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Transport and metabolic functions in cultured renal tubule cells

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Cultured renal tubule epithelia are widely recognized as a most effective tool by which to study individual factors involved in the regulation of growth and function in polarized cells. The principal advantage of this tool lies in its availability, that is, large homogeneous cell populations and direct access to their monolayers, within a rigorously controlled environment for long-term monitoring of metabolic and biophysical events. This article's[1] focus is the review of certain current principal lines of study, to which renal epithelial cultures have contributed in broadening our understanding of corresponding in vivo processes. These include the control of cell growth, the regulation of transepithelial solute transport, the induction of differentiated function, the control of metabolic pathways, and the expression of epithelial sidedness of these functions.

Control of growth in cultured tubule epithelia

Recent studies on the control of growth of renal epithelia have rekindled the hypothesis of Cone [3] that monovalent cations may be important regulators of cell mitotic activity. Using renal cell lines (BSC-1 and -2),[2] it was demonstrated that low K-concentration [5] or elevated Na [6] in the medium stimulate cell mitotic growth, probably via an autocrine, growth-inhibitor-mediated control system [7], in which the inhibitor protein may act upon cell Na content [8]. The transient enhanced Na influx, for example, during low potassium in the medium, may be responsible for initiation of kidney epithelial cell growth [9]. Like other established cell systems, the BSC-1 line responds to the addition of NaCl (5 to 25 mM) with increased DNA-synthesis in a concentration-dependent manner [6]. The synthesis depended on serum and cell density. Thus, the net flux of Na or its intracellular concentration appears to be mediators or messengers involved in the mechanism by which "growth factors" regulate epithelial cell proliferation. The BSC-1 cells secrete a growth inhibitor protein ($M_r \approx 24{,}000$) which may depress growth via control of cell Na content [8]. It is of particular interest that the presence of the inhibitor can be neutralized by either serum or EGF suggesting that an autoregulation of growth may be located in this epithelial line.

Growth regulation of the MDCK line, similarly, depends on serum-mediated Na-uptake, stimulation of Rb-uptake (an indicator of K-movement through the ATPase carrier), and subsequent stimulation of DNA-synthesis [10]. The serum-initiated DNA-synthesis was prevented by ouabain. These facts emphasize the possible role of monovalent ion entry and of intracellular "ionic composition" in growth regulation, as outlined [3] and shown previously for other cell types. Chemical inducers of differentiated properties such as brushborder enzyme activities or dome formation in MDCK were found to also increase intracellular Na content, probably by differing mechanisms [11].

Vasopressin. It was initially reported in fibroblasts to increase growth and Na-entry [12]. Subsequently, epithelia of the line BSC-1, when grown in confluent cultures, were shown to respond to vasopressin by increased growth [13]. Not only was the cell Na content of BSC-1 elevated by vasopressin, but, vasopressin rapidly stimulated the uptake of AIB and enhanced the binding of EGF. However, to my knowledge, none of these effects has been established yet as a mediator of cell proliferation.

Serum-free growth of renal epithelia. Without altering cell properties in comparison to serum-dependent growth, this would be an advantage for studies on determinants of growth and differentiation into terminal biophysical and biochemical function. Also, substitution of serum by defined hormonal components or related factors should reveal the role of serum constituents in numerous regulatory mechanisms of epithelial transport and metabolism.

To my knowledge, requirements for growth of epithelial lines in serum-free medium were first reported by Hayashi and Sato [14]. This approach has been applied to MDCK [15], to LLC-PK_1, to cells obtained from purified proximal tubule suspensions [17], and to epithelial cultures derived in vitro from defined nephron segments [18–20]. It appears that cultures of nondefined (MDCK and LLC-PK_1) and defined nephronal segmental origin have some requirements for medium components in common. For example, vasopressin, transferrin, and insulin, when deleted alone from the medium, reduce proliferation in LLC-PK_1 [16]. In MDCK, prostaglandin E_1, transferrin, and insulin appear to be critical for maintenance and growth of the monolayer [15]. Unlike these lines of nondefined renal origin, cell cultures derived in vitro from individual distal nephron segments appear to depend on hormones and "factors" for proliferation and on others for the expression of differentiated functions [21]. Cell number in segmental nephron cultures depended on selective hormonal supplements of the serum-free

[1] Work on transport and metabolism in cultured tubule epithelia which has been reported since 1980 [1, 2] will be emphasized.
[2] Terminology and definitions in this article correspond to those suggested by the Tissue Culture Association [4].

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medium [18, 19], as was DNA-synthesis [20]. Cells of the cortical and medullary segments of collecting tubule (CT) and thick ascending loop of Henle retained properties characteristic of the epithelium in vivo; in particular, the differentiated and polar ultrastructure [22], hormone-sensitive adenylate cyclase [20], Na-K-ATPase distribution [20], and mitochondrial heterogeneity, that is, two cell types, in cortical CT cell cultures [23]. It was suggested that the hormonal control of proliferation differs from that of the terminal differentiation in segmental renal cultures [24]. In fact, physical and biochemical transport functions of cultured collecting tubule epithelia (CCT) are induced selectively by steroid and thyroid hormones in serum-free medium [22, 24].

Solute transport and metabolism in cultured tubule epithelia

Epithelial lines derived from cell suspensions of the mammalian kidney have been utilized as in vitro model systems for studies on ion transport, enzyme biochemistry, and substrate metabolism in epithelia. Two of these lines, MDCK and LLC-PK_1, express principal features of renal epithelial morphology, that is, basalolateral membrane infoldings, junctional complexes, and apical microvilli. Both lines exhibit functional parameters of their tissue of origin such as vectorial ion and water transport associated with transepithelial voltage and electrical resistance. While the MDCK line resembles some characteristics of the distal nephron [25], LLC-PK_1 retains properties such as Na-dependent glucose uptake [26] reminiscent of the proximal nephron.

Transepithelial salt transport. In cultured epithelia this is evaluated best by direct measurements across cell monolayers grown on permeable support (filter, collagen membrane, and amnion) and mounted into Ussing-type chambers. MDCK cells express voltage and electrical resistance values within the range of those occurring in natural distal nephron epithelia. However, maximum Na transport rates, as estimated from short-circuit current, Na pump sites, and ATP hydrolysis [27], are considerably lower in MDCK. This fact most likely reflects the comparatively low degree of basolateral organization [28].

Luminal Na uptake. In Na-depleted monolayers of MDCK, luminal Na uptake revealed that the rate of Na uptake, but not the intracellular steady-state concentration, was increased significantly by external K, whereas Li and amiloride inhibited Na uptake only in the presence of K. These data suggest two noninteracting transport modes: an amiloride-sensitive, K-independent, and a furosemide-sensitive, K-stimulated component of luminal Na uptake [29]. In addition, Na stimulated the uptake of Rb. Thus, MDCK appears to express a (NA,K) cotransport system, as evaluated from luminal uptake studies, which does not depend on the (Na,K)ATPase but depends on ATP activation. ATP, when applied to the basolateral cell surface of MDCK monolayers, results in a rheogenic Cl secretion from basal to apical, as evidenced by a furosemide-sensitive increase in short-circuit current without changes in Na flux [30]. The K-stimulated Na uptake depended on the concentration of Cl in the medium [31]. Other anions were unable to substitute for Cl, suggesting that Cl participates in the cation cotransport system to render it electroneutral with a ratio of two Cl coupled to the two cotransported cations. Basolateral K uptake in MDCK displays a component which depends on external Na and Cl, is insensitive to ouabain, and sensitive to furosemide [32]. In addition, K influx is activated by Ca^{2+} [33] and sensitive to adrenalin.

Adrenalin. It stimulated the transepithelial short circuit-current in MDCK acutely by a factor of almost 30; this increase corresponded to a rise in the baso-apical net transfer of Cl [34], which was amiloride-insensitive but inhibited by furosemide. This and other data suggest that MDCK expresses a mode of Cl secretion in which a basolateral, Na-energized Cl transport is followed by an apical passive exit, similar to the mechanism described for in vivo epithelia. In addition, epinephrine stimulated the adenylate cyclase activity and intracellular cyclic AMP accumulation. The extent to which these receptor-mediated processes may relate to an increase Cl conductance at the apical side of MDCK is as yet unknown.

In MDCK, two strains with different functional and structural characteristics have been described [35]. $MDCK_I$ cells are high-resistance, low short-circuit current (SSC) monolayers; their SCC is insensitive to adrenalin, PGE_1, and AVP. By contrast, $MDCK_{II}$ is a low-resistance epithelium, and SCC is insensitive to those factors which stimulate in $MDCK_I$. However, specific Na-K-ATPase is higher by a factor of two in $MDCK_{II}$, and this strain is equipped with alkaline phosphatase and γ-glutamyl transpeptidase activities, suggesting that $MDCK_{II}$ may express some properties of the proximal tubule whereas those of $MDCK_I$ resemble some of the collecting tubule epithelia.

Differentiated transport functions. In renal epithelia derived from defined nephron segments, these were first shown in monolayers grown in vitro from medullary thick ascending loops of Henle [36]. These primary cultures expressed a transepithelial voltage for some 3 weeks, which was positive toward the apical cell surface, which was reduced reversibly by furosemide but not by amiloride, indicating that specific in vivo properties had been maintained in vitro. Segmental differentiated transport functions in response to selective hormones substituted in the medium were demonstrated in monolayer cultures derived in vitro from the cortical collecting tubule [21]. Transmonolayer voltage and Na-K-ATPase activity were expressed differentially when either dexamethasone or aldosterone were the sole steroids in the medium. Utilizing the nephron segmental culture system [18], cortical collecting tubule cells were also shown to retain their response of cyclic AMP to antidiuretic hormone in the presence of a phosphodiesterase inhibitor [37].

Continuous cell lines established from the medullary thick ascending loops of rabbit [38] and mouse [39] express differentiated segmental functions; the principal maneuver in this important advance was an amnion matrix [38] used for initial passages, or a type I collagen gel [39] on which the cells were kept without separation for more than 2 years. It is of particular importance that the mouse cell line [39], following subcutaneous implantation into mice of monolayers in a diffusion chamber, re-expressed characteristics of the segment of origin which had been lost during continuous culture [39]. MDCK cells when maintained in nude mice, develop a higher rate of net Na flux when compared to the in vitro monolayer [40]. These findings [39, 40] may re-emphasize the significance of undefined factors for the expression of differentiated function in culture.

Ca^{2+} transport kinetics have been evaluated in renal tubule cell cultures. Ca^{2+} uptake into LLC-MK_2 is characterized by

two distinct influx rates. The fast Ca^{2+} influx and its compartment size increase linearly with medium Ca^{2+} concentration suggesting that this component is Ca^{2+} diffusion. The slow Ca^{2+} influx exhibits the pattern of a saturable component with a K_m of 0.3 mM and a V_{max} of 0.065 μmoles cm^{-2} s^{-1}, indicating an active carrier transport [41]. PTH increases the carrier-mediated Ca^{2+} uptake, whereas the fast Ca^{2+} influx is not affected: PTH increases the K_m and V_{max} by factors of 4 and 20, respectively [41]. Kinetic parameters of $^{45}Ca^{2+}$ desaturation in LLC-MK_2 cells revealed three exchangeable calcium pools with different time constants [42]. Two of the efflux rate constants are comparable with the uptake rate constants, representing the extracellular and cytoplasmic compartment, respectively. The third component, with a very low efflux rate constant is completely abolished by two specific inhibitors of mitochondrial Ca^{2+} uptake, antimycin A, and Warfarin; this component, then, represents the mitochondrial calcium pool. Intracellular free calcium in LLC-MK_2 is some 57 nmoles l^{-1} [43].

Phosphate uptake. The kinetics of phosphate uptake were studied in LLC-PK_1 [44], where phosphate uptake results from a saturable sodium-dependent, and a nonsaturable sodium-independent component. The apparent K_m of the sodium-dependent phosphate uptake in LLC-PK_1 was 0.025 mM, similar to that in renal brushborder vesicles [45]. Phosphate uptake was four times higher from the apical than from the basolateral cell side. Sodium increases V_{max} without altering the apparent K_m, and arsenate competitively inhibits this sodium-dependent phosphate transport [44]. The stoichiometric analysis revealed that two sodium ions are cotransported with one HPO_4^{2-} ion in an electroneutral manner [46]. The Na-dependent phosphate uptake is inhibited markedly by ouabain, carbonylcyanide-p-trifluoromethoxyphenylhydrazone, and p-chloromercuribenzoate [47]. Furthermore, PTH, db-cyclic AMP and forskolin, a diterpene activator of adenylate cyclase, significantly decreased Na-dependent phosphate uptake in LLC-PK_1, whereas vasopressin and isoproterenol had no effect [47]. Detailed analysis of the Na-dependent phosphate transport, utilizing apical membrane vesicles from LLC-PK_1, revealed that an apparent K_m of 99 μmoles l^{-1}, and an apparent K_i of 1.9 mmoles l^{-1} for the arsenate inhibitory effect. For Na activation of phosphate uptake, an apparent K_m of 32 mmoles l^{-1} was present [48]. The LLC-PK_1 line expresses components of the regulatory system for phosphate transport.

The regulation of calcium and phosphate homeostasis partially depends on the hormonally active major metabolite of 25-hydroxyvitamin D_3, the 1,25-dihydroxyvitamin D_3. Renal biotransformation of 25-hydroxyvitamin D_3 was characterized in renal tubule cells in culture, and the hydroxylation reactions appear to be identical in cultures from different species [49–52]. In primary cultures from normal rhesus monkey kidney, 25-hydroxyvitamin D_3 is transformed preferentially to either 24,25-dihydroxyvitamin D_3, or to 1,25-dihydroxyvitamin D_3 depending on the experimental conditions. PTH in the culture medium suppressed 24, 25-dihydroxyvitamin D_3 formation, whereas 1,25-dihydroxyvitamin D_3 in the medium produced a marked increase in 24,25-dihydroxyvitamin D_3 formation. When Ca^{2+} was 3 mM instead of 1 mM in the medium, a significant increase of 24,25-dihydroxyvitamin D_3 ensured [50]. Corresponding results were obtained in primary cultures of renal cells derived from vitamin D-deficient chicken, where 1,25-dihydroxyvitamin D_3 resulted in an inhibition of 25-hydroxyvitamin D_3-1α-hydroxylase activity and a stimulation of the 25-hydroxyvitamin D_3-24R-hydroxylase [51]. Estradiol inhibits the 25-hydroxyvitamin D_3-1α-hydroxylase activity. When 25-hydroxyvitamin D_3-1α-hydroxylase was suppressed and the 25-hydroxyvitamin D_3-24R-hydroxylase was induced by 1,25-dihydroxyvitamin D_3, estradiol had no effect on 1,25-dihydroxyvitamin D_3 formation; the production of 24,25-dihydroxyvitamin D_3 was not affected by estradiol. Similar results were obtained when either testosterone or insulin was present in the medium [52]. PTH, by contrast, increased 1,25-dihydroxyvitamin D_3 formation, but only in the presence of insulin [53]. The cultured cells, then, provide a system by which direct epithelial regulation may be separated from feedback mechanisms involving extrarenal systems.

Transepithelial D-glucose transport. In LLC-PK_1 it represents a model system of glucose reabsorption in the proximal tubule. Net apical to basolateral flux of α-methyl-D-glucoside, a nonmetabolizable glucose, is Na-energized [26], inhibited by phlorizin [54, 55], by ouabain and p-trifluoro-methoxyphenyl-hydrazone [56], moderately inhibited by phloretin, and only weakly inhibited by 3-0-methyl-D-glucose [57]. Short-circuit current was used as an estimate of the cotransport of Na and α-methyl-D-glucoside. An increase of short-circuit current is associated with an increment in oxygen consumption and stimulation of LLC-PK_1 metabolism increases Na/D-glucose cotransport [58]. ^{3}H-phlorizin binding studies in LLC-PK_1 revealed the existence of two distinct types of binding sites. Phlorizin binding for high affinity sites is Na-dependent, whereas its binding for low affinity sites is Na-independent [59]. In apical membrane vesicles prepared from LLC-PK_1, the stoichiometry of Na-dependent phlorizin binding was 1:1, and that of Na/hexose symport was 2:1 [60], suggesting that one Na ion and one glucose molecule interact with the carrier resulting in an uncharged complex. The binding of a second Na ion results in a single positive charge of the complex, thereby inducing the transformation step [60]. In accordance with these findings, a stoichiometry of 2:1 Na/glucose was shown in primary cultures of rabbit renal proximal tubule cells [61]. Kinetic analysis of α-methyl-D-glucoside transport demonstrated an apparent K_m of some 0.7 mM in intact LLC-PK_1 cells [26, 55]. In primary cultures of rabbit proximal tubule cells an apparent K_m of 0.8 mM and a V_{max} of 0.015 nmoles mg^{-1} min^{-1} were calculated [61]. In apical membrane vesicles prepared from LLC-PK_1, however, kinetic parameters differed considerably. For the Na-dependent component an apparent K_m of 2 mM [62], 10 mM [63], and 0.3 mM [64] was calculated; V_{max} ranged from 3 to 6 nmoles/mg of protein/min [62–64]. The uptake of α-methyl-D-glucoside into LLC-PK_1 is lower during the early growth phase after plating, and it increases gradually as growth rate decreases in confluent cultures. This pattern was modified by various factors. Dibutyryl-cAMP, hexamethylen bisacetamide, theophylline, and 1-methyl-3-isobutylxanthine produced an increase, whereas 12-0-tetradecahoylphorbol-13-acetate resulted in an inhibition of α-methyl-D-glucoside uptake. These studies, moreover, demonstrated that cyclic AMP, among others, is necessary to induce the development of α-methyl-D-glucoside transport [65]. The Na-dependent α-methyl-D-glucoside transport in LLC-PK_1 is regulated by the

glucose concentration in the medium. Epithelia grown in a low glucose medium have a much higher uptake rate and phlorizin binding than cells grown in high external glucose concentration [66]. Fructose and mannose which are not transported by the Na/glucose transporter, substitute for glucose in carbohydrate metabolism. The modification of their concentrations in the medium had a glucose-like effect on the α-methyl-D-glucoside transport. By contrast, α-methyl-D-glucoside did not affect its own transport [67], indicating that the trigger is not a direct effect of glucose on its transporter but possibly a component of carbohydrate metabolism. Indeed, ^{14}C glucose, after luminal uptake, is decarboxylated to a considerable extent, as demonstrated in primary cultures of proximal tubule cells [61].

Insulin. In addition to its basolateral effect on net Na transport, it stimulates the incorporation of glucose into glycogen [68]. The number of insulin binding sites appears to be related to the glucose concentration of the medium, as shown in a line of Chinese hamster kidney cells. The number of binding sites was higher in cells grown in high glucose concentration when compared to those in low glucose. Tunicamycin, an inhibitor of protein glycosylation, decreased the number of insulin binding sites in high glucose cells [69]. The stimulatory effect of glucagon on cyclic AMP production was reduced markedly in virus transformed MDCK cells when compared to the control MDCK. The sensitivity to glucagon could be restored by the addition of PGE_1 or butyrate to the culture medium [70].

Amino acid transport systems in the proximal tubule have been studied by clearance techniques, cortical slice analysis, microperfusion, and membrane vesicle preparations [71]. LLC-PK_1 cells have served to further characterize the transport of neutral and acidic amino acids, and different transport systems for neutral amino acids [72, 73] have been demonstrated. Of these, one transports alanine in a strictly Na-dependent manner, it mediates alanine exchange, and it is inhibited preferentially by serine, cystein, and α-amino-n-butyric acid. Another system transports leucine without Na-dependency, it mediates leucine exchange, and it is inhibited by 2-aminonorborane-2-carboxillic acid, or by hydrophobic amino acids [72]. The uptake of 2-aminoisobutyric acid and the accumulation of cycloleucine in LLC-PK_1 are mediated by a saturable Na-dependent, and by a nonsaturable Na-independent transport component. The Na-dependent uptake of 2-aminoisobutyric acid is partially inhibited by 2-methyl-aminoisobutyric acid. This latter transport fraction is mediated via the A system, whereas the uninhibited fraction is linked to the ASC system (see below). The Na-dependent uptake of cycloleucine is competitively inhibited by 2-aminobutyric acid, but not by 2-methyl-aminoisobutyric acid, and it is probably mediated by the ASC system. The Na-independent component of cycloleucine uptake is inhibited competitively by 2-amino-bicyclo (2,2,1)-heptane-2-carboxylic acid, a specific substrate of the L-system [73]. In MDCK cells, four transport systems for neutral amino acids have been described in detail [74]. The systems were characterized by Na-dependent α-methylaminoisobutyric acid uptake (A), the Na-dependent alanine uptake in the presence of 0.1 mM α-methylaminoisobutyric acid (ASC), the Na-independent leucine or methionine uptake (L), and the Na-dependent glutamine uptake (N). The A system was stimulated by insulin, inhibited by PGE_1; it was particularly stimulated in chemically transformed MDCK. The ASC system was stimulated only slightly by insulin and PGE_1 but it was unchanged in the transformed cell line. The activity of σ-glutamyltransferase, apparently, is not involved in the regulation of L-alanine and L-leucine transport in LLC-PK_1 [75]. Based on findings in LLC-PK_1, transport sites for L-alanine appear to be located predominantly, if not exclusively, at the basolateral cell side [76]. For the uptake of L-alanine into BHK21-C13 cells, a K_m of 0.81 mM and a V_{max} of 57.7 pmoles/μg protein · min were calculated [77], and K_m and V_{max} for the uptake of glutamate were 46 μM and 7.5 pmoles/μg protein · min, respectively [78]. Furthermore, Na-coupled α-methylaminoisobutyric acid uptake in confluent MDCK monolayers and isolated membrane vesicles revealed that the functional A system carriers are localized in the basolateral membrane [79]; the transport is electrogenic. α-Methyl-aminoisobutyric acid transport in basolateral membrane vesicles prepared from MDCK exhibited a K_m of 0.59 mM and a V_{max} from 1.5 to 5.3 nmoles/mg · min [79]. Renal epithelial cells in culture thus have been demonstrated to express distinct amino acid transport systems, their respective subspecies, and regulatory phenomena.

Hormonal regulation and induction in cultured tubule epithelia

Among the assumptions to be proven in cultured renal epithelia is the capacity of the cell to respond to physiological hormonal stimuli by adequate regulation and the expression of differentiated functions. The response may be defined in terms of any step interposed between receptor binding of the substance and its final effect.

Vasopressin, parathyroid hormone, calcitonin, and epinephrine increase the activity of adenylate cyclase in membrane fractions from whole kidney. The response of the enzyme, moreover, in single nephron segments is differential [80]. Continuous renal cell lines serve in characterizing additional steps in the action of hormones on epithelia. Adenylate cyclase activity in LLC-PK_1 cells increases after exposure to vasopressin and to salmon calcitonin [81] but not to epinephrine or PTH, a response which resembles to some extent that of the medullary thick ascending loop. When whole kidney cultures were pretreated for 24 hr in vitro with salmon calcitonin, a desensitization of adenylate cyclase ensued which was correlated with reduced specific calcitonin receptors; the pretreatment induced a tight binding of the hormone to its receptor, with persistent activation of the adenylate cyclase even after the hormone had been removed from the medium [82]. Vasopressin induces a probenecid-sensitive release of cyclic AMP from LLC-PK_1 monolayers into the medium bathing the apical cell surface [83]. The release into the basal compartment was smaller and not probenecid-sensitive suggesting a functional polarity of the LLC-PK_1 cell for this parameter. An elegant approach to further evaluate this functional polarity in cultured collecting tubule cells demonstrated that cyclic AMP was released in response to antidiuretic hormone when added to the basolateral but not to the apical surface of the confluent monolayer [84]. Moreover, an interaction of vasopressin with its receptor was demonstrated in LLC-PK_1 cultures, in that the hormone induced an alteration in binding affinity (receptor transition) which, by itself, may have a regulatory role at the level of coupling between hormone-receptor complex and adenylate

cyclase [85]. Vasopressin receptors were also examined [86] by ^{3}H-(Lys^8) vasopressin binding in LLC-PK$_1$ monolayers, a line derived from LLC-PK$_1$. In response to insulin or serum in the totally defined medium, the number of receptors and the vasopressin-sensitive adenylate cyclase activity were induced without a change in the apparent receptor affinity or cell growth. Other constituents of the defined medium, such as hydrocortisone, thyroid hormone, or dexamethasone did not affect the receptor binding of (Lys^8) vasopressin in LLC-PK$_1$ cells, in contrast to the in vivo renal tissue. ADH-stimulated production of adenylate cyclase in MDCK was characterized by the effects of ADH on the protein kinase [87]. Lysine-vasopressin activated the cyclic AMP-dependent protein kinase of MDCK in the presence of theophylline, indicating a role for the phosphorylation of some membrane constituents in the physiological action of ADH. PGE$_2$ inhibits the response of vectorial water transport to ADH in MDCK [88], suggesting that MDCK may lend itself for further studies on the regulation of ADH induced water transport. In view of the modulations that natural epithelia undergo during continuous culture in vitro, it is of considerable interest that vasopressin did not alter the short-circuit current and the transepithelial resistance, nor osmotic water flow and adenylate cyclase activity in two toad bladder lines, TB-6c and TB-M [89]. This is most likely due to the loss of AVP receptors or of the subsequent coupling step. The intermediate cyclic AMP, however, induced an increase in Na transport and in short-circuit current which was amiloride-insensitive.

Aldosterone. In the A6 epithelial line, it reversibly stimulated net transepithelial Na transport which was inhibited by the mucosal application of amiloride [90]. The kinetics of unidirectional apical Na influx in A6 have been defined [91] as a saturable function of Na concentration with a K_m of 18 mM and a V_{max} of 2.5 nmoles/min · cm^2. The entry step is inhibited by amiloride and activated threefold by aldosterone; the aldosterone-stimulated increase is inhibited by amiloride. The A6 epithelia express this amiloride-sensitive apical Na influx only when grown on permeable basolateral supports. First direct measurements of amiloride-sensitive Na channel activities were made in single isolated channels after incorporation into planar lipid bilayers [92]. Channel conductance was cation–selective with a low Na/K selectivity ratio of 2:1, and conductance in the open state of the channel was blocked very fast by cis amiloride. Methylation of A6 apical vesicles increases the rate of amiloride-sensitive sodium transport, implying that channel constituents may be part of the mechanism by which aldosterone regulates sodium flux [93]. Aldosterone regulates Na transport, in different hormone-sensitive epithelia, via the induction of citrate synthetase activity, implying that mitochondrial metabolism may be involved in this regulatory adaptation. It is of interest that continuous aldosterone-responsive cell lines (TB-6c and TB-M) did not respond with a change in citrate synthetase activity after incubation with aldosterone [94], indicating that the enzyme in these epithelia is not involved in aldosterone-induced Na transport. Insulin, after 24 to 30 hr of preincubation of A6 monolayer epithelia with aldosterone, caused a response of the short-circuit current which was additive to that of aldosterone when compared with the aldosterone-free preincubation [95]. This synergism may suggest an interaction of both hormones in the regulation of epithelial ion transport. The adequacy of the A6 cell for studies on steroid action in epithelia was emphasized further by the demonstration of co-occupancy, aldosterone, and corticosterone in nuclear-enriched fractions, of two classes of high-affinity binding sites [96], one of which is involved in the stimulation of Na transport. Epithelia derived in culture from the cortical collecting tubule express segment-specific functions following postmitotic induction by hormones [97]. The transmonolayer voltage, when measured in 2-day intervals in confluent cultures between days 6 and 14, was induced selectively by long-term incubation in triiodothyronine (10^{-11} M), dexamethasone (10^{-8} M), or aldosterone (10^{-9} M). The effect of aldosterone was amiloride-sensitive. Transepithelial electrical resistance was not dependent on the type of hormonal inducer. These cultures also express aldosterone receptor binding characteristic for the in vivo segment. It is proposed that hormones which regulate epithelial transport functions in vivo may operate as epigenetic factors in the expression of differentiated epithelial functions during in vitro culture [97]. The Na-K-ATPase in MDCK, as in LLC-PK$_1$ [98], is localized in the basolateral membranes only, and the sodium pump sites per cell depend on cell density in culture [99]. Chemical, non-physiological inducers of dome formation, an estimate of vectorial ion and water transport in attached monolayers, increase cellular Na content implying that this event, as in other epithelial lines, is regulated [99]. Na-K-ATPase activity is expressed in cultured nephron epithelia in response to hormones; using a microradioisotope assay [100], tridiodothyronine (10^{-11} M), dexamethasone (10^{-8} M), or aldosterone (10^{-9} M), in ascending order of their effect, were shown to selectively induce the Na carrier enzyme in cultured collecting tubule epithelia [22, 97]. The effect of aldostereone on the enzyme was amiloride-sensitive, suggesting a mediator role for intracellular Na content.

Summary

The study tool of cultured tubule epithelia has been applied to new areas in nephron cell biology, such as the evolution of epithelial membrane asymmetry. Studies utilizing monoclonal antibodies against plasma membrane glycoproteins in MDCK revealed that the development of surface cell polarity is a continuous process requiring intact tight junctions and their electrical resistor function [101]. The role of the junctional complex to establish and maintain distinct membrane protein domains had been suggested earlier from work utilizing the apical aminopeptidase [102] and fluorescent membrane probes [103]. Cultured tubule epithelia lend themselves for the evaluation of cell-specific membrane protein synthesis [104] and antigenic determinants [105]. Human renal epithelia, from normal [106, 107] and defined abnormal kidney [108], have been maintained functional in primary and passage culture [106]. Pathophysiological mechanisms may be examined in cultured tubule epithelia, as shown first [109] by studies on the recovery from ischemic failure, where anoxia and substrate deprivation resulted in cell swelling which was prevented in culture by an oncotic agent.

This article has not attempted to give an exhaustive account of the studies in which cultured tubule cells have served as a tool. Instead, the investigations quoted herein represent some principal lines of study, as seen from renal physiology, which may disclose details in culture of complex in vivo phenomena.

It was Bernard [110] who, in 1865, suggested that "physiological events must be isolated outside the organism . . . to better understand the deepest associations of the phenomena."

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