Osmosis in Cortical Collecting Tubules

ADH-Independent Osmotic Flow Rectification

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ABSTRACT The present experiments were designed to evaluate the effects of varying the osmolality of luminal solutions on the antidiuretic hormone (ADH)-independent water and solute permeability properties of isolated rabbit cortical collecting tubules. In the absence of ADH, the osmotic water permeability coefficient (cm s^{-1}) $P_f^{l \to b}$, computed from volume flows from hypotonic lumen to isotonic bath, was $20 \pm 4 \times 10^{-4}$ (SEM); the value of $P_f^{b \to l}$ in the absence of ADH, computed from volume flows from isotonic bath to hypertonic lumen, was $88 \pm 15 \times 10^{-4}$ cm s^{-1}. We also measured apparent urea permeability coefficients (cm s^{-1}) from ^{14}C-urea fluxes from lumen to bath ($P_{D_{urea}}^{l \to b}$) and from bath to lumen ($P_{D_{urea}}^{b \to l}$). For hypotonic luminal solutions and isotonic bathing solutions, $P_{D_{urea}}^{l \to b}$ was $0.045 \pm 0.004 \times 10^{-4}$ and was unaffected by ADH. The ADH-independent values of $P_{D_{urea}}^{l \to b}$ and $P_{urea}^{b \to l}$ were, respectively, $0.216 \pm 0.022 \times 10^{-4}$ cm s^{-1} and $0.033 \pm 0.002 \times 10^{-4}$ cm s^{-1} for isotonic bathing solutions and luminal solutions made hypertonic with urea, i.e., there was an absolute increase in urea permeability and asymmetry of urea fluxes. Significantly, $P_{D_{urea}}^{l \to b}$ did not rise when luminal hypertonicity was produced by sucrose; and, bathing fluid hypertonicity did not alter tubular permeability to water or to urea. We interpret these data to indicate that luminal hypertonicity increased the leakiness of tight junctions to water and urea but not sucrose. Since the value of $P_f^{b \to l}$ in the absence of ADH, when tight junctions were open to urea, was approximately half of the value of $P_f^{l \to b}$ in the presence of ADH, when tight junctions were closed to urea, we conclude that tight junctions are negligible paracellular shunts for lumen to bath osmosis with ADH. These findings, together with those in the preceding paper, are discussed in terms of a solubility-diffusion model for water permeation in which ADH increases water solubility in luminal plasma membranes.

INTRODUCTION

Antidiuretic hormone (ADH) increases the permeability of luminal surfaces of isolated rabbit cortical collecting tubules to water, but not to small hydrophilic species such as urea, thiourea, or acetamide (1–4). The diffusional resistance of the epithelial cell layer, exclusive of luminal surfaces, to lipophilic species is 15–25 times greater than in an equivalent thickness of water (2). To rationalize these data, we proposed that the mode of water transport across luminal surfaces, with or without ADH, was diffusional in nature, and that the disparity between the ADH-dependent values of the osmotic (P_f, cm s^{-1}) and diffusional (P_{D_w}, cm s^{-1}) water permeability coefficients in cortical collecting tubules was referable to cellular constraints to diffusion, which were approximately 25-fold greater than in an equivalent layer of water (2). The preceding paper (5) provided evidence which indicated that these cellular diffusion constraints might be due to a reduction in the area of the cell layer available for water transport, and that such a geometric restriction to water transport did not result in appreciable underestimates of P_f from steady-state osmotic flows. However, the analysis provided no information concerning the fractional area available for water flow in the luminal surfaces, or, in other words, whether osmotic volume flow traversed luminal plasma membranes or tight junctions.

In that regard, a number of reports have indicated that hypertonic solutions may alter appreciably the solute (6–14) and water (13, 15–17) permeability properties and electron microscopic appearance (18–21) of epithelial tight junctions. The present paper describes the effects of luminal solutions made hypertonic with sucrose or urea on the permeability properties of cortical collecting tubules for water and solutes. The results are consistent with the view that hypertonic luminal solutions increase the permeation of water and urea, but not sucrose, through tight junctions of cortical collecting tubules; and that osmosis from lumen to bath in this tissue involves water diffusion through plasma membranes with negligible paracellular shunting of water and solute flows by tight junctions.

METHODS

The experiments described in this paper include water and solute fluxes in isolated cortical collecting tubules obtained by freehand dissection from rabbit kidney slices. The details of the experimental techniques, including specifically: the measurement of osmotic volume flows from lumen to bath ($J_v^{l\to b}$, $cm^3\ s^{-1}\ cm^{-2}$) or from bath to lumen ($J_v^{b\to l}$, $cm^3\ s^{-1}\ cm^{-2}$), and the measurement of solute permeability coefficients ($P_{D_s}^{l\to b}$, cm s^{-1}) from lumen to bath solute fluxes, have been described previously (2, 4, 5). The present experiments were carried out in an identical manner, with the exceptions described below.

First, in certain instances, we carried out solute fluxes from bath to lumen. In these experiments, sufficient ^{14}C-tracer was added to the bath so that, during any flux period, the tracer concentration in the bath was, at a minimum, 10^2 times greater than the tracer concentration in the lumen. Since the tracer concentration in the bath remained very nearly constant, the apparent solute permeability coefficient ($P_{D_s}^{b\to l}$, cm s^{-1}) was computed from the bath to lumen tracer flux and the expression:

$$P_{D_s}^{b\to l} = \frac{Q \cdot C_l^*}{t \cdot A \cdot X_b \cdot C_b}, \quad (1)$$

where Q = volume of collected fluid (cm^3), C_l^* = tracer concentration in collected fluid (cpm cm^{-3}), t = duration of flux period (seconds), A = inner surface area of tubule (cm^2), X_b = specific activity of tracer in the bathing solution (cpm mol^{-1}), and C_b = solute concentration in the bath (mol cm^{-3}).

Second, the composition of the perfusing solutions was altered during the course of some experiments. This was accomplished by opening a gas-tight valve (Hamilton Instrument Co., Whittier, Calif.) fitted to the rear of the perfusion pipet and introducing a length of polyethylene tubing (Intramedic PE 10, Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N.J.) to the tapered portion of the perfusion pipet. The old perfusing solution was removed and the perfusion pipet was flushed at least twice with the perfusing solution. The time required for the new perfusing solution to reach the collecting pipet, estimated from the appearance in collected fluid of ^{3}H-inulin, which had been added to new but not original perfusing solutions, was approximately 5–10 min. Accordingly, flux periods involving a new perfusion solution were started approximately 20 min after a solution change.

All experiments were carried out at pH 7.4, 25 ± 0.5°C. The isotonic solutions (290 mosmol $liter^{-1}$), Krebs Ringer phosphate (KRP) and Krebs-Ringer bicarbonate (KRB), used in the present studies were identical in composition to those described in the preceding paper (5). As in previous studies (2, 4, 5), the osmolalities of these solutions were varied by altering NaCl, urea, or sucrose concentrations without modifying the concentrations of other constituents.

Measurements in a given tubule were used to compute a mean value for that tubule. Mean values for individual tubules were then used to calculate a mean value ± standard error for the mean (SEM) for a number of tubules.

RESULTS

Effect of Varying Luminal Osmolality on P_f

The effects of varying luminal osmolality on P_f, the osmotic water permeability coefficient, are shown in Figs. 1 and 2 and Table I. It should be noted in this regard that the results in the previous paper (5) indicated that cellular constraints to diffusion in the epithelial cell layer of cortical collecting tubules resulted in a relatively small, approximately 15%, under-estimation of P_f when the latter was computed from steady-state flows and the traditional expression:

$$J_v = \sigma P_f \bar{V}_w [C_l - C_b], \quad (2)$$

where σ is the reflection coefficient of the solute used to generate osmotic flow, $\bar{V}_w$ is the partial molar volume of water, and C_l and C_b are, respectively, the measured osmolalities of the luminal and bathing solutions. Accordingly, the values of P_f reported in this paper were calculated according to Eq. 2, assuming a σ of unity for urea, NaCl, and sucrose ([4, 5]; Table I). Values of P_f computed from lumen to bath and from bath to lumen osmotic fluxes will be termed, respectively, $P_f^{l\to b}$ and $P_f^{b\to l}$. For the purposes of the present paper, we shall use the term "ADH-independent" and "ADH-dependent" to refer, respectively, to volume flow (or P_f values) in the absence and in the presence of ADH.

A representative experiment illustrating the effect of varying luminal osmolality on ADH-independent osmotic water flow is illustrated in Fig. 1. In agreement with earlier observations (1, 2), $P_f^{l\to b}$, for lumen to bath flows, declined to values near zero at 120–180 min. However, when the perfusing solution was made hypertonic with urea, $P_f^{b\to l}$ rose to approximately 50 $\times$ 10^{-4} cm s^{-1}.

The precise nature of the temporal decline in osmotic volume flow from lumen to bath is not understood. However, when ADH is added to bathing solutions at 180 min, both $P_f^{l\to b}$ and $P_f^{b\to l}$ rise to values in the range 180–200 $\times$ 10^{-4} cm s^{-1} (1, 2, 5). Thus, it seems reasonable to assume that osmotic volume fluxes at 180 min provided an index to the ADH-independent water permeability properties of the luminal surfaces of these tubules. According to

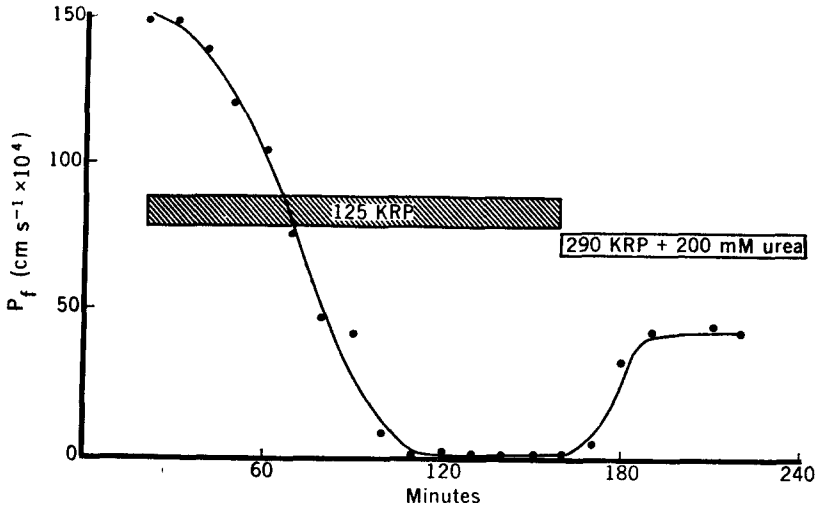

FIGURE 1. The effect of luminal hypertonicity on P_f. The bath contained isotonic KRB. At 30 minutes, isotonic KRP perfusing solution was replaced with hypotonic KRP solution (125 mosmol $liter^{-1}$); at 160 min, the perfusing solution was changed to isotonic KRP containing 200 mM urea. P_f was calculated according to Eq. 2 from $J_v^{l\to b}$, when the perfusing solution was hypotonic, and from $J_v^{b\to l}$, when the perfusing solution was hypertonic. Zero time was taken as the time of decapitation.

this view, Fig. 1 indicates that luminal hypertonicity produced by urea increased the rate of ADH-independent water permeation from bath to lumen in these tubules.

To evaluate this issue more quantitatively, we compared the temporal changes in $P_f^{l \to b}$ with those in $P_f^{b \to l}$ in a large number of tubules. The results are shown in Fig. 2 (closed circles, $P_f^{l \to b}$, lumen to bath osmotic fluxes; open circles, $P_f^{b \to l}$, bath to lumen osmotic fluxes). It is evident that, at 180 min, the values of P_f computed from $J_v^{b \to l}$ were appreciably greater than those calculated from $J_v^{l \to b}$. Moreover, although the data are not shown in Fig. 2, there

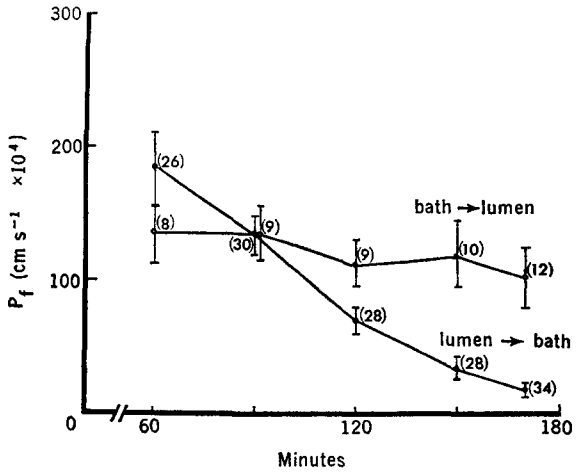

FIGURE 2. The time-course of P_f. The bathing solution was isotonic KRB. P_f was calculated from lumen to bath osmotic volume fluxes when the perfusing solution was 125 mosmol liter^{-1} KRP (closed circles) or from bath to lumen fluxes when the perfusing solution was 290 mosmol liter^{-1} KRB containing either 100 mM NaCl, 200 mM urea, or 200 mM sucrose. The results are expressed as the mean values ± SEM for the number of tubules indicated in the figure.

was no change in the values of P_f computed from $J_v^{b \to l}$ over the time interval 180–200 min. Table I summarizes the data from Fig. 2 for oppositely directed volume flows at 180 min. The values of P_f for bath to lumen flows were approximately four times greater ($P < 0.001$) than for lumen to bath flows. Stated in another way, the results in Figs. 1 and 2 and Table I indicate that luminal solutions made hypertonic either with urea, NaCl, or sucrose increased the water permeability of cortical collecting tubules, thereby producing rectification of osmotic water flow.

The reflection coefficient for either sucrose, NaCl, or urea in these tubules, estimated from lumen to bath volume flows with or without ADH, is unity (4). Table I shows that there were no significant differences among the values of P_f for bath to lumen fluxes when either NaCl, urea, or sucrose was used to

TABLE I

VASOPRESSIN-INDEPENDENT OSMOTIC FLOW RECTIFICATION IN CORTICAL COLLECTING TUBULES

Lumen	Bath	Test solute	P_f
mosmol/liter			*cm s^{-1} × 10^4*
125	290	NaCl	20 ± 4 (34)
490	290	NaCl, urea, sucrose	88 ± 15 (12)
490	290	urea	83 ± 26 (5)
490	290	sucrose	96 ± 25 (5)
490	290	NaCl	81 (2)

This table summarizes the mean values ± SEM from Fig. 2 for the ADH-independent values of P_f at 180 min. In the upper half of the table, P_f was calculated from all bath to lumen osmotic fluxes, regardless of whether NaCl, urea, or sucrose was used to make the luminal solutions hypertonic. In the lower half of the table, the values of P_f calculated from bath to lumen fluxes were grouped according to the particular solute used to produce luminal hypertonicity.

raise the osmolality of the luminal solutions. Thus, it is likely that, in the case of ADH-independent bath to lumen flows, σ was approximately the same and presumably near unity, for NaCl, urea, and sucrose. It should be stressed that these observations are not inconsistent with the possibility that luminal hypertonicity resulted in an increase in the permeability of these tubules to urea (see below). More specifically, since the standard deviation for P_f determinations was approximately 15–20% (Table I), it is unlikely that the osmotic volume flow measurements could have detected reproducibly a reduction in σ_{urea} from 1.0, in the case of lumen to bath flows (4), to approximately 0.9–0.95, in the case of bath to lumen flows.

Several other factors relating to ADH-independent osmotic flow rectification should also be noted. First, the ADH-independent values of P_f listed in Table I for bath to lumen flows, i.e., 88 × 10^{-4} cm s^{-1}, are approximately half as great as the ADH-dependent values of P_f calculated from lumen to bath flows, i.e., 190–200 × 10^{-4} cm s^{-1} (1, 2, 5). Second, osmotic flow rectification in these tubules was limited to the ADH-independent case; as noted in the preceding paper (5), the ADH-dependent values of $P_f^{b\to l}$ for hypertonic luminal solutions and isotonic bathing solutions were the same, within experimental error as the values of $P_f^{l\to b}$ when the luminal solutions were hypotonic and the bathing solutions were either isotonic or hypertonic. Third, at least in cortical collecting tubules, the addition of hypertonic solutions to bathing media does not alter appreciably osmotic water permeability. Thus, we observed previously that both the ADH-dependent and ADH-independent values of P_f calculated from lumen to bath fluxes were not affected, within experimental error, when the luminal and bathing solutions were, respectively, either hypotonic and isotonic or isotonic and hypertonic (2). In contrast,

Ripoche et al. (18) noted that hypertonic serosal solutions increased the rate of ADH-independent mucosal to serosal osmotic flow in frog urinary bladder. Finally, ADH-independent osmotic flow rectification entirely comparable to that illustrated in Table I has been observed in other ADH-sensitive tissues. In the toad urinary bladder without ADH, Urakabe et al. (13) observed that osmotic volume flows from serosa to mucosa produced by mucosal solutions made hypertonic with urea were substantially greater than osmotic flows from mucosa to serosa produced by serosal solutions made hypertonic with urea; moreover, under such conditions of hypertonicity, the ADH-independent serosal to mucosal flows were more than half as great as the comparable ADH-dependent flows, while the ADH-independent mucosal to serosal flows were negligibly small with respect to the comparable ADH-dependent flows.

Effect of Luminal Hypertonicity on Urea Permeability

Ussing (8, 22) first observed that exposure of the outer surface of amphibian skin to hypertonic solutions increased the passive skin permeability to small hydrophilic solutes such as Na^+, K^+, and sucrose. The inward sucrose flux was considerably greater than the outward sucrose flux when the sucrose concentrations on either side of the skin were identical, despite the fact that net osmotic volume flow was in the outward direction (22).

Table II lists the results of a series of experiments designed to evaluate the possibility of urea flux asymmetry in cortical collecting tubules. In order to facilitate comparison between these data and earlier observations, the unidirectional urea fluxes in the present experiments have been expressed in terms of apparent permeability coefficients. In this regard, it should be noted that, in earlier experiments with identical cortical collecting tubules (4): first,

TABLE II

EFFECT OF VARYING TONICITY ON THE APPARENT PERMEABILITY COEFFICIENT FOR UREA IN CORTICAL COLLECTING TUBULES

Lumen	Bath	ADH	P_{Durea} $l \rightarrow b$	P_{Durea} $b \rightarrow l$
			$cm\ s^{-1} \times 10^4$	
125 KRP	290 KRB	−	0.045±0.004 (4)	
125 KRP	290 KRB	+	0.050±0.003 (4)	
290 KRB + 200 urea	290 KRB	−	0.216±0.022 (4)	0.033±0.002 (4)
290 KRB + 200 sucrose	290 KRB	−	0.046±0.013 (4)	

The apparent permeability coefficient for urea was calculated from unidirectional ^{14}C-urea fluxes from lumen to bath ($l \rightarrow b$) or from bath to lumen ($b \rightarrow l$) as described in Methods. The compositions of the luminal and bathing solutions are indicated in the table. The values of P_{Durea} are expressed as the mean value ± SEM for the number of tubules listed in parentheses. The experiments without ADH were always carried out at more than 180 min after decapitation (cf. Figs. 1 and 2). ADH, when present, was added to the bathing solutions (250 $\mu U\ ml^{-1}$).

the ADH-independent value of $P^{l\to b}_{D_{urea}}$ at zero volume flow was 0.03 × 10^{-4} cm s^{-1}; second, ADH did not alter the values of $P^{l\to b}_{D_{urea}}$ at zero volume flow; and, third, $P^{l\to b}_{D_{urea}}$ was not affected by osmotic volume flow from isotonic luminal solutions to hypertonic bathing media.

In the present experiments (Table II), the ADH-independent value of $P^{l\to b}_{D_{urea}}$ was 0.045 × 10^{-4} cm s^{-1} for the case of hypotonic luminal solutions and isotonic bathing solutions, and was not affected significantly by adding ADH to the bathing media. However, when bath to lumen volume flows were produced by luminal solutions made hypertonic with urea, $P^{l\to b}_{D_{urea}}$, but not $P^{b\to l}_{D_{urea}}$, was increased more than four-fold. However, $P^{l\to b}_{D_{urea}}$ was not increased when sucrose was added to the luminal solutions. Stated in another way, the results in Table II indicate that luminal hypertonicity produced by urea, but not sucrose, resulted in both asymmetrical ratios for unidirectional ^{14}C-urea fluxes and a striking increase in the absolute value of $P^{l\to b}_{D_{urea}}$. In other experiments, not shown in Table II, there was no increase in $P^{l\to b}_{D_{sucrose}}$ when the perfusing solution was made hypertonic either with urea or with sucrose. Specifically, tubules were bathed with 290 mosmol $liter^{-1}$ KRB. The tubules were perfused first with 125 mosmol $liter^{-1}$ KRP, then with 290 mosm $liter^{-1}$ KRP plus either 200 mM urea or 200 mM sucrose, and finally with 125 mosmol $liter^{-1}$ KRP. In two tubules, luminal hypertonicity was produced with urea and in one tubule with sucrose. In all instances, $P^{l\to b}_{D_{sucrose}}$ remained less than 0.02 × 10^{-4} cm s^{-1} and did not change significantly, in a given tubule, when the luminal solutions were either hypotonic or isotonic.

DISCUSSION

The experiments described in this paper indicate clearly that, in the ADH-independent case, osmotic flow rectification occurred as a consequence of increases in $P_f^{b\to l}$, with respect to $P_f^{l\to b}$, which were produced when luminal solutions were made hypertonic either with urea, NaCl, or sucrose (Figs. 1 and 2; Table I). Coincidentally, luminal solutions made hypertonic with urea, but not with sucrose, resulted in both asymmetrical ^{14}C-urea fluxes and more than a four-fold increase in the apparent permeability coefficient for lumen to bath urea fluxes (Table II), and luminal solutions made hypertonic either with sucrose or with urea did not increase $P^{l\to b}_{D_{sucrose}}$. Significantly, in these cortical collecting tubules, bathing solutions made hypertonic with NaCl did not affect either the osmotic water permeability coefficient for lumen to bath flows, or the apparent urea or thiourea permeability coefficients estimated from lumen to bath tracer fluxes (2, 4, 5).

It is instructive to compare the results of the present experiments with comparable data in other epithelia. Following the terminology of Frömter and Diamond (23): epithelia such as gall bladder or small intestine, which have relatively low transepithelial electrical resistances (6–100 ohm-cm^2), generate

small spontaneous transepithelial potential differences (0–10 mV), and carry out isotonic fluid reabsorption, may be termed "leaky" epithelia; tissues such as frog skin and toad urinary bladder, which have relatively high transepithelial electrical resistances (300–2,000 ohm-cm^2), develop high spontaneous transepithelial potential differences (30–90 mV), and maintain steep transepithelial salt gradients, may be termed "tight" epithelia. Significantly, the effects of luminal (for gall bladder, urinary bladder, and renal tubules) or outside (for frog skin) hypertonicity on tissue permeability are quite different in the two types of epithelia. In leaky epithelia such as gall bladder or small intestine, the responses to luminal hypertonicity include: a collapse in size of intercellular spaces (24–26), an increase in transepithelial electrical resistance (24, 25), a decrease in the permeability of the tissue to small hydrophilic molecules such as urea or sucrose (24, 25), and coefficients of hydraulic conductivity which are appreciably lower for osmotic flow from isotonic serosal to hypertonic mucosal media than for volume flow from hypotonic mucosal to isotonic serosal solutions (26). In contrast, luminal or outside hypertonicity in tight epithelia generally results in increases in tissue permeability. Thus, outside hypertonicity in frog skin or mucosal hypertonicity in toad urinary bladder results both in increases in the transepithelial electrical conductance (6–8, 21) and in morphologically detectable deformations, presumed to represent widening, of tight junctions (20, 21) in these tissues.

Of particular relevance to the present experiments, hypertonicity also results in asymmetrical solute fluxes and increases in solute permeability in tight epithelia. A number of investigators (8–12, 14, 22) have shown that, in amphibian skin, outside hypertonicity produced by hydrophilic solutes such as urea, glucose, galactose, and sucrose, but not raffinose or inulin, results in: first, increases in the permeability of amphibian skin to test solutes such as urea, mannitol, and sucrose and second, anomalous flux ratios for these test solutes, such that inward solute flux exceeds outward solute flux, despite net osmotic volume flow in the outward direction and the absence of chemical driving forces in bathing solutions for the fluxes of test solutes.

Two classes of explanations have been proposed to account for the asymmetrical solute fluxes observed under these conditions. Ussing assumed that the osmotic gradient produced by hypertonic outside solutions increased the leakiness of tight junctions between epithelial cells, so that solute from the hypertonic outside solution penetrated tight junctions and created an osmotic flow from cells to lateral intercellular spaces, which drained in the inward direction (22). Ussing proposed that flux asymmetry for solutes such as sucrose was due to coupling of solute and solvent flows within lateral intercellular spaces, and termed the phenomenon "anomalous solvent drag" (22). Alternatively, Franz, Galey, and Van Bruggen (9, 11, 12), as well as Biber and Curran (10), have suggested that acceleration of solute flow in a direction

opposite to osmotic water flow produced by hypertonic solutions on the outer surface of amphibian skin was the consequence of coupling of flows between test solute and hypertonic solute, the latter moving down its concentration gradient after permeating tight junctions (9–12).

In the present context, it is particularly relevant to stress that, in terms of either explanation, i.e., anomolous solvent drag (8, 22) or solute-solute coupling (9–12) in lateral intercellular spaces, asymmetrical fluxes of test solutes in amphibian skin require permeation of tight junctions by the solute used to produce outside hypertonicity. Franz and Van Bruggen (9) noted in frog skin that outside solutions made hypertonic with sucrose, but not with raffinose, resulted in flux asymmetry for solutes such as urea or sucrose; thus, these workers, as well as Ussing (8, 22), concluded that outside hypertonicity opened tight junctions sufficiently to admit sucrose but not raffinose. Similarly, in the toad bladder, the observations of DiBona and Civan imply that hypertonic mucosal solutions increased the permeability of tight junctions to mannitol to a greater extent than to raffinose (21). In the present experiments, the results in Table II indicate clearly that asymmetrical urea fluxes and absolute increases in $P_{D_{\text{urea}}}^{l \to b}$ occurred when urea, but not sucrose, was used to generate luminal hypertonicity; and, when either urea or sucrose was used to produce luminal hypertonicity, there was no increase in $P_{D_{\text{sucrose}}}^{l \to b}$. Accordingly, we conclude that, in cortical collecting tubules, hypertonic luminal solutions opened tight junctions sufficiently to admit water (Figs. 1 and 2; Table I) and urea, but not sucrose (Table II).

According to this view, the ADH-independent value of $P_f^{b \to l}$, measured when luminal solutions are made hypertonic with urea, provides an estimate of the coefficient of hydraulic conductivity through tight junctions when the latter are sufficiently permeable to urea to permit asymmetrical urea fluxes (Table II). From Table I, the ADH-independent value of $P_f^{b \to l}$ is 95×10^{-4} cm s^{-1}. During ADH-dependent lumen to bath osmosis, when luminal fluids contain either hypotonic or isotonic fluids, urea permeation through tight junctions must be negligible, since $P_{D_{\text{urea}}}^{l \to b}$ is vanishingly small, $0.03–0.05 \times 10^{-4}$ cm s^{-1} (1, 3, 4; Table II); and, it is reasonable to infer that hydraulic conductivity through tight junctions is also less than during osmosis from bath to lumen, when as indicated above, tight junctions may be open to urea and $P_f^{b \to l}$ is 88×10^{-4} cm s^{-1}. Since the ADH-dependent value of $P_f^{l \to b}$ for lumen to bath osmosis is relatively large, in the range $180–200 \times 10^{-4}$ cm s^{-1} (1, 2, 4), we suggest that, in cortical collecting tubules, a small fraction of ADH-dependent lumen to bath osmosis involves bulk flow through tight junctions. Stated in another way, ADH-dependent lumen to bath osmosis may involve water flow primarily through the luminal plasma membranes of cortical collecting tubules. Similar models for transepithelial osmosis have been proposed

by other workers for ADH-sensitive epithelia such as the amphibian urinary bladder (19, 21, 27).

We suggested previously that the mode of ADH-dependent water transport through luminal surfaces during lumen to bath osmosis was diffusional in nature (2, 5). And, the preceding paper (5) provided evidence in support of the view that cellular constraints to diffusion did not result in more than a 15% under-estimation of $P_f^{l\to b}$ from steady-state osmotic flows. The results of the present experiments indicate that the fractional area of the luminal surfaces available for lumen to bath osmosis may approach unity, i.e., that lumen to bath osmosis involves primarily luminal plasma membranes.

To our knowledge, the composition and structure of these membranes is unknown. However, if it is assumed that the lipids of luminal plasma membranes are oriented in a lipid bilayer configuration, existing primarily in a liquid crystal rather than crystalline state, a comparison of experimental $P_f^{l\to b}$ values with those predicted from the solubility-diffusion properties of water in bulk lipid phases may be instructive in evaluating the ADH-dependent increase in water permeability of cortical collecting tubules.

If the mode of water transport through luminal membranes during lumen to bath osmosis is diffusional in nature, $P_f^{l\to b}$ may be expressed as:

$$P_f^{l\to b} = \frac{\beta_w D_w}{\Delta x}, \tag{3}$$

where β_w is the partition coefficient for water, D_w is the diffusion coefficient of water, and Δx is membrane thickness. For hexadecane, β_w and D_w are, respectively, 0.6×10^{-4} and 4.1×10^{-5} cm^2 s^{-1} (28, 29); thus, for a 60-Å thick hexadecane phase, P_f would be 41×10^{-4} cm s^{-1}. Finkelstein and Cass (30) and Price and Thompson (31) have pointed out the close similarities between P_f values computed in this manner and those observed in planar synthetic lipid bilayer membranes, where the mode of water transport during osmosis appears to be diffusional in nature (30–32).

In the present context, it is particularly relevant to note that the ADH-independent and ADH-dependent values of $P_f^{l\to b}$, respectively, 22×10^{-4} (Table I) and $180–200 \times 10^{-4}$ (1, 2, 4) cm s^{-1}, differ by approximately a factor of five from the values of P_f predicted for comparable thicknesses of bulk hexadecane or observed in planar lipid bilayer membranes formed from a variety of different lipids (30–33). It is also noteworthy in this connection that D_w for bulk lipid phases (28, 29) is approximately the same as the free diffusion coefficient of water in water (34). Since the experimentally observed values of P_f in planar lipid bilayer membranes may be rationalized in terms of the solubility-diffusion properties of water in bulk lipid phases (30–32), one may infer that the diffusion coefficient of water in organized lipid bilayers is not very different from D_w in water or bulk lipids. Accordingly, we speculate that

the ADH-dependent increments in the water permeability of cortical collecting tubules may be referable to an increased solubility of water (β_w, Eq. 3) in luminal membranes. Such a change in β_w may require a relatively short time interval, since the effects of ADH on water permeability occur within 5–10 min after exposing the outer surfaces of cortical collecting tubules to the hormone (1, 2). However, whether this proposed ADH-dependent increase in β_w is the consequence of reversible changes in the composition, packing or conformation of lipids in luminal plasma membranes, or the result of other factors, is wholly indeterminate at present.

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