit cortical collecting ducts (Petty, Kokko & Marver, 1981). The effect of aldosterone on Na,K-ATPase appears to require or may merely be secondary to the stimulation of sodium transport.

The apical entry step for sodium has been studied in A6 epithelia grown on millipore filters (Sariban-Sohraby *et al.* 1983). The entry step is a saturable function of sodium concentration ($K_m = 18$ mM, $V_{max} = 2{\cdot}5$ nmol min^{-1} cm^{-2}). Amiloride is a potent competitive inhibitor of sodium entry ($K_i = 50$ nM), as it is in naturally-occurring high resistance epithelia. Incubation with aldosterone leads to a 200% increase in the rate of sodium uptake. The time course for the increase in sodium uptake and the time course for the increase in short-circuit current are similar. The results are consistent with an aldosterone-induced increase in the number of apical membrane sodium channels, as proposed on the basis of studies of naturally-occurring epithelia using electrical techniques (Nagel & Crabbe, 1980; Palmer, Li, Lindemann & Edelman, 1982).

A6 epithelia demonstrate an important principle in epithelial cell culture. In most epithelia, nutrient uptake and interaction with hormonal and other signals is a function that resides in the basolateral plasma membrane. In view of the evidence of normal polarity in cultured epithelia, this is probably true in culture as well. Therefore, as epithelial cells grow on a culture dish and form an epithelium with tight junctions between cells, the basal surface, which is the attachment surface, becomes sealed off from the growth medium. We believe that because of this, differentiation is limited in A6 epithelia on plastic Petri dishes or on filters permitting only limited access of medium to the basal surface of the epithelium as in the initial study of A6 epithelia (Perkins & Handler, 1981). The latter were non-ciliated and adenylate cyclase was unresponsive to hormones. When A6 epithelia are grown on filters arranged to allow free access of solution to the filter, they have active motile cilia, higher potential difference and short-circuit currents, and adenylate cyclase is stimulated by vasopressin, isoproterenol and adenosine (Handler, Preston & Steele, 1983; Lang, Forrest, Preston & Handler, 1983). The foregoing effects of basal feeding are not the first to be recognized in cultured epithelia. Epithelia formed by MDCK cells (*vide infra*) develop a basement membrane when grown on hydrated collagen gels but not when grown on plastic Petri dishes (Valentich, 1982). A variety of primary cultures [e.g. rat lingual epithelia (Lillie, MacCallum & Jepsen, 1980), rat urinary bladder epithelia (Chlapowski & Haynes, 1979)] develop differentiated morphology on collagen rafts in contrast to a simpler, less differentiated morphology obtained on plastic Petri dishes. Primary cultures of porcine thyroid epithelia demonstrate the same point. Confluent cultures on Petri dishes, with or without collagen coating, are polarized and form domes. However, the epithelia are not well differentiated morphologically, are unable to concentrate and organify iodide, and have a poor cAMP response to thyrotropin. In contrast, cells seeded on filters concentrate iodide and have a significant cAMP response to thyrotropin (Chambard, Verrier, Gabrion & Mauchamp, 1983). Some epithelia in culture differentiate further if the porous surface on which they are growing can contract after the epithelium is formed. Floating collagen gels allow contraction as well as exchange at the basal surface. Such ge[illegible]

roduced for hepatocytes by Michalopoulos & Pitot (1975), lead to increased differentiation of thyroid (Chambard *et al.* 1983) and also of mammary epithelia (Shannon & Pitelka, 1981).

MDCK epithelia

Epithelia formed by MDCK cells, a continuous line derived from dog kidney (Madin & Darby, 1958), have been studied in many laboratories. Apparently, there are two (or more) strains of MDCK cells. Strain I, obtained as a lower passage number, forms epithelia with a high resistance (4000 Ωcm^2) (Richardson, Scalera & Simmons, 1981); strain II forms epithelia with a lower resistance (80 Ωcm^2) (Richardson *et al.* 1981; Cereijido *et al.* 1978; Misfeldt *et al.* 1976). The strains also differ morphologically (Richardson *et al.* 1981; Valentich, 1981). Both strains are affected by hormones and other agents. These effects and other characteristics of the epithelia are summarized in Table 2. MDCK epithelia synthesize prostaglandins. Prostaglandin synthesis is altered by a variety of agents (Hassid, 1982). Apparently, there are strain differences in prostaglandin synthesis too (Lewis & Spector, 1981).

Cereijido and his colleagues have studied the electrical resistance of tight junctions in low resistance (strain II) MDCK epithelia, as well as the kinetics of tight junction

Table 2. *MDCK epithelia*

Characteristic	Strain I	Strain II	Reference
Passage number	60–70	> 100	(a, b)
PD (mV), apical surface negative	0·1–2·5	0·5–2·0	(a, c, d)
Resistance (Ωcm^2)	4000	80	(a, c, d)
Epithelial morphology	flattened two or three cell types	cuboidal single cell type with single cilium	(a) (b)
Response to hormones	(short-circuit current)	(cAMP production)	
adrenaline	stimulates	NT (3)	(a)
vasopressin	stimulates	stimulates	(a, e)
prostaglandin E	NT	stimulates	(e)
glucagon	NT	stimulates	(e)
Effect of:			
ATP (1)	increased apical to basal Cl flux		(f)
furosemide	blocks ATP effect (2)	alters cell flux of Na, K, Cl	(f, g)
amiloride	lowers PD	lowers PD	(h, e)

(1) ATP added to apical or basal solution, presumably acting *via* receptors for adenosine.
(2) Furosemide effective only when added to the basal solution.
(3) NT–not tested.

(a) Richardson, Scalera & Simmons (1981).
(b) Valentich (1981).
(c) Midfeldt, Hamamoto & Pitelka (1976).
(d) Cereijido *et al.* (1978).
(e) Rindler, Chuman, Shaffer & Saier (1979).
(f) Simmons (1981*a*).
(g) McRoberts, Erlinger, Rindler & Saier (1982).
(h) Simmons (1981*b*).

formation. Those studies are summarized elsewhere in this symposium (Cereiji[redacted] 1983).

EPITHELIAL CELL BIOLOGY

The development and maintenance of epithelial cell polarity have been studied in several ways. One promising approach is based on the observation that certain enveloped viruses (e.g. semliki forest virus) bud from the apical surface of infected epithelial cells whereas another enveloped virus, vesicular stomatitis virus, buds from the basolateral surface (Rodriguez Boulan & Sabatini, 1978). The viruses bud from all surfaces of infected non-polar cells. Because of the information already available about virus envelope proteins, they have become valuable models for studying the polar distribution of plasma membrane proteins in epithelia. It is possible to locate specific viral envelope proteins in a cell using immunofluorescence and immunoelectron microscopy. Before viral budding, the envelope proteins are exclusively localized in the same plasma membrane from which the virus buds (Rodriguez Boulan & Pendergast, 1980). Apparently, virus coat proteins are synthesized and inserted in the plasma membrane of infected cells before the nucleocapsid emerges from the cell through the plasma membrane. Garoff and his associates (Kondor-Koch, Riedel, Soderberg & Garoff, 1982) have injected into the nucleus of baby hamster kidney cells (a fibroblastic cell) mRNA for the three structural proteins of semliki forest virus as well as cloned cDNA prepared from the mRNA. Using immunofluorescence, they found the three structural proteins were expressed in the injected cells. The envelope protein designated p62 was located in the plasma membrane in cells injected with mRNA or with cDNA. The cDNA can be altered to determine which characteristics of the gene and its products are important for the various steps in the synthesis of plasma membrane proteins (including their insertion in membranes and their polar distribution in epithelia).

Epithelial cells lose their polarity when they are separated into single cells. There is disagreement as to the immediate fate of the plasma membrane when epithelial cells are separated from an epithelium. Pisam & Ripoche (1976) prepared single epithelial cells by exposing frog urinary bladder to divalent cation-free Ringer's solution containing EDTA. Single cells maintained in divalent cation-free solution lost polarity. Over a period of 2 h, a variety of apical and basolateral plasma membrane markers were found redistributed over the entire cell in a global fashion. Fujimoto & Ogawa (1982) performed similar experiments with frog urinary bladder. They confirmed that the apical plasma membrane microvilli redistributed in a global fashion, but reported that the basolateral plasma membrane was endocytosed. The reason for the difference in results is not apparent. Redistribution of membrane markers has also been observed when cultured epithelia are separated into single cells by chelation of divalent cations, without separation from their attachment surface (Dragsten, Blumenthal & Handler, 1981; Sang, Saier & Ellisman, 1979).

Since the tight junction is located at the junction of apical and basolateral plasma membranes, it is convenient to assign it a functional role in the maintenance of polarity. This has not been established. Other cell constituents may perform this function. It is possible that however chelation disrupts tight junctions, it also disru[redacted]

[illegible]er cell constituents that maintain polarity. For example, tight junctions are not directly involved in the distribution of certain lectin-binding sites on the apical membrane of cultured epithelia. In intact epithelia, fluorescent lectins bound to the apical plasma membrane are immobile in the plane of the plasma membrane as detected by photobleaching recovery. Following chelation of the epithelium, the bound lectin moves around the surface of the cell, but in an organized, non-random fashion (Dragsten *et al.* 1981). The binding sites are probably anchored to the cell cytoskeleton. Louvard and his colleagues (Louvard, 1980; Reggio, Coudrier & Louvard, 1982) used immunofluorescence to follow an apical membrane enzyme, aminopeptidase, in MDCK epithelia. After binding to sites on the apical plasma membrane, the antibody is endocytosed in coated pits. Subsequently, the antibody appears within the cell in a region near the nucleus. Endocytosed antibody, as well as new antigenic sites, appear in the apical plasma membrane at the periphery of the cell, near the tight junction. It is not clear whether this is a normal cycle for apical membrane aminopeptidase and other membrane proteins, or a cycle provoked by the binding of polyclonal antibodies.

Plasma membrane lipids are also distributed in a polar fashion in epithelia. Van Meer & Simons (1982) took advantage of the polar budding of enveloped viruses to sample apical and basolateral plasma membranes of cultured epithelia. There are differences in the lipids of viruses that bud from opposite surfaces of an epithelium, but no differences when the infected epithelium is chelated and allowed to become non-polar before viral budding. The mechanism of segregation of lipids in the plasma membrane of epithelia has been studied by following the fluorescence of lipid-like fluorescent probes incorporated in the plasma membrane of cultured epithelia (Dragsten, Handler & Blumenthal, 1982). Some probes pass the tight junction when incorporated in the apical or basolateral plasma membrane, whereas other lipid probes do not. Those that pass the tight junction flip-flop between leaflets of the plasma membrane bilayer of cells or liposomes and are more mobile in the plasma membrane than lipid probes that do not pass. It was proposed that movement of lipids in the outer leaflet of the plasma membrane bilayer is restricted by the tight junction, whereas movement in the inner leaflet is not restricted.

PROSPECTS

In view of the increasing interest in cultured epithelia, I anticipate that a greater variety of transporting epithelia will become available for study. In addition to the work summarized in Table 1, other laboratories (Horster, 1979; Grenier, Rollins & Smith, 1981) are developing new epithelial cultures. When cells can be grown in a defined medium without serum, control of culture conditions is increased and cost reduced. Defined media have been described for MDCK (Taub, Chuman, Saier & Sato, 1979) and for LLC-PK_1 (Saier, Erlinger & Boerner, 1982) cells, and will probably become available for other epithelial cultures. Methods will be developed to promote differentiation and a wider range of techniques will be applied to study transport and its control in cultured epithelia. I look forward to the time when the techniques of cell biology are applied directly to the study of transport and its [illegible]trol.

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