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Apico-Basal Osmotic Gradient Induces Transcytosis in Cultured Renal Collecting Duct Epithelium

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Summary. The present experiments report the existence of an apico-basal plasma membrane shuttle in cultured renal collecting duct principal cell epithelium. Apical and basal perfusion under isotonic conditions, 290 mosm phosphate-buffered saline (PBS), has no effect on the shape of the epithelium. In contrast, gradient perfusion bf the epithelium with 75 mosm PBS on the apical side and 290 mosm PBS on the basal side for 10 min alters the morphology of the epithelium by causing the originally columnar epithelial cells to become lower, the intercellular spaces to dilate, and the intracellular vesicles to enlarge. Perfusion of the epithelium with isotonic PBS in the presence of electron-dense cellular markers such as gold-coupled $GP_{CD}I$ antibody, recognizing a glycoprotein in the plasma membrane of collecting duct cells (W.W. Minuth, G. Lauer, S. Bachman and W. Kriz, *Histochemistry* **80**:171–182, 1984), cationized ferritin (CF), horseradish peroxidase (HRP) and native ferritin (NF) for 10 min reveals their binding at the apical plasma membrane. Little endocytosis is observable. However, after labeling the luminal side by the cellular markers and following exposure to apical hypotonicity, 75 mosm PBS for 10 min, endocytosis of all markers is enhanced to a high degree. Furthermore, the gold-coupled $GP_{CD}I$ antibody and cationized ferritin are transported within vesicles unidirectionally through the epithelium and are exocytosed at the basolateral aspect, indicating the retrieval and possible translocation of apical plasma membrane. In contrast, volume markers such as NF and HRP are also endocytosed under osmotic gradient exposure, but are not seen to be transcytosed. Therefore, the function of this membrane pathway seems not to be related to water reabsorption, but may be part of a cellular response as protection against the osmotic gradient.

Key Words mammalian kidney · collecting duct · cultured cells · endocytosis · transcytosis · osmotic gradient

Introduction

In addition to the aldosterone-dependent Na^+ reabsorption (Morel & Doucet, 1986), the main function of the collecting duct system is the reabsorption of water, dependent on a luminal-antiluminal osmotic gradient, and triggered by the antidiuretic hormone vasopressin (Morel et al., 1976; Kokko, 1987; Morel, Imbert-Teboul & Chabardes, 1987). Studies of microdissected mammalian collecting duct tubules applying a luminal-antiluminal osmotic gradient of approx. 200 mosm have provided qualitative and quantitative data elucidating the mechanism of water reabsorption mediated by the vasopressin-sensitive adenylate cyclase system (Ganote et al., 1968; Grantham et al., 1969; Kirk, Schafer & DiBona, 1984; Strange & Spring, 1987). Investigations on amphibian toad bladder using tracer substances have demonstrated that cellular endocytotic processes may be additionally involved (Masur, Cooper & Rubin, 1984; Ding, Franki & Hays, 1985; Beauwens et al., 1986; Hays, Franki & Ding, 1987). On the other side, information about transcellular pathways, independent on the adenylate cyclase systems has already been documented in studies both on continuous cell lines and other epithelial cells by labeling with specific markers such as immunoglobulin G (IgG; Abrahamson & Rodewald, 1981), implanted plasma membrane proteins (Matlin et al., 1983; Pesonen & Simons, 1983; Mostov & Simister, 1985), as well as with unspecific markers like cationized ferritin (CF; van Deurs, von Bülow & Möller, 1981; von Bonsdorff, Fuller & Simons, 1985), and horseradish peroxidase (HRP; Abrahamson & Rodewald, 1980).

Recently, we demonstrated that cultured collecting duct cells grown on a specific renal support can be maintained in a differentiated state (Minuth et al., 1986). The cultured cells develop many characteristics of adult Principal cells of the renal collecting duct, as shown by morphological (Minuth & Kriz 1982; Gross et al., 1986), immunological (Minuth et al., 1984; Minuth & Gilbert 1987), and electrophysiological methods (Gross et al., 1986). In the present investigation we analyzed an apico-basal transcellular pathway in cultured renal collecting duct epithelia. We studied the distribution of electron-dense markers in the cells perfused in a microchamber system under isotonic and osmotic

gradient conditions. These experiments were performed according to previous investigations (Ganote et al., 1968; Kirk et al., 1984; Strange & Spring, 1987) and according to the conditions in the kidney, where a luminal-antiluminal osmotic gradient across the collecting duct exists. This transepithelial gradient increases from the cortex towards the medulla.

Materials and Methods

Cell Culture

Cortical explants of kidneys from neonatal New Zealand rabbits were isolated as previously described (Minuth & Kriz, 1982; Gross et al., 1986). The thin tissue layer consists of collecting duct anlagen and *S*-shaped bodies adhering to the capsula fibrosa (Fig. 1). It was mounted on a shallow plastic cylinder (R_1) of 3-mm inner diameter (Fig. 1*a*) and fixed by a tight-fitting plastic ring (R_2). This tissue setting was cultured in Dulbecco's MEM containing 10% fetal calf serum (Gibco Biocult, Karlsruhe, FRG) in humid atmosphere with 95% air and 5% CO_2. Within the first 24 hr of cultivation the collecting duct cells grew out of the collecting duct anlagen (Fig. 1*b*), forming a polarized differentiated epithelium (Fig. 1*c*). After 4 to 5 days of cell culture the cells were used for microperfusion experiments.

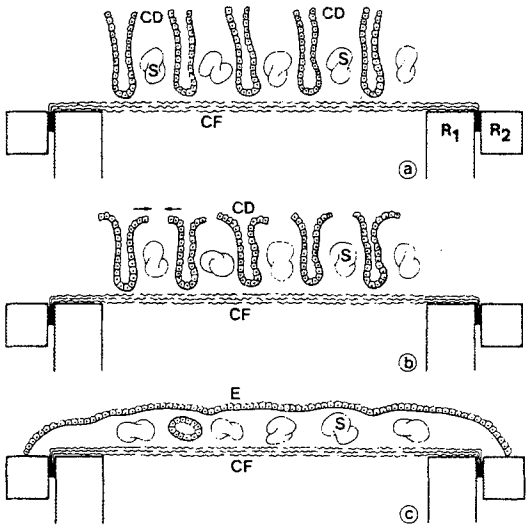

Fig. 1. (*a-c*) Schematic illustration of the outgrowth and development of renal collecting duct epithelium. (*a*) The thin cortical explants of neonatal rabbit kidney consisting of collecting duct anlagen (*CD*), S-shaped bodies (*S*) and capsula fibrosa (*CF*) were mounted on a plastic cylinder (R_1) with an inner diameter of 3 mm and fixed by a tight-fitting ring (R_2). (*b*) In the DMEM containing 10% fetal calf serum, the collecting duct cells grow (arrows) out of the collecting duct anlagen. (*c*) Within 24 hr of culture a single-layered, polarized and differentiated collecting duct epithelium (*E*) is formed

Perfusion Chamber for the Collecting Duct Epithelium

For studying morphological and cell biological aspects of the cultured collecting duct epithelium under perfusing conditions, the cells were transferred into a perfusion chamber (Fig. 2), which was designed for this purpose by Erich Botz (Institute of Anatomy I, University of Heidelberg, FRG). The epithelium, fixed on the plastic cylinder, was mounted in the basal chamber part and was covered with a tight-fitting apical chamber lid equipped with a rubber ring seal. Thus, a separate apical and basal perfusion of the epithelium by inserted cannulae was possible. The apical and basal chamber was perfused independently with media at a constant flow of 3 ml/hr at room temperature of 22°C.

Perfusion Experiments

The collecting duct epithelium installed in the chamber was perfused using different protocols. For morphological studies the epithelium was perfused either under isotonic conditions or under an apico-basal osmotic gradient using diluted apical medium. In order to investigate transcytosis of membrane, a second approach was made. Tracer substances were added to the apical side of the collecting epithelium cells under isotonic conditions. Then the cells were either perfused isotonically or exposed to an apico-basal osmotic gradient for 10 min.

Isotonic Perfusion

The epithelia (n = 19) were perfused simultaneously both on the basal and the apical side under isotonic conditions. The basal and the apical perfusion medium was phosphate-buffered saline (PBS) with an osmotic pressure of 290 mosm. Measurements were performed with a Knauer half-micro osmometer (Heidelberg Eppelheim, FRG).

Osmotic Gradient Perfusion

To produce an apico-basal gradient, the apical side of the epithelia (n = 24) was perfused with PBS diluted to an osmotic pressure of 75 mosm by adding sterile H_2O. The basal perfusion medium remained unaltered PBS (290 mosm).

Applications of Tracers

As volume markers, horseradish peroxidase (HRP, 5 mg/ml) or native ferritin (NF, 10 mg/ml) were added to the apical perfusion medium. Isotonic as well as gradient perfusions were carried out.

Membrane Markers

Functioning as adsorptive membrane marker either cationized ferritin (CF, 10 mg/ml) or the specific collecting duct $GP_{CD}I$ antibody (Minuth et al., 1984) with IgG-coupled gold (Janssen, Belgium) were applied. Isotonic and osmotic gradient perfusion ensued.

Light and Electron Microscopy

The epithelia were fixed by simultaneous apical and basal perfusion of 3% buffered glutaraldehyde for 10 min. The cells were

removed from the chamber assembly, postfixed in 1% OsO_4, dehydrated using increasing concentrations of ethanol, and finally embedded in a flat rubber chamber in Epon 812. Ultrathin sections strongly perpendicular to the surface of the epithelia were cut and examined with a Philips 301 electron microscope. All chemicals were obtained from Sigma, Munich, FRG.

Results

In the first set of experiments cultured collecting duct epithelia were perfused under isotonic conditions with 290 mosm PBS both on the luminal and basal side for 10 min. Light- and electron microscopy showed columnar shape of the cells (Fig. 3*a,b*). Narrow and sharply outlined intercellular spaces separated neighboring cells (Fig. 3*b*). In contrast, perfusion of the epithelia under an osmotic gradient of 75 mosm PBS at the luminal and 290 mosm PBS at the basal side revealed consistent changes in morphology (Fig. 3*c,d*). Compared to controls (Fig. 3*a,b*), these epithelia became flattened to about half their original height. At the light-microscopical level dilated intercellular spaces were visible (Fig. 3*c*). In electron-microscopical cross sections the area of each intercellular space was approximately equal to one-quarter of the related cell profile (Fig. 3*d*). The tight junctions of gradient-treated epithelia displayed the same intact shape as seen in epithelia under isotonic perfusion. However, at the apical cell side, protruding processes of neighboring plasma membrane into the apical medium often were noticed (Fig. 9*a*). Compared to control epithelia, the vacuoles in the apical cytoplasm appeared to be swollen and increased in number after osmotic gradient exposure.

To gain further insight into the membrane processes involved in these morphological alterations, experiments were carried out in the presence of volume markers in the apical perfusion medium. Volume tracers were horseradish peroxidase (HRP; Fig. 4; Graham & Karnovsky, 1966) and native ferritin (NF; Farquhar, 1981). These markers are known to be internalized into the cells by fluid-phase pinocytosis (Steinman et al., 1983). On collecting duct epithelia perfused under isotonic conditions, HRP was found to be attached to the apical plasma membrane, while NF could only be detected close to some microvilli. Only very few cells contained labeled vesicles. However, the number of cells containing labeled vesicles increased, when an osmotic gradient was set up across the perfused epithelium (Fig. 4). Almost every cell showed several vesicles labeled with HRP in the apical cell pole. Roughly estimated, NF could be seen less frequently than HRP. However, neither HRP nor NF could be detected within the intercellular spaces

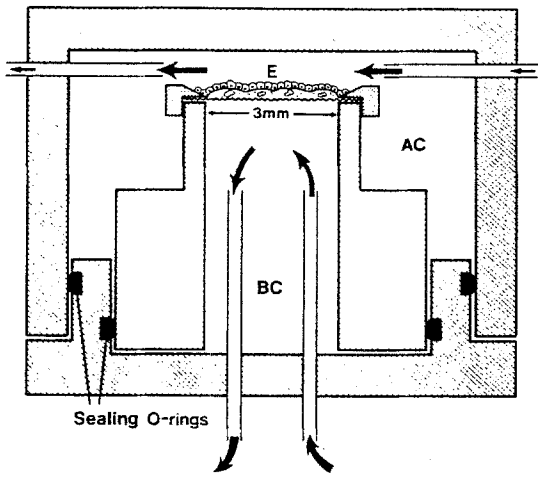

Fig. 2. Schematic illustration of the perfusion chamber. The cultured epithelium (*E*) mounted on the plastic cylinder seals off an apical (*AC*) from a basal chamber (*BC*). Both chambers are perfused separately through inserted cannulae

and at the basal aspect of the epithelium under any perfusion conditions.

In the third experimental approach, markers were used that bind to the apical plasma membrane. Perfusion experiments were carried out with the antibody against $GP_{CD}I$, a glycoprotein specific of collecting duct cells (Minuth et al., 1984). This antibody was applied to the apical side of the epithelium and visualized by IgG coupled with gold (Fig. 5*a*). The apical cell membrane of the epithelium reacted with the antibody in the form of irregular clusters, as revealed by the distribution of gold particles. Up to 100 gold grains were counted per cell profile. Under isotonic perfusion conditions, only a small degree of internalization of the antigen-antibody complex was observed. The exposure of labeled $GP_{CD}I$-antibody epithelia to an apico-basal osmotic gradient resulted in the internalization of apical plasma membrane (Fig. 5*a*). Anti-$GP_{CD}I$-gold complexes were detected in coated pits, coated vesicles (Fig. 5*b*) and in the cytoplasm (Fig. 6). The gold complexes were also found to be exocytosed at the basal aspect of the epithelium (Fig. 5*c*). In epithelia not exposed to an osmotic gradient, transcytosis of the $GP_{CD}I$-antibody gold complexes was not observed.

CF has been widely used as a probe for studying membrane cycling (Farquhar, 1983; Steinman et al., 1983). It was applied to the cultured collecting duct cells (Figs. 7, 8, 9) in order to confirm the results obtained by using the specific $GP_{CD}I$ antibody label (Fig. 5). After CF had bound to the apical plasma membrane (Fig. 7*a*), it was widely endocytosed Figs. 7*b*, 8*a,b*, 9*b*) when the epithelia were exposed to an osmotic gradient. The endocytic pits

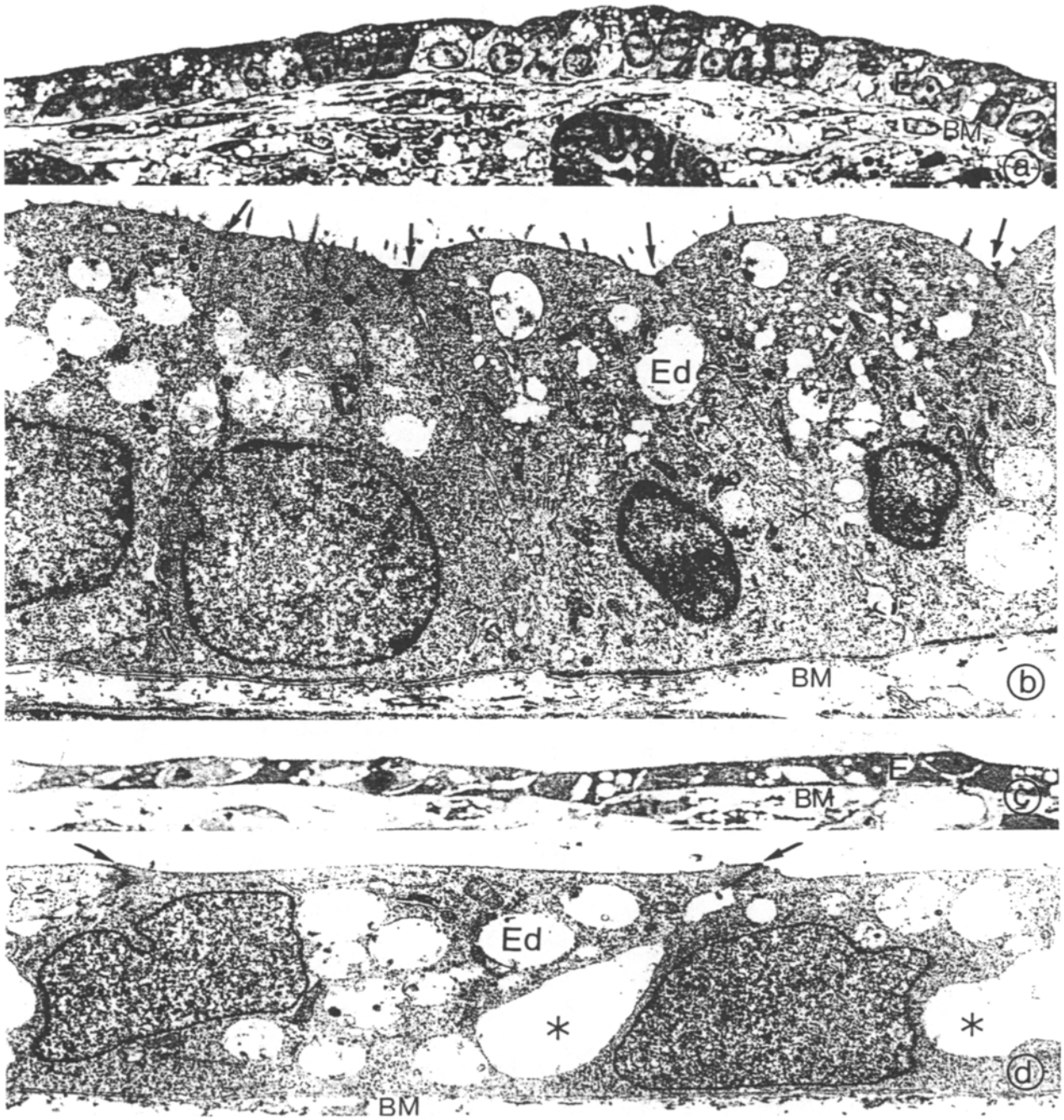

Fig. 3. (*a-d*) Light- and electron microscopy of (*a*, *b*) isotonically perfused and (*c*, *d*) osmotic gradient exposed collecting duct epithelium (*E*), 10 min each. (*a*) The collecting duct cells reveal a columnar shape with a basally located nucleus. (*b*) The epithelium rests on a basement membrane. (*BM*). The tight junctions (arrows) separate the sharply outlined intercellular spaces (asterisk) from the luminal side. The apical cytoplasm contains few endosomes (*Ed*). (*c*) After osmotic gradient exposure for 10 min, the epithelium (*E*) appears lower down to half of the original height. The intercellular spaces and endosomes are dilated. (*d*) After gradient perfusion of the epithelial cells for 10 min, the cytoplasm contains numerous endosomes (*Ed*). Above the dilated intercellular spaces (asterisk) the tight junctions (arrows) seem to be well preserved. (*a*) ~980×; (*b*) ~7220×; (*c*) ~980×; (*d*) ~7220×

and vesicles containing CF were obviously not coated (Fig. 8*a,b*). In the apical cytoplasm small labeled vesicles were either fusing with or separating from vacuoles (Fig. 9*b,c*). The gradient influenced the localization of vacuoles. As compared to controls, relatively more labeled vesicles close to the basolateral cell membrane were noticed after osmotic gradient exposure (Figs. 7*b*, 8*c*, 9*d*). CF

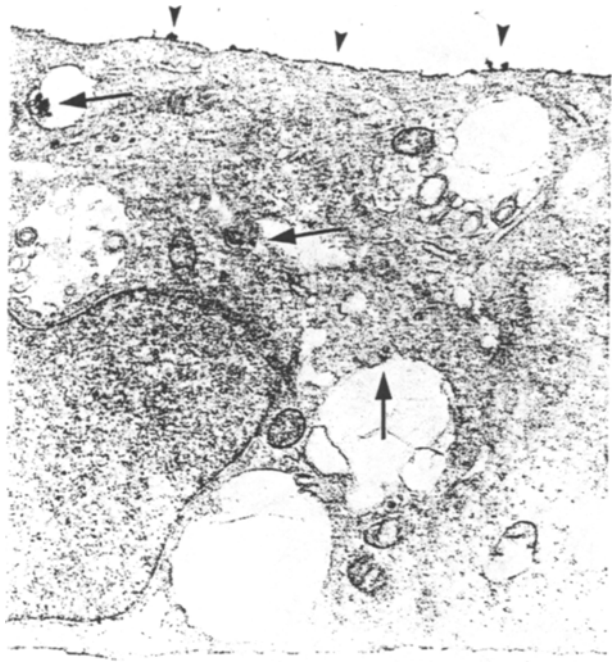

Fig. 4. Collecting duct epithelium in vivo labeled with the fluid phase marker HRP and exposed to an osmotic gradient for 10 min. There is a discontinuous label at the apical plasma membrane (arrowheads). Several vesicles in the cytoplasm contain HRP reaction product (arrows). ~17,000×

could often be detected in the dilated intercellular spaces (Figs. 7*b,c*, 8*c*) and exocytosed at the basal aspect (Fig. 8*d,e*) after gradient exposure. Vesicles containing CF were perceived close to the basolateral plasma membrane (Figs. 8*c*, 9*b*). Exocytotic pits were seen at the lateral plasma membrane area and at the basal surface of the cells facing towards the basement membrane (Fig. 8*d,e*). CF was not seen in the narrow intercellular spaces of epithelia (Fig. 7*a*) perfused under isotonic conditions. A shift of CF into the Golgi complex was not observed (Fig. 9*c*).

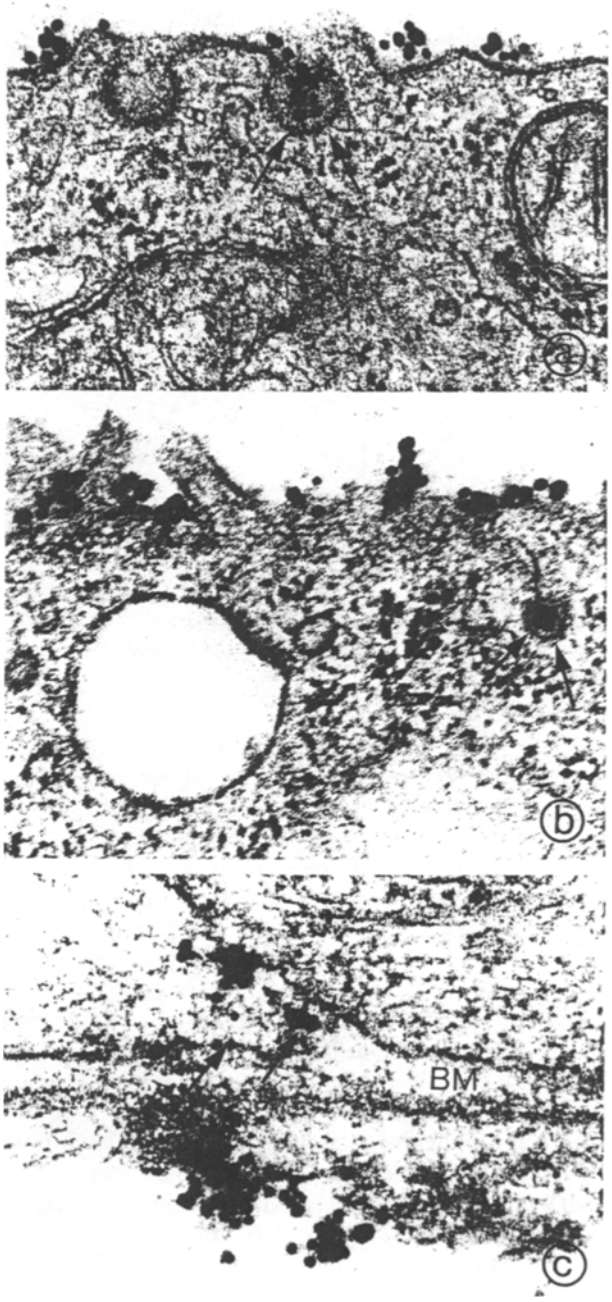

Fig. 5. (*a-c*) Collecting duct epithelium cultured for 5 days was incubated with $GP_{CD}I$ antibody and gold label followed by exposing to 75 mosm PBS at the luminal side for 10 min. (*a*) The $GP_{CD}I$ antibody reacts with the apical plasma membrane and the label is internalized in coated pits (arrows). (*b*) Labeled vesicles (arrows) are seen in the apical cytoplasm. (*c*) The label is exocytosed at the basal aspect of the epithelium. *BM*, basement membrane. (*a*) ~82,900×; (*b*) ~82,500×; (*c*) ~60,100×

Discussion

Based upon the observations that membrane cycling is related to water reabsorption (Ding et al., 1985; Hays et al., 1987), we studied endo- and exocytotic processes in cultured renal collecting duct epithelium. As an osmotic gradient has been proven to be important for ADH-regulated endocytosis on toad bladder (Masur, Cooper & Rubin, 1984) and for ultrastructural alteration on perfused collecting duct tubules (Ganote et al., 1968), the influence of an apico-basal osmotic gradient across the epithelium was first tested with respect to its morphology.

After 10-min exposure to an apico (75 mosm PBS)-basal (290 mosm PBS) osmotic gradient, the

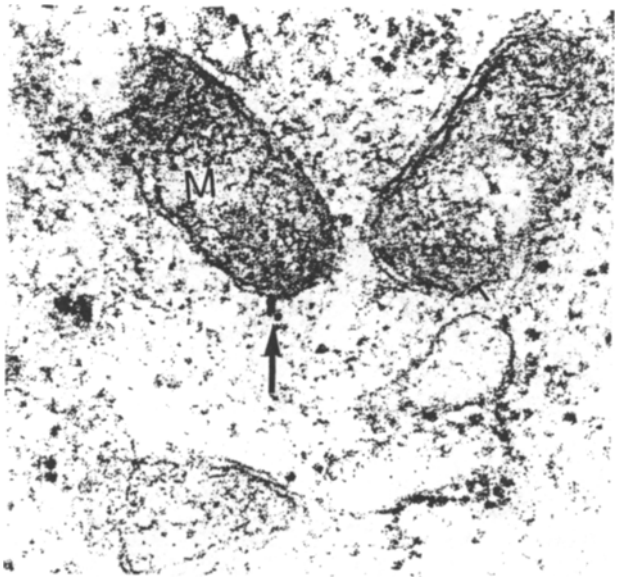

Fig. 6. Collecting duct cells incubated with $GP_{CD}I$ antibody and gold label. After exposing to an osmotic gradient for 10 min, gold grains were discovered away from vesicles (arrow). *M*, mitochondria. ×60,000×

cultured epithelium flattened to about half of its original height (Fig. 3). Compared to controls, the cytoplasm of epithelia exposed to a gradient contained more and larger endosomes, and the intercellular spaces were dilated (Fig. 3*d*). Thus, the dilatation of the endosomal vesicle compartments and the dilation of the intercellular spaces, which was observed on perfused collecting duct cells however in presence of ADH (Strange & Spring, 1987), may be caused by a transfer of fluid out of the cytoplasm. Further, a decrease in cell thickness was seen after 5 to 10 min during the initial hypotonic perfusion of collecting duct tubules in absence of ADH (Ganote et al., 1968). Shrinkage in cell height was stated otherwise on cells of the renal cortical thick ascending limb (Hebert, 1986) and of the collecting duct (Grantham et al., 1969) applying an apico-basal osmotic gradient only without hormone.

To elucidate these morphological changes on cultured collecting duct cells, tracers with different physiological and biochemical properties were ap-

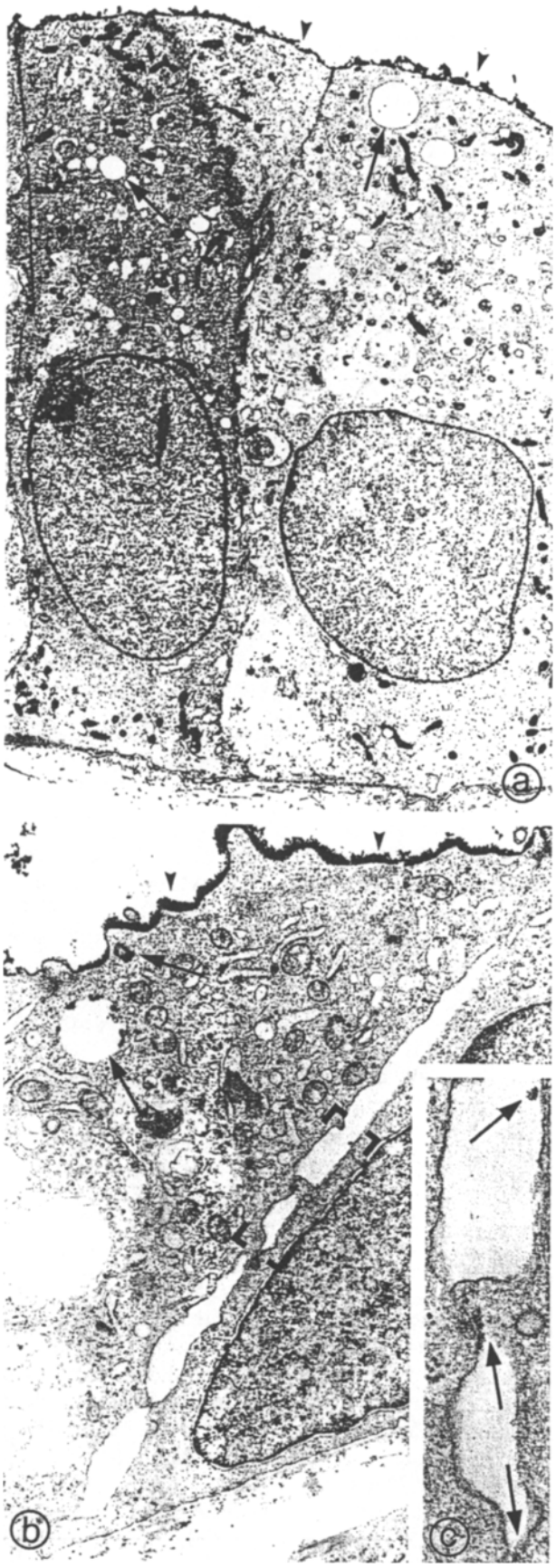

Fig. 7. (*a-c*) Cultured collecting duct epithelium was labeled with cationized ferritin. The label is seen at the apical plasma membrane (arrowheads). (*a*) Isotonic perfusion for 10 min. In the cytoplasmatic vesicles (arrows) and in the narrow intercellular spaces cationized ferritin could not be detected. (*b*) Perfusion under an apico-basal osmotic gradient for 10 min. Numerous vesicles (arrows) contain CF. (*c*) In the dilated intercellular spaces, CF is exocytosed. Section out of (*b*) as marked. (*a*) ~4600×; (*b*) ~13,100×; (*c*) ~45,000×

plied. Membrane bindings markers were the specific $GP_{CD}I$ antibody (Minuth et al., 1984) and cationized ferritin (CF; Beauwens et al., 1986). Volume markers were native ferritin (NF) and horseradish peroxidase (HRP; Farquhar, 1981; Steinman et al., 1983). All tracers were internalized into the epithelial cells to a small degree under isotonic perfusion. However, exposure of the cells to an apico-basal osmotic gradient enhanced endocytosis of all substances. Roughly estimated, there was a lesser uptake of volume markers than of membrane binding markers. This is in line with findings on choroid plexus (van Deurs et al., 1981), kidney proximal tubular cells (Christensen, Carone & Rennke, 1981; Goligorsky & Hruska, 1986), and distal tubular cells (Madsen, Harris & Tisher, 1982). Furthermore, CF as a membrane binding tracer was internalized to a relatively higher degree than the gold-labeled $GP_{CD}I$ antibody. Only the $GP_{CD}I$ antibody was always found to be endocytosed in coated pits (Fig. 5*a*,*b*). This could favor a model of a receptor-mediated uptake (Goldstein, Anderson & Brown, 1979). Thus, different internalization routes for the $GP_{CD}I$ antibody and CF were observed, the CF never being present in coated vesicles (Fig. 8*a*,*b*).

NF and HRP (Fig. 4) remained in apically located endosomal vesicles, whereas the $GP_{CD}I$ antibody complex and CF were transcytosed as already shown by other models for transcellular transport, such as choroid plexus cells (van Deurs et al., 1981), newborn rat intestine cells (Abrahamson & Rodewald, 1980, 1981), altered thyroid cells (Herzog, 1983), MDCK cells (Matlin et al., 1983; Pesonen & Simons, 1983; von Bonsdorff et al., 1985) and renal proximal tubular cells (Goligorsky & Hruska, 1986). Both CF and $GP_{CD}I$-antibody gold grains could be detected in vesicles close to the basolateral cell membrane and in the extracellular matrix at the basal aspect of the epithelium (Figs. 5*c*, 8*d*,*e*, 9*d*). In addition, intracellular gold particles could be seen also away from the cell membrane (Fig. 6), favoring the assumption that degradation of the $GP_{CD}I$-antibody complex in part took place in a similar way as that shown for epidermal growth factors (EGF) and transferrin and other receptor molecules (Pastan & Willingham, 1985).

Different trigger mechanisms of membrane retrieval and transfer have been established. Exo- and

Fig. 8. (*a-c*) Transcytosis in cultured collecting duct epithelium after exposure to an osmotic gradient for 10 min. (*a*) CF is attached to the luminal plasma membrane (arrowheads) and in endocytic pits (arrows). (*b*) By exposure of the epithelium to an osmotic gradient, CF is internalized (arrow), and (*c-e*) exocytosed at the basolateral and basal aspect of the epithelium (arrows). BM, basement membrane. (*a*) ~67,800×; (*b*) ~73,600×; (*c*) 83,300×; (*d*) ~130,000×; (*e*) ~90,000×

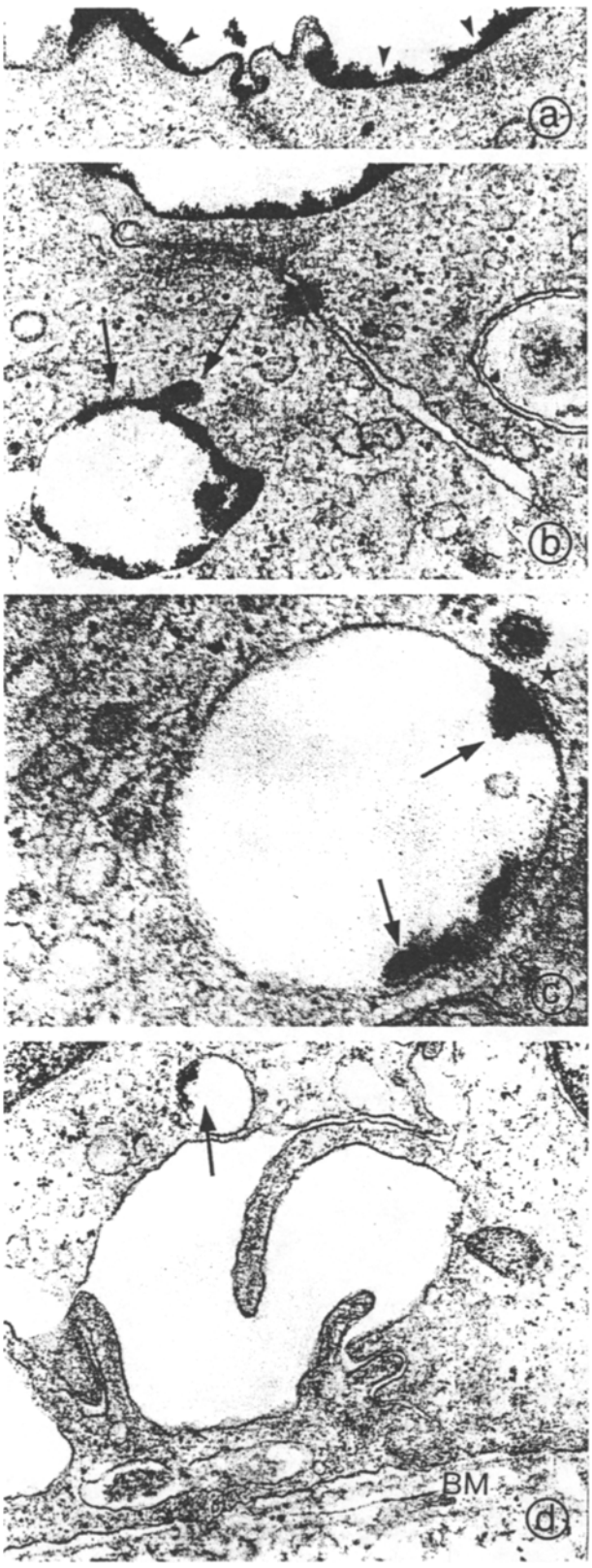

Fig. 9. (*a-d*) Transcytosis in cultured collecting duct epithelium after exposure to an osmotic gradient for 10 min. (*a*) CF is attached to the luminal cell surface (arrowheads). The plasma membrane of neighboring cells protrudes processes into the apical medium. (*b*) and (*c*) By exposure of the epithelium to an osmotic gradient, CF is internalized into endosomal vesicles (arrows) involving smaller vesicles (asterisk) in this process. (*d*) Labeled endosome (arrow) is seen close to the basal aspect of the epithelium. *BM*, basement membrane. (*a*) ~31,600×; (*b*) ~43,700×; (*c*) ~81,200×; (*d*) ~28,700×

endocytosis are initiated or enhanced in thyroid cells by ISM (Herzog, 1983) and in toad urinary bladder cells by ADH (Gronowicz, Masur & Holtzman, 1980; Ding et al., 1985; Hays et al., 1987). Beside these hormonal stimulations the pH gradient is known to trigger the shuttle of cell membrane in collecting duct cells (Schwartz, Barasch & Al-Awqati, 1985). However, to our knowledge, endo-, trans-, and exocytosis in cultured collecting duct cells induced by an apico-basal osmotic gradient have not been described yet.

The cultured collecting duct cells established characteristics of a tight epithelium with apico-basal Na^+-transport properties (Gross et al., 1986), but failed to demonstrate a substantial transport of fluid phase markers as indicated by our results with HRP and NF. However, osmotic gradient-induced endo- and transcytosis was demonstrated. Endocytosis was suggested to be involved in the decrease of water permeability in other water-transporting epithelia (Masur et al., 1984; Beauwens et al., 1986), exposed to an apico-basal osmotic gradient. Thus, we conclude that the endocytosis observed in the cultured collecting duct cells after applying an apico-basal osmotic gradient, might have a similar function sealing up the epithelium by apically retrieving plasma membrane.

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References

Abrahamson, D.R., Rodewald, R. 1980. Selective transfer of vesicle contents during endocytosis in the rat neonate rat intestine. *J. Cell Biol.* **87**:310a T2405 (*Abstr.*)

Abrahamson, D.R., Rodewald, R. 1981. Evidence for the sorting of endocytic vesicle contents during the receptor mediated transport of IgG across the newborn rat intestine. *J. Cell Biol.* **91**:270–280

Beauwens, R., Te Kronnie, G., Snauwaert, J., In't Veld, P. 1986. Polycations reduce vasopressin-induced water flow by endocytic removal of water channels. *Am. J. Physiol.* **250**:C729–737

Bonsdorff, C.H. von, Fuller, S.D., Simons, K. 1985. Apical and basal endocytosis in Madin-Darby canine kidney (MDCK) cells grown on nitrocellulose filters. *EMBO J.* **4**:2781–2792

Christensen, E.I., Carone, F.A., Rennke, H.G. 1981. Effect of molecular charge on endocytic uptake of ferritin in renal proximal tubule cells. *Lab. Invest.* **44**:351–358

Deurs, B. van, Bülow, F. von, Möller, M. 1981. Vesicular transport of cationized ferritin by the epithelium of rat choroid plexus. *J. Cell Biol.* **89**:131–139

Ding, G., Franki, N., Hays, R.M. 1985. Evidence for cycling of aggregate-containing tubules in the toad urinary bladder. *Biol. Cell* **55**:213–218

Farquhar, M.G. 1981. Membrane recycling in secretory cells: Implications for traffic of products and specialized membranes within the Golgi complex. *Methods Cell Biol.* **23**:399–427

Farquhar, M.G. 1983. Multiple pathways of exocytosis, endocytosis and membrane recycling: Validation of a Golgi-route. *Fed. Proc.* **42**:2407–2413

Ganote, C.E., Grantham, J.J., Moses, H.L., Burg, M.B., Orloff, J. 1968. Ultrastructural studies of vasopressin effect on isolated perfused renal collecting tubules of the rabbit. *J. Cell Biol.* **36**:355–367

Goldstein, J.L., Anderson, R.G.W., Brown, M.S. 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature (London)* **279**:679–685

Goligorsky, M.S., Hruska, K.A. 1986. Transcytosis in cultured proximal tubular cells. *J. Membrane Biol.* **93**:237–247

Graham, R.C., Karnovsky, M.J. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. *J. Histochem. Cytochem.* **14**:291–302

Grantham, J.J., Ganote, C.E., Burg, M.B., Orloff, J. 1969. Paths of transtubular water flow in isolated renal collecting tubules. *J. Cell Biol.* **41**:562–576

Gronowicz, G., Masur, S.K., Holtzman, E. 1980. Quantitative analysis of exocytosis and endocytosis in the hydroosmotic response of toad bladder. *J. Membrane Biol.* **52**:221–235

Gross, P., Minuth, W.W., Kriz, W., Frömter, E. 1986. Electrical properties of renal collecting duct principal cell epithelium in tissue culture. *Pfluegers Arch.* **406**:380–386

Hays, R.M., Franki, N., Ding, G. 1987. Effects of antidiuretic hormone on the collecting duct. *Kidney Int.* **31**:530–537

Herbert, S.C. 1986. Hypertonic cell volume regulation in mouse thick limbs. I. ADH dependency and nephron heterogeneity. *Am. J. Physiol.* **250**:C907–C919

Herzog, V. 1983. Transcytosis in thyroid follicle cells. *J. Cell Biol.* **97**:607–617

Kirk, K.L., Schafer, J.A., DiBona, D.R. 1984. Quantitative analysis of the structural events associated with antidiuretic hormone-induced volume reabsorption in the rabbit cortical collecting tubule. *J. Membrane Biol.* **79**:65–74

Kokko, J.P. 1987. The role of the collecting duct in urinary concentration. *Kidney Int.* **31**:606–610

Madsen, K.M., Harris, R.H., Tisher, C.C. 1982. Uptake and intracellular distribution of ferritin in the rat distal convoluted tubule. *Kidney Int.* **21**:354–361

Masur, S.K., Cooper, S., Rubin, M.S. 1984. Effect of an osmotic gradient on antidiuretic hormone-induced endocytosis and hydroosmosis in the toad urinary bladder. *Am. J. Physiol.* **247**:F370–F379

Matlin, K., Bainton, D.F., Pesonen, M., Louvard, D., Genty, N., Simons, K. 1983. Transepithelial transport of a viral membrane glycoprotein implanted into the apical plasma membrane of Madin-Darby canine kidney cells. I. Morphological evidence. *J. Cell Biol.* **87**:627–637

Minuth, W.W., Gilbert, P. 1987. Specific proteins in the renal collecting duct system and in cultured collecting duct cells. *Histochemistry (in press)*

Minuth, W.W., Gilbert, P., Lauer, G., Aktories, K., Gross, P. 1986. Differentiation properties of renal collecting duct cells in culture. *Differentiation* **33**:156–167

Minuth, W.W., Kriz, W. 1982. Culturing of renal collecting duct epithelium as globular bodies. *Cell Tissue Res.* **224**:335–348

Minuth, W.W., Lauer, G., Bachmann, S., Kriz, W. 1984. Immunocytochemical localization of a renal glycoprotein ($GP_{CD}I$) synthesized by cultured collecting duct cells. *Histochemistry* **80**:171–182

Morel, F., Chabardes, D., Imbert, M., Montegut, M., Clique, A. 1976. Functional segmentation of the rabbit distal tubule by microdetermination of hormone-dependent adenylate cyclase activity. *Kidney Int.* **9**:264–277

Morel, F., Doucet, A. 1986. Hormonal control of kidney function at the cell level. *Physiol. Rev.* **66**:377–468

Morel, F., Imbert-Teboul, M., Chabardes, D. 1987. Receptors to vasopressin and other hormones in the mammalian kidney. *Kidney Int.* **31**:512–520

Mostov, K.E., Simister, N.E. 1985. Transcytosis. *Cell* **43**:389–390

Pastan, J., Willingham, M.C. 1985. The pathway of endocytosis. *In:* Endocytosis. J. Pastan and M.C. Willingham, editors. pp. 1–44. Plenum, New York, London

Pesonen, M., Simons, K. 1983. Transepithelial transport of a viral membrane glycoprotein implanted into the apical plasma membrane of Madin Darby canine kidney cells. II. Immunological quantitation. *J. Cell Biol.* **87**:638–643

Schwartz, G.J., Barasch, J., Al-Awqati, Q. 1985. Plasticity of functional epithelial polarity. *Nature (London)* **318**:368–371

Steinman, R.M., Mellman, J.S., Mutler, W.A., Lohn, Z.A. 1983. Endocytosis and the recycling of plasma membrane. *J. Cell Biol.* **23**:399–427

Strange, K., Spring, K.R. 1987. Absence of significant cellular dilution during ADH-stimulated water reabsorption. *Science* **235**:1068–1070

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