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Quantitative Analysis of the Structural Events Associated with Antidiuretic Hormone-Induced Volume Reabsorption in the Rabbit Cortical Collecting Tubule

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Summary. We quantitatively examined the influence of antidiuretic hormone (ADH)-dependent volume reabsorption on the morphology of the rabbit cortical collecting tubule. Estimates of cell volume and the geometry of the lateral intercellular spaces were extracted from differential interference contrast images of perfused nephron segments using the morphometric procedures described in the preceding paper (K.L. Kirk, D.R. DiBona and J.A. Schafer, *J. Membrane Biol.* **79**:53–64, 1984). The results indicate that ADH addition in the presence, but not absence, of a lumen-to-bath osmotic gradient (130 to 290 mOsm) stimulated transepithelial volume flow and simultaneously increased the volumes of both the cells (+28%) and the lateral intercellular spaces (+78%). In addition, the formation of cytoplasmic vacuoles could be observed during the latter stages of the swelling response, and vacuole formation continued well after new steady-state values for transepithelial water flow and cell volume had been reached. Two main conclusions can be drawn from these results. First, the cytoplasmic vacuoles comprise a slowly filling compartment that lies in parallel to the transepithelial pathway for ADH-stimulated volume reabsorption. Second, from the magnitude of the cell volume increase, we estimate that the hydraulic conductivities of the opposing cell membranes are nearly equal during maximal ADH stimulation.

Key Words antidiuretic hormone · rabbit cortical collecting tubule · transepithelial volume flow · differential interference contrast microscopy · cell volume · lateral intercellular spaces · cytoplasmic vacuoles

Introduction

Previous ultrastructural studies of the mammalian collecting duct have provided a qualitative picture of the morphologic changes associated with antidiuretic hormone (ADH)-stimulated water reabsorption (Ganote et al., 1968; Grantham, Ganote, Burg & Orloff, 1969; Tisher, Bulger & Valtin, 1971; Woodhall & Tisher, 1973). During the onset of ADH-stimulated volume flow, there is an apparent increase in the volume of the epithelium which is accompanied by apical rounding of the cells, dilation of the lateral intercellular spaces and cytoplasmic vacuolation. This description of flow-induced alterations in epithelial structure is based primarily on comparisons of electron microscopic images, obtained from different preparations before and during ADH-induced volume flow, in which the quantitative aspects of the morphologic response to flow were not evaluated. Thus, there remain unresolved a number of issues that are potentially important to a quantitative understanding of the pathway for ADH-dependent water reabsorption, including the relative contributions of cellular swelling and lateral space-dilation to the observed increase in epithelial volume.

The purpose of the present study was to characterize quantitatively the influence of ADH-stimulated volume flow on the structure of the collecting duct with the broad goal of providing a more detailed understanding of the transepithelial pathway for hormone-dependent volume reabsorption. The specific aims of these studies included: *i*) determination of the temporal sequence of the structural events associated with the onset of flow and *ii*) estimation of the magnitudes of the cell volume—increase and the lateral space—dilation associated with ADH-stimulated flow. Extended observations of the isolated and perfused rabbit cortical collecting tubule were performed with differential interference contrast (DIC) microscopy during experiments in which transepithelial water flow and transepithelial voltage were measured simultaneously. Estimates of cell and lateral space volumes were extracted from recorded DIC images using the morphometric procedures described in the preceding paper (Kirk, DiBona & Schafer, 1984). The results demonstrate that ADH addition in the presence, but not absence, of a lumen-to-bath osmotic gradient stimulated transepithelial water flow and, with a similar time course, reversibly increased the

volumes of both the cells and the lateral intercellular spaces. In addition, the formation of cytoplasmic vacuoles, which in most experiments was quite marked, could be observed during the latter stages of cellular swelling and lateral space-dilation, and vacuolation continued well after new steady-state values for cell volume and transepithelial water flow had been reached.

Regarding the pathway for transepithelial volume flow, two main conclusions can be drawn from these studies. First, the lateral intercellular spaces comprise a rapidly filling compartment within the pathway for ADH-dependent flow, whereas the cytoplasmic vacuoles comprise a more slowly filling compartment which lies in parallel to the volume flow pathway. Second, we estimate, from the magnitude of the relative increase in cell volume (+28%) and the osmometric behavior of collecting tubule cells described in the preceding paper (Kirk et al., 1984), that the hydraulic resistances of the opposing cell membranes are nearly equal during maximal ADH stimulation. This result implies that the luminal cell membrane, which is the site for hormonal regulation of transepithelial water permeability (Ganote et al., 1968; Schafer & Andreoli, 1972; Harmanci, Kachadorian, Valtin & DiScala, 1978), is also the rate-determining barrier to transcellular volume flow at submaximal ADH concentrations.

Materials and Methods

All aspects of the experimental design and morphometric procedures were identical to those described in the preceding paper (Kirk et al., 1984) with the exceptions noted below. During the course of the experiments described in this paper the isotonic perfusing solution was replaced with a hypotonic solution (130 mOsm) which was identical in composition except for the deletion of 90 mM NaCl. The perfusate was changed by means of a concentric glass capillary mounted within the perfusion pipette. By switching a valve connecting the perfusion pipette to the hydrostatic pressure reservoir, the new solution could be injected through the glass capillary and into the perfusion pipette, flushing the old solution out of the rear of the perfusion pipette. The time required to completely exchange the luminal perfusate was less than 60 sec, as determined by the time course of the lumen-positive deflection in transepithelial voltage ($\Delta\psi_T$) that was observed during the dilution of intraluminal NaCl. $\Delta\psi_T$ was routinely measured in all experiments by continuously monitoring on a strip chart recorder (model A2-5; Varian) the voltage difference between two calomel cells connected by 0.9% NaCl-agar bridges to the perfusate and bath.

As described previously (Schafer & Andreoli, 1972; Al-Zahid, Schafer, Troutman & Andreoli, 1977), transepithelial water flow (J_v) was calculated as the difference between simultaneously measured perfusion and collection rates. Perfusion rate ($\dot{V}_o$) was determined from the rate of collection of ^{3}H-methoxy-inulin, which had been dialyzed as previously reported (Al-Zahid et al., 1977). This solute was added as a volume marker to the perfusing solution to a final isotope concentration of approximately 30 cpm/nl. The leakage rate of the volume marker, determined by continuously monitoring the appearance of inulin in the bathing solution, was less than 1 to 2% of the initial perfusion rate. Collection rate ($\dot{V}_L$) was estimated from timed (every 8 to 10 min) collections of the perfused fluid using a constant bore capillary (~1.25 nl mm^{-1}) calibrated with perfusing solution prior to each experiment (Al-Zahid et al., 1977). During the collections the tubule and pipette were observed using the low power, bright field optics described in the preceding paper (Kirk et al., 1984). Collections and experimental observations with DIC microscopy could not be performed simultaneously because of the high magnification at which we conducted our DIC observations and because of the geometric constraints imposed by the DIC condenser. The osmotic water permeability coefficient (P_f: cm sec^{-1}), normalized to the inner surface area of each tubule, was calculated from the perfusion and collection rates according to the following equation (DuBois, Verniory & Abramow, 1976; Al-Zahid et al., 1977):

$$P_f = -\frac{\dot{V}_o C_o}{\pi \overline{V}_w D_i L}\left[\frac{(C_o - C_L)}{C_o C_b C_L} + \frac{1}{C_b^2}\ln\frac{(C_L - C_b)}{(C_o - C_b)}\frac{C_o}{C_L}\right] \quad (1)$$

where $\overline{V}_w$ has its usual significance, L is tubule length, D_i is inside tubule diameter and C_o, C_L and C_b are the osmolalities of the perfusate, collectate and bath, respectively. C_L was calculated from C_o and the perfusion and collection rates assuming that net solute loss or gain from the lumen was negligible compared to the water loss or gain (Al-Zahid et al., 1977). From P_f, we calculated the hydraulic conductivity (L_p: cm^3/sec atm mm tubule length) according to Eq. (2):

$$L_p = (\pi \overline{V}_w D_i / RT) P_f \quad (2)$$

where R and T have their usual significances. In all experiments $\dot{V}_o$ was maintained at a level (10 to 20 nl/min) sufficient to prevent a greater than 2 to 5% dissipation of the osmotic gradient between the perfusion pipette and the site of experimental observations, typically 300 to 700 μm from the tip of the perfusion pipette.

The protocol for each experiment included the initial 38°C incubation period described in the preceding paper during which the initially high water permeability of the dissected collecting tubule was reduced (Al-Zahid et al., 1977). All experimental observations and physiologic measurements were then conducted at 25°C. ADH (Pitressin: Parke-Davis) was added to the bathing solution to a final concentration of 250 μU/ml, a concentration which elicits a maximal water permeability response in the rabbit cortical collecting tubule (Al-Zahid et al., 1977).

Results

Structural Changes Associated with ADH Exposure in the Presence of an Osmotic Gradient

Figures 1 and 2 show for individual cortical collecting tubules the morphologic changes observed following the addition of exogenous ADH in the presence of a lumen-to-bath osmotic gradient (130 to 290

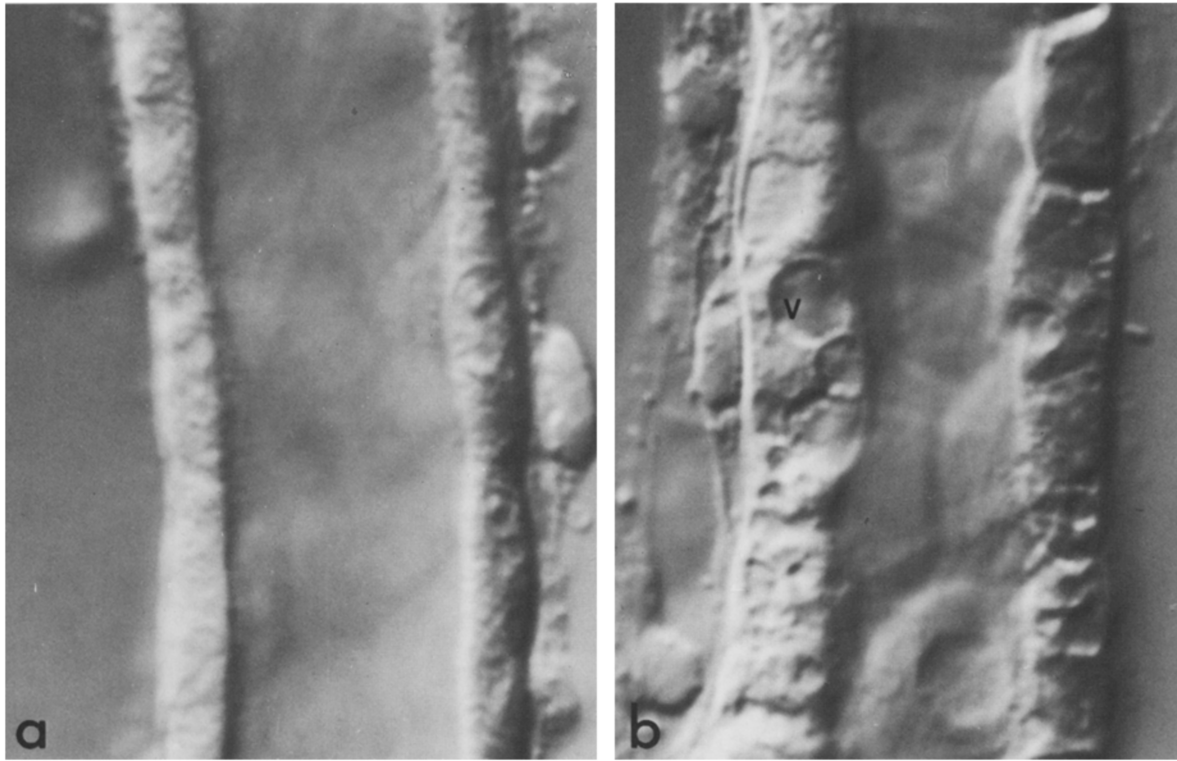

Fig. 1. DIC images of a single cortical collecting tubule observed before and after ADH addition in the presence of an osmotic gradient (130 mOsm perfusate/290 mOsm bath). (*a*) Reference focal position (longitudinal axis); pre-ADH. (*b*) Reference focal position; post-ADH. Note the cell bulging and the formation of vacuoles (*see V*) observed following ADH addition. 1765×

mOsm). Figure 1 shows two DIC images of a single collecting tubule that were obtained at the reference focal plane (longitudinal axis of the tubule) before and after the addition of ADH. Switching to the dilute perfusate prior to ADH addition had no noticeable effect on epithelial morphology (*not shown*). The subsequent addition of ADH resulted in marked bulging of the cells into the lumen and, in some cells, the formation of large vacuoles (diameter ~1 to 5 μm). Note that apical rounding was characteristic of all the cells lining the tubule, i.e., there was no evidence for a subpopulation of cells which was structurally unresponsive to ADH.

Dilation of the lateral intercellular spaces is also a feature of the structural response to ADH addition in the presence of the osmotic gradient. As shown in Fig. 2, this event is most clearly observed when the focal plane optically sections the epithelium, i.e., when the plane of focus is adjusted downward from reference into the cell layer nearest the chamber bottom. Figure 2 compares two DIC images (2*a*, *b*) of an individual collecting tubule, that were obtained prior to ADH addition at the reference focal plane and 14 μm below reference, with two images (2*c*, *d*) which were obtained at the same focal positions 10 min following ADH addition. Again, cell bulging is apparent at the level of the reference focal plane (compare Figs. 2*a* and 2*c*). And, as can be seen in the optical sections, the lateral spaces dilated markedly and fairly uniformly in response to ADH addition.

Influence of ADH on Epithelial Volume and Transepithelial Water Flow

The ADH-induced changes in epithelial morphology are associated with simultaneous increases in epithelial volume (V_T) and transepithelial water flow (J_v). Figure 3 compares for a single collecting tubule, exposed to the lumen-to-bath osmotic gradient, the influences of ADH on V_T, J_v and the transepithelial voltage ($\Delta\psi_T$). V_T, normalized to that observed prior to ADH addition, increased following exposure to ADH and reached a new value within 30 min which was about 30% larger than the pre-ADH value. Coincident with the increase in V_T was a transient stimulation of the lumen-negative $\Delta\psi_T$ and a nearly tenfold increase in transepithelial volume flow. The time course of the increase in J_v,

which has been examined in previous experiments (Schafer & Andreoli, 1972 and *unpublished observations*), is similar to the time course of the increase in V_T observed in these studies.

Table 1 summarizes the magnitudes of the ADH-induced changes in V_T, the outer (D_o) and inner (D_i) tubule diameters and the osmotic water permeability (P_f) measured in four experiments. The

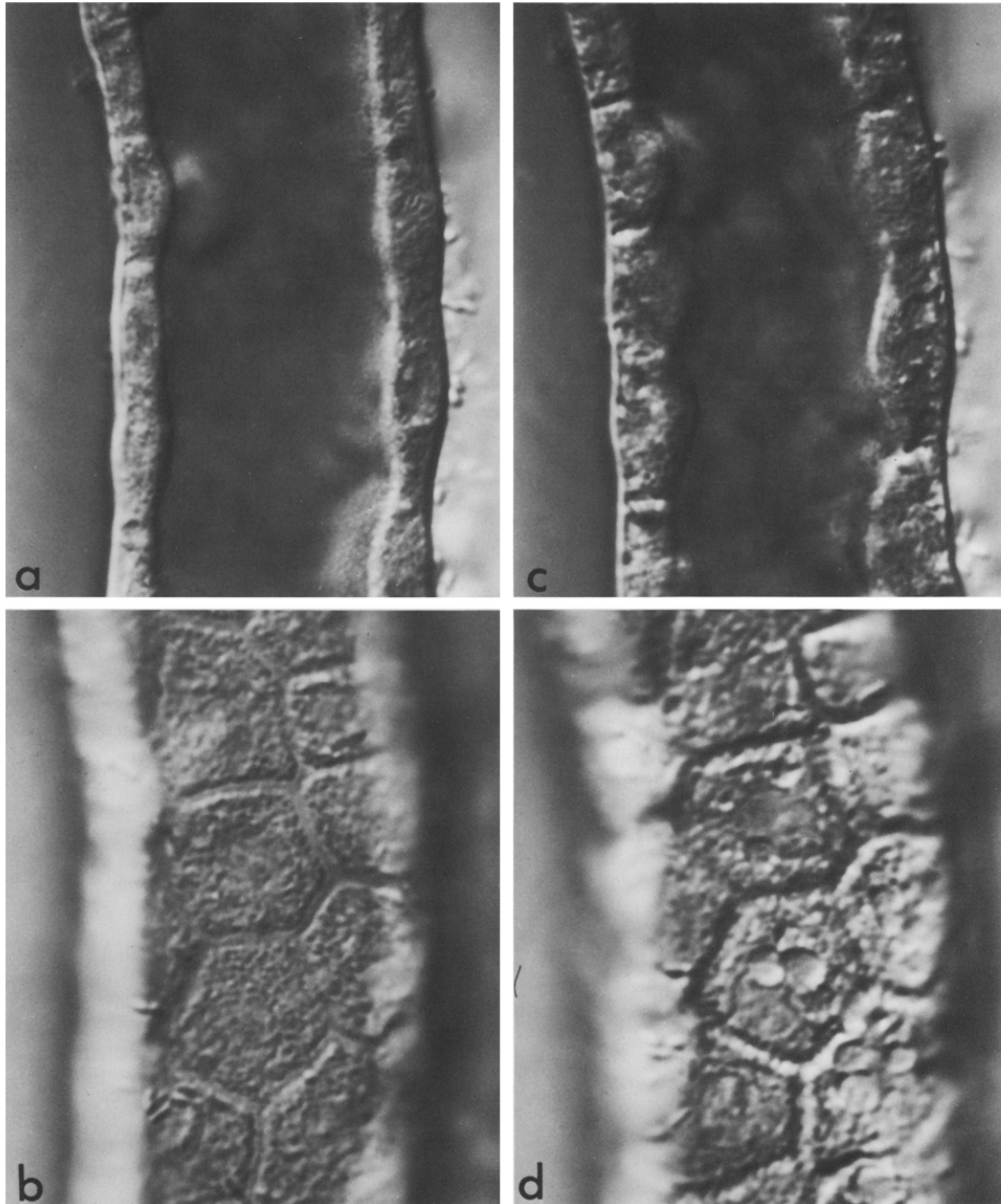

Fig. 2. Dilation of the lateral intercellular spaces observed following ADH addition in the presence of an osmotic gradient (130 mOsm perfusate/290 mOsm bath). (*a*) Reference focal position; pre-ADH. (*b*) Focal position: -14 μm; pre-ADH. (*c*) Reference focal position; post-ADH. (*d*) Focal position: -14 μm; post-ADH. 1765×

mean, ADH-induced increase in P_f was similar to that previously reported (Schafer & Andreoli, 1972) and, although the increase varied from about 120 to 250×10^{-4} cm sec^{-1}, there was no apparent correlation between the ADH-stimulated P_f and the increase in epithelial volume. Note that the increase in V_T, which averaged about 30% of the pre-ADH value, was accomplished largely by a reduction in D_i; D_o increased only slightly. Thus, as was the case following peritubular dilution (Kirk et al., 1984), epithelial volume increased following ADH addition by swelling of the cell layer into the tubule lumen rather than outwardly against the relatively noncompliant basement membrane (Welling & Grantham, 1972).

Osmotic Gradient-Dependence of the Morphologic Response to ADH

Although the data summarized in Fig. 3 and Table 1 correlate the morphologic changes with the onset of ADH-dependent volume flow, they do not preclude the possibility that these structural events are induced, not by volume flow, but by a more direct action of ADH on epithelial morphology. Table 2 summarizes data from 10 experiments which demonstrate that the influence of ADH on V_T (and epithelial structure, in general) requires the presence of the lumen-to-bath osmotic gradient. ADH addition in the presence of isotonic perfusate and bath had no effect on epithelial morphology or the measured V_T. Subsequently changing to the dilute perfusate (130 mOsm) resulted in the same structural changes as depicted in Figs. 1 and 2 and increased V_T by about 30%. As expected, the time course of the epithelial volume increase under these conditions was considerably more rapid than that observed following ADH addition in the presence of a previously applied osmotic gradient (compare Figs. 3 and 5 below). Removal of the gradient returned V_T toward pre-gradient values and reversed the lateral space dilation and cytoplasmic vacuolation. Thus, the structural events observed during the onset of ADH-dependent volume flow are, in fact, flow-dependent events and are not induced by the presence of ADH, *per se*. The increase in V_T associated with the onset of hormone-dependent flow supports two conclusions: 1) in the presence of a favorable osmotic gradient ADH enhances water entry from the lumen into the epithelial cell layer and 2) there exist barriers distal to the luminal membrane entry step which offer resistance to ADH-dependent flow.

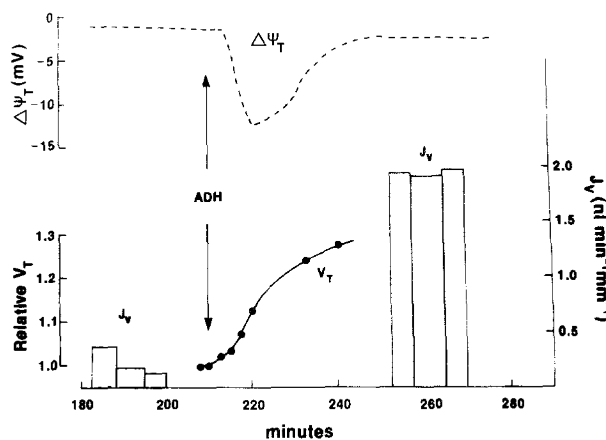

Fig. 3. ADH-induced changes in epithelial volume (V_T), transepithelial water flow (J_v) and transepithelial voltage ($\Delta\psi_T$). The data were determined for a single tubule that was exposed to a lumen-to-bath osmotic gradient (130 to 290 mOsm). V_T was normalized to that value obtained prior to ADH addition. $\Delta\psi_T$ has not been corrected for liquid junction potentials at the bridge—solution interfaces, which introduce a lumen—positive offset in the observed $\Delta\psi_T$ of approximately 3 mV (calculated from the Henderson diffusion equation). The time axis refers to the number of minutes elapsed after killing the rabbit

Table 1. Effect of ADH on epithelial volume and osmotic water permeability during hypotonic perfusion

	Outside tubule diameter (D_o) (μm)	Inside tubule diameter (D_i) (μm)	Epithelial volume (V_T) (nl mm^{-1})	Osmotic water permeability (P_f) (cm sec^{-1} × 10^4)	Hydraulic conductivity (L_p) (cm^3 sec^{-1} atm^{-1} mm^{-1} × 10^9)
−ADH	33.4 ± 3.0	21.5 ± 2.5	0.52 ± 0.08	5 ± 5	0.2 ± 0.2
+ADH	34.2 ± 2.9	18.2 ± 2.5	0.66 ± 0.09	170 ± 27	6.8 ± 0.8
+ADH/−ADH	1.02 ± 0.02	0.84 ± 0.04	1.27 ± 0.04	—	—
P	>0.20	<0.05	<0.02	—	—

$n = 4$; perfusate, 130 mOsm; bath, 290 mOsm.

Table 2. Osmotic gradient-dependence of the ADH-stimulated increase in epithelial volume (V_T)

	Δ Osmolality (mOsm)	ADH (μU ml^{-1})	V_T (nl mm^{-1})	Percent change (from control)	P (*vs.* control)
Control	0	0	0.49 ± 0.03	—	—
	0	250	0.49 ± 0.03	0 ± 1%	>0.50
	160	250	0.65 ± 0.5	32 ± 4%	<0.001
Recovery	0	250	0.51 ± 0.03	4 ± 3%	>0.50

n = 10; bath, 290 mOsm; perfusate, 130 or 290 mOsm.

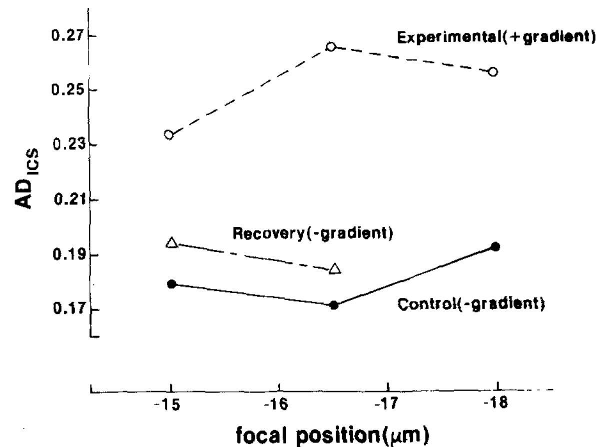

Fig. 4. Influence of ADH-stimulated volume flow on the areal density of the lateral intercellular spaces (AD_{ICS}). The measurements were performed on optical sections obtained from a single tubule which was exposed to ADH throughout the experiment. Images were recorded at 2 to 3 focal positions in the presence of isotonic (290 mOsm) perfusate and bath (control), then after switching to the hypotonic (130 mOsm) perfusate (experimental) and finally after returning to the isotonic perfusate (recovery). The focal positions of the luminal and basal surfaces of the epithelium observed in the absence of the osmotic gradient were −13.5 and −19.5 μm, respectively

Influence of ADH-Dependent Flow on Lateral Intercellular Space-Geometry and Cellular Volume

In order to characterize the influence of ADH-dependent flow on the geometry of the lateral intercellular spaces, we utilized the procedure described in the preceding paper (Kirk et al., 1984) to estimate the areal density of lateral spaces (AD_{ICS}) observed in optical sections through the cell layer which were obtained before and during hormone-stimulated volume reabsorption. Figure 4 is a plot of AD_{ICS}, determined for a single collecting tubule exposed to ADH in the presence and absence of an osmotic gradient, as a function of focal position within the cell layer. Using AD_{ICS} as an index of relative space width (Kirk et al., 1984), the data in Fig. 4 illustrate that imposition of the lumen-to-bath osmotic gradient in the presence of ADH increased lateral space width to approximately the same degree along the height of the lateral spaces from apex to base. Subsequently removing the gradient reversed the increase in space width and returned AD_{ICS} toward pre-gradient values. Averaged over eight experiments, in which AD_{ICS} was determined at 1 to 3 focal positions for each experiment, the areal density of lateral spaces increased by about 40% (*see* Table 3) following the onset of ADH-dependent volume flow. In addition, the relative increase in AD_{ICS} did not vary significantly as a function of focal position within the epithelial cell layer (all P values > 0.20).

As discussed in the preceding paper (Kirk et al., 1984), we can estimate the increases in cell and lateral space volumes associated with the onset of ADH-dependent flow from the flow-induced changes in V_T, AD_{ICS} and the estimated height of the lateral spaces. Table 3 summarizes for eight collecting tubules the calculated volumes of the lateral intercellular spaces (V_{ICS}) and the cells (V_{cell}) before and during ADH-stimulated flow. The onset of flow induced approximately a 75% increase in the volume of the lateral spaces. This marked increase was referable to a mean increase in lateral space width (AD_{ICS}) of 44% and a 22 ± 5% ($\bar{X}$ ± SEM) increase in lateral space height (estimated for each tubule from the flow-induced changes in epithelial thickness at 4 to 6 junctions between adjacent cells). Note that the magnitude of the lateral space swelling accounts for only about a 10% increase in epithelial volume; the remaining increase in V_T is attributable to a 28% increase in cell volume. Thus, the onset of ADH-stimulated flow is associated not only with marked dilation of the lateral intercellular spaces, but also with moderate swelling of the cells themselves.

Temporal Sequence of the Structural Events Associated with ADH-Stimulated Flow

Figure 5 compares the time courses of the cell swelling and lateral space dilation observed during the onset of ADH-dependent volume flow for an individual tubule. AD_{ICS}, determined for a single focal position throughout the experiment, and V_T are plotted as functions of time following application of the osmotic gradient in the presence of ADH. The values for each parameter have been

Table 3. Effect of ADH-dependent volume flow on epithelial volume, cellular volume and the volume of the lateral intercellular spaces

	Epithelial volume (V_T) (nl mm^{-1})	Area density of lateral spaces (AD_{ICS})	Cell volume (V_{cell}) (nl mm^{-1})	Lateral space volume (V_{ICS}) (nl mm^{-1})
Zero volume flow (−ADH; ± osmotic gradient)	0.49 ± 0.02	0.15 ± 0.02	0.42 ± 0.02	0.07 ± 0.01
ADH-dependent flow (+ADH; + osmotic gradient)	0.66 ± 0.04	0.21 ± 0.02	0.53 ± 0.02	0.13 ± 0.02
Percent change	+35 ± 5%	+44 ± 9%	+28 ± 5%	+78 ± 11%
P	<0.001	<0.01	<0.001	<0.01

$n = 8$. V_{ICS} and V_{cell} were calculated for the "zero flow" condition according to Eqs. (4) and (5) in the preceding paper (Kirk et al., 1984). The relative, flow-induced increase in V_{ICS} was estimated for each tubule as the product of the relative increases in lateral space height and lateral space width (AD_{ICS}). V_{cell} for the "ADH-dependent flow" condition was then estimated as the difference between V_{ICS} and V_T determined for the ADH-stimulated case.

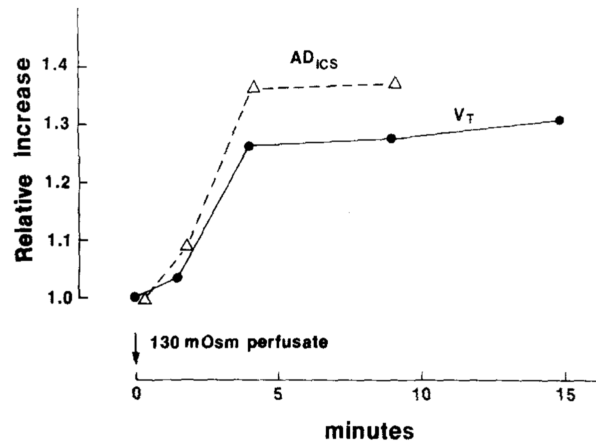

Fig. 5. Comparison of the time courses of the increases in epithelial volume (V_T) and areal density of lateral intercellular spaces (AD_{ICS}) associated with the onset of ADH-dependent volume reabsorption. The measurements were performed on images of a single tubule that were obtained before and at several time points after changing the perfusate from the isotonic (290 mOsm) to the hypotonic (130 mOsm) solution. The AD_{ICS} measurement was performed on optical sections obtained at a single focal position. ADH was present throughout the course of the experiment

normalized to that value determined for the zero gradient condition. Using AD_{ICS} as an index of lateral space width and V_T as an approximation of cell volume, the data in Fig. 5 demonstrate that the lateral spaces dilate with approximately the same time course as the increase in cell volume and, therefore, with the same time course as the increase in transepithelial volume flow. For three such experiments the average time required to reach new steady-state values for V_T and AD_{ICS} were 5.0 ± 1.0 and 5.5 ± 0.9 min, respectively. In contrast, an average of 3.7 ± 0.2 min was required for the first vacuole to be observed and vacuole formation continued for at least 20 to 30 min after AD_{ICS}, V_T and transepithelial volume flow had reached new steady-state values. The latter result implies that cytoplasmic vacuole formation is a secondary response to transcellular volume flow and does not represent the expansion of a preferential route for the movement of water from the luminal to the basolateral cell membranes.

Discussion

Implications for the Transepithelial Pathway for ADH-Dependent Flow

The results of this study confirm and extend the qualitative description of ADH-induced changes in epithelial morphology derived from previous ultrastructural observations of the mammalian collecting tubule (Ganote et al., 1968; Grantham et al., 1969; Tisher et al., 1971; Woodhall & Tisher, 1973). Associated with the onset of ADH-dependent flow was a marked increase in the volume occupied by the lateral intercellular spaces, a simultaneous, more modest increase in cell volume and a slower, more prolonged vacuolation of the cytoplasm. As suggested by Ganote et al. (1968), the fact that these morphologic events required an osmotic gradient in addition to ADH implies that they represent a passive expansion of intraepithelial compartments induced by flow and not an "active," hormone-stimulated rearrangement of epithelial architecture in anticipation of flow.

The temporal sequence of the flow-induced perturbations in collecting tubule structure provides several insights as to the arrangement of these compartments within the pathway for transepithelial volume flow (*see* Fig. 6). First, the rapid swelling of the cells associated with the onset of ADH-dependent volume flow is consistent with the notion that ADH increases the water permeability of the luminal cell membrane (Ganote et al., 1968; Schafer & Andreoli, 1972; Harmanci et al., 1978) and argues against a completely paracellular route for transepithelial volume reabsorption. Second, the coincidental dilation of the lateral spaces implies that a fraction of the volume exit from the cells into the peritubular surroundings traverses these intercellular spaces. Third, because cytoplasmic vacuole formation was not observed until the latter stages of

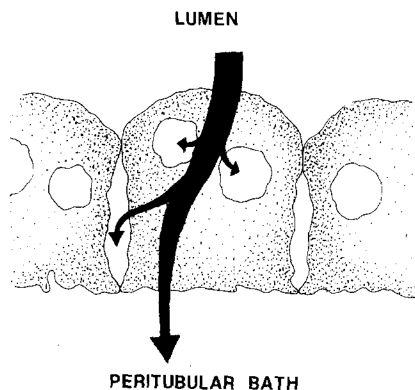

Fig. 6. Schematic representation of the pathway for ADH-stimulated water reabsorption

cell volume expansion and the water flow increase, these structures probably do not comprise a preferential route for transcellular flow and, instead, more likely represent a slowly filling compartment which lies in parallel to the volume flow pathway.

Cellular Specificity of the Structural Response to ADH-Dependent Flow

In their electron microscopic examination of the rabbit cortical collecting tubule, Ganote et al. (1968) observed that the principal and intercalated cells responded similarly to ADH-stimulated volume flow. Although we could not distinguish between the two cell types of the collecting duct, the uniformity of the structural changes observed in the present studies also implies that both the principal and intercalated cells are structurally responsive to hormone-stimulated flow. These observations, which are in contrast to evidence for the toad urinary bladder that implicates that granular cell as the sole cell type responsive to ADH-stimulated flow (DiBona, Civan & Leaf, 1969), suggest that both cell types of the rabbit collecting duct may be target cells for the action of ADH on water permeability. However, it must be emphasized that distortion and vacuolation of cells which are not directly responsive to ADH could be effected by the accumulation of fluid within the lateral intercellular spaces—fluid which has entered these spaces from adjacent, hormone-responsive cells. Therefore, we cannot dismiss the possibility that one of the two cell types is insensitive to the water permeability increase induced by ADH. And, the preponderance and the magnified basolateral membrane surface of the principal cell (Kaissling & Kriz, 1979; LeFurgey & Tisher, 1979; Wade, O'Neil, Pryor & Boulepeap, 1979; Welling, Evan & Welling, 1981) argues that this cell type is more important for the reabsorption of water along the mammalian collecting tubule.

Lateral Intercellular Space-Dilation in ADH-Dependent Flow

A principal issue is the relative importance of the lateral intercellular spaces as a pathway for the movement of water from the cells to the peritubular surroundings. It seems reasonable that, barring any differences in intrinsic permeability characteristics, the relative volume flows across the lateral and basal cell membranes should reflect the surface areas and geometries of each of these membranes. On these grounds, Welling et al. (1981) have argued that the highly infolded basal cell membranes are the preferential route for volume exit from the cells to the peritubular surroundings, but that the lateral spaces should be able to deliver as much as one-third of the total reabsorbed volume. Considering the relatively small volume occupied by these spaces ($\sim$0.13 nl mm^{-1} tubule length (*see* Table 3)) and a typical ADH-dependent, transepithelial volume flow of 2 nl min^{-1} mm^{-1} tubule length (*see* Fig. 3), a flow rate through these spaces which is one-third of the total transepithelial flow would completely "turn over" the volume occupied by lateral spaces every 10 sec. Such a brisk flow rate through the lateral spaces is supported by the evidence of Wade and DiScala (1971) who observed, within 4 min after the onset of ADH-stimulated volume reabsorption, a complete washout of horseradish peroxidase from the lateral spaces of the toad urinary bladder.

Dilation of the lateral spaces during the onset of ADH-dependent flow presumably results from an accumulation of fluid (and hydrostatic pressure) within the spaces as a result of increased volume entry from the cells and restricted volume exit into the peritubular surroundings. The most likely anatomical sites for significant resistance to fluid outflow from the lateral spaces are the basilar slits, where the lateral spaces narrow to 1 to 2 $\times$ 10^{-2} μm in width (Grantham et al., 1969). However, as discussed by Grantham et al. (1969), one can calculate from the physical dimensions of these slits a hydraulic conductivity per unit length of slit (6.2 $\times$ 10^{-7} cm^3/sec atm cm slit length) which, when multiplied by the total length of basilar slit per mm length of tubule ($\sim$2 cm slit/mm tubule length)[1], provides

[1] A value of 1.9 cm basilar slit/mm tubule length can be calculated for the "model" cortical collecting tubule of Welling et al. (1981) in which there are 825 cells/mm tubule length, each of which has a circumference of 44.9 μm at the base.

an estimate of the total hydraulic conductivity of the basilar slits which is almost 200 times greater than the transepithelial hydraulic conductivity measured during ADH stimulation (1.2×10^{-6} as compared to 6.8×10^{-9} cm^3/sec atm mm tubule length). Thus, even if in the face of a transepithelial osmotic pressure difference of 4.2×10^3 cm H_2O (160 mOsm) all of the steady-state volume exit from the cells proceeded via the lateral spaces, the required driving force for flow across the basilar spaces would be less than 20 cm H_2O, or in equivalent units of osmolality difference, less than 1 mOsm. This implies that lateral space dilation is referable, not to a substantial resistance to volume exit across the basilar spaces but, instead, to the distensible, lateral cell membranes which deform in response to the development of moderate transmembrane hydrostatic pressures.

Cytoplasmic Vacuolation in ADH-Dependent Flow

Vacuoles could not be observed following peritubular dilution in the absence of ADH (Kirk et al., 1984) nor following exposure to ADH in the absence of an osmotic gradient. Possibly, these vacuoles comprise a compartment whose water permeability is enhanced by ADH and which then expands in response to the cytoplasmic dilution associated with hormone-stimulated water entry. However, two lines of evidence argue against this possibility. First, we have not been able to detect vacuolation in experiments in which both the perfusate and bath were diluted equally (290 → 190 mOsm) in the presence of ADH (*unpublished observations*). Second, the degree to which the vacuoles expand during flow is considerably greater than would be expected for osmotic swelling of intracellular compartments. Thus, the cytoplasmic vacuoles, which are probably unimportant for the transcellular movement of water from the luminal membrane to the peritubular space, are nonetheless directly induced by this flow and are not simply the consequences of cytoplasmic dilution.

Relative Hydraulic Resistances of the Luminal and Basolateral Cell Membranes

If we model the ADH-stimulated cells as a single compartment bounded by the luminal and basolateral cell membranes, we can estimate the ratio of the hydraulic resistances of the opposing cell membranes according to the following equation (*see* Appendix):

$$\frac{R_B}{R_L} = \frac{\alpha - 1}{(1 - F_I) - (\alpha - F_I)\dfrac{\pi_p}{\pi_b}}. \tag{3}$$

R_B and R_L are the hydraulic resistances of the basolateral and luminal cell membranes, respectively, π_p/π_b is the ratio of the perfusate and bath osmotic pressures, α is the relative increase in cell volume induced by ADH-dependent volume flow and F_I is that fraction of the control cell volume which is osmotically inactive. The latter was estimated by assuming that the osmometric behavior of collecting tubule cells during ADH-induced flow is identical to that observed in the preceding paper (Kirk et al., 1984) during cell swelling (of about the same magnitude) induced by peritubular dilution. Inserting into Eq. (3) values for π_p/π_b, α and F_I of 0.45, 1.28 and 0.22, respectively, we calculate that R_B/R_L is about unity (0.92). Thus, we estimate that, in the presence of a supermaximal ADH concentration, the hydraulic resistances of the opposing cell membranes are nearly equal—a result which is consistent with similar estimates for the toad urinary bladder (Levine & Kachadorian, 1981). The significance of this result is not that the basolateral cell membranes offer resistance to volume exit from the cells but, instead, that they do not comprise the rate-determining step for transepithelial volume flow, even during maximal ADH stimulation of the water permeability of the luminal membranes. Thus, as Welling et al. (1981) have argued on the basis of the highly magnified surface area of the basal cell membranes, the cells of the collecting duct are well suited to the task of reabsorbing large quantities of volume with a minimum of cytoplasmic dilution and, presumably, cell damage.

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Appendix

Treating the ADH-stimulated cells as a single compartment bounded by the opposing luminal and basolateral cell membranes, we can estimate the relative hydraulic resistances of the two membranes as follows. In the steady state, the volume flows across the luminal (J_L) and basolateral (J_B) cell membranes are equal:

$$J_L = J_B. \tag{A1}$$

Considering the Starling forces which drive volume flow, we can rewrite Eq. (A1) as:

$$\frac{\Delta\pi_L + \Delta P_L}{R_L} = \frac{\Delta\pi_B + \Delta P_B}{R_B} \tag{A2}$$

where $\Delta\pi$ and ΔP represent the osmotic and hydrostatic pressure differences, respectively, across each membrane and R_B and R_L are the hydraulic resistances, or the reciprocal hydraulic conductivities, across the basolateral and luminal cell membranes, respectively. Assuming that hydrostatic pressure contributes negligibly to the total driving force for volume flow across each membrane, we can rearrange Eq. (A2) to obtain:

$$\frac{R_B}{R_L} = \frac{\Delta\pi_B}{\Delta\pi_L} \tag{A3}$$

or

$$\frac{R_B}{R_L} = \frac{\pi_b - \pi_{\text{cell}}}{\pi_{\text{cell}} - \pi_p} \tag{A4}$$

where π_{cell}, π_p and π_b represent the osmotic pressures within the cell, the perfusate and the bath, respectively. Equations (A3) and (A4) formalize our intuitive notion that the distribution of the driving forces for volume flow across the opposing cell membranes and, hence, the degree of cytoplasmic dilution, are directly related to the relative hydraulic resistances of these barriers.

According to Eq. (A4), if we can relate π_{cell} for the ADH-stimulated condition to some measurable quantity, we can estimate R_B/R_L. Rearranging Eq. (6) of the preceding paper (Kirk et al., 1984), we can estimate the relative decrease in π_{cell} ($\pi_{\text{cell}}/\pi^i_{\text{cell}}$) associated with the onset of ADH-dependent volume flow from the relative increase in cell volume (α) and that fraction of the control cell volume which is osmotically insensitive (F_I):

$$\frac{\pi_{\text{cell}}}{\pi^i_{\text{cell}}} = \frac{1 - F_I}{\alpha - F_I} \tag{A5}$$

where π^i_{cell} is the initial cell osmotic pressure prior to ADH-dependent flow. Since prior to flow, the cell osmotic pressure was equilibrated with that of the bath, Eq. (A5) can be rewritten as:

$$\pi_{\text{cell}} = \pi_b \frac{(1 - F_I)}{(\alpha - F_I)} \tag{A6}$$

and, substituting Eq. (A6) into Eq. (A4) and rearranging, we obtain:

$$\frac{R_B}{R_L} = \frac{\alpha - 1}{(1 - F_I) - (\alpha - F_I)\dfrac{\pi_p}{\pi_b}}. \tag{A7}$$