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Topical Review

The Effects of Antidiuretic Hormone (ADH) on Solute and Water Transport in the Mammalian Nephron

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The biochemical and physiological events responsible for osmotic homeostasis range from vasopressin (antidiuretic hormone, ADH) synthesis and release from the central nervous system to the action of ADH on specific renal tubular cells, the latter being ultimately responsible for water conservation by the kidney. Although a general review of this subject would appear timely, the explosion of knowledge in this field over the last decade has been great; thus covering all recent events would permit only a superficial analysis. Consequently, this review has been restricted to an examination of the effects of antidiuretic hormone on solute and volume flows in the mammalian nephron. Although such a choice might appear myopic, we believe it to be justifiable for several reasons.

First, an understanding of the physiological effects of ADH on renal tubular epithelia forms a basis for analyzing the pathophysiology of clinical disorders of renal water transport. Second, recent experimental observations on the temperature-dependence of tracer water diffusion and net volume flow in cortical collecting tubules, both in the presence and absence of ADH, provide a way of assigning specific characteristics to the water permeation sites in apical membranes of these tubules, and to the way ADH affects these regions. In addition, this type of analysis demonstrates the importance of explicit knowledge of the major barriers which impede transport across epithelia when interpreting apparent "breaks" in Arrhenius activation energy relations, either for water transport or for solute transport. Third, the discovery of an ADH-induced NaCl absorptive mechanism (Hall, 1979; Hall & Varney, 1979; Hebert et al., 1980*b*) in the mouse medullary thick ascending limb of Henle's loop provides a way of investigating the effect of ADH on neutral sodium chloride transport in this nephron segment, and may lead to a better understanding of how mammalian species preserve outer medullary interstitial hypertonicity during ADH-induced antidiuresis.

Finally, a substantial body of information, beyond the scope of the present review, has been gathered on the effects of antidiuretic hormone on transport processes in amphibian epithelia. Therefore these issues will not be covered in any detail. Rather we will discuss, in brief terms only, apparent differences and important similarities between amphibian epithelia. Before describing the ADH-induced water and solute transport processes in the mammalian nephron, we shall present a brief anatomical and histological description of the ADH-responsive nephron segments.

Anatomy and Histology of Nephron Segments with ADH-Responsive Adenylate Cyclase Activity

Thick Ascending Limb of Henle

Until very recently, the only physiologically significant effect of ADH in the mammalian nephron was considered to be an increase in the water permeability of the luminal membranes of collecting duct cells (Grantham & Burg, 1966; Frindt & Burg, 1972; Schafer & Andreoli, 1978; Hebert & Andreoli, 1980). However, Morel and co-workers, in a recent series of elegant studies, have shown unambiguously that ADH increases adenylate cyclase activity not only in the collecting duct and in the most distal segment of the distal convoluted tubule or connecting tubule (*see below*), but also in medullary, but not cortical, thick ascending limbs of Henle's loop (Imbert et al., 1975*a*, *b*; Chabardès et al., 1977; Imbert-Teboul et al., 1978). Morel et al. (1976) have also found this latter effect to be variable among different species; among species

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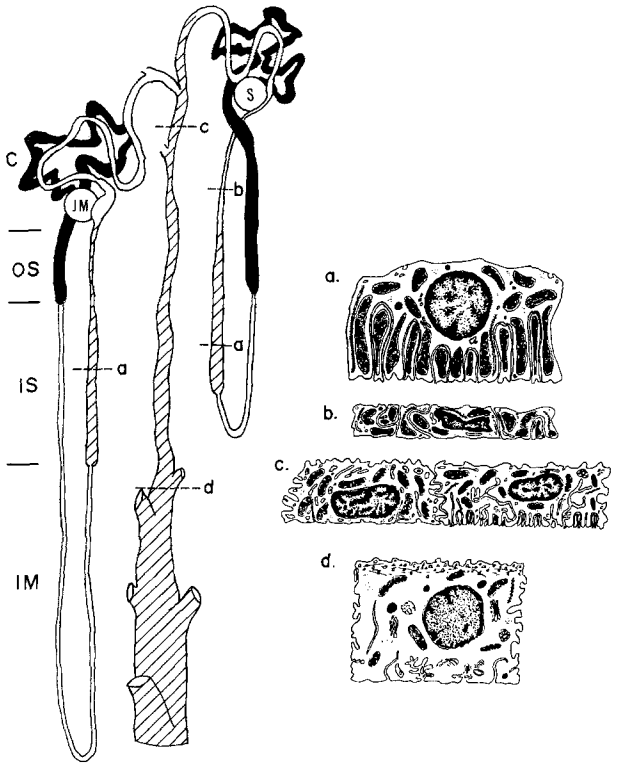

Fig. 1. Schematic representation of the arrangement of a juxtamedullary nephron, *JM*, and a superficial nephron, *S*, in the kidney. The cortex is labeled *C*: the medulla is divided into the outer strip (*OS*), the inner strip (*IS*), and the inner medullary (*IM*) areas. The hash-marked segments have been shown to respond to ADH. Also shown are a number of cell-types from inside nephron segments indicated by the corresponding letters *a–d:* cell-type *a*, medullary thick ascending limb cell; cell-type *b*, cortical thick ascending limb cell; cell-type *c*, cortical collecting tubule cells, an intercalated cell on the left and a principal cell on the right; cell-type *d*, a principal cell from the medullary collecting duct

tested to date, the ADH-mediated increase in adenyl cyclase activity has been most pronounced in the mouse.

Now the effects of ADH on transport processes in hormone-sensitive epithelia are considered to be mediated in accord with the second messenger hypothesis (Sutherland, 1961): the hormone binds to receptors on basolateral surfaces of responsive cells and results in an adenylate cyclase-mediated acceleration of cAMP production from ATP, and cAMP effects changes which modify transport processes through these cells. Thus the observations of Morel and his co-workers imply that, in addition to the effect of the hormone on water flows in the collecting duct, ADH may also alter transport events in the mouse medullary thick ascending limb.

The arrangement of these tubule segments in the nephron and their relations in the kidney, for both superficial and juxtamedullary nephrons, are shown schematically in Fig. 1. The latter is illustrative of general interrelations, and it should be recognized that many differences among species also occur. For example, in the rabbit, the ratio of long-looped nephrons (those with loops of Henle which penetrate deep into the medulla) to short-looped nephrons is 3:2 (Kaissling & Kriz, 1979). Kriz and Koepsell (1974) have found just the opposite in the mouse, where the ratio of long- to short-looped nephrons is 1:7; in man, the ratio is 1:7 (Peter, 1909; Valtin, 1977). In addition, within a given species, major anatomical and histological differences occur between short- and long-looped nephrons. In the rabbit, all juxtamedullary nephrons are long-looped, as are the majority of mid-cortical nephrons, while short-looped nephrons arise from the superficial and outer mid-cortical nephrons (Kaissling & Kriz, 1979). The long-looped nephrons in the mouse, however, come almost exclusively from juxtamedullary nephrons, while superficial and mid-cortical nephrons produce only short loops (Kriz & Koepsell, 1974).

The short loops in rabbit (Kaissling & Kriz, 1979), rat and mouse (Dieterich et al., 1975) are composed of a descending thin limb lined with a flat, noninterdigitating epithelium and a thick ascending limb. No thin ascending limb is present in the short loops. There is, however, a major difference in the organization of the loops between the rabbit and the rat and mouse; the latter two species have their short loops integrated into the medullary vascular bundles (Kriz et al., 1972; Kriz & Koepsell, 1974; Dieterich et al., 1975), while in rabbits the short loops are randomly distributed between the vascular bundles. The short loops of superficial and mid-cortical nephrons have thick ascending limbs composed of both medullary and cortical segments, with the lengths of each segment seemingly dependent upon the position of the glomerulus in the cortex (e.g., the superficial nephrons have the longest cortical segments of thick ascending limbs).

The epithelia of these two segments are quite different and have been extensively studied in the rabbit by Kaissling and Kriz (1979) and in the rat by Allen and Tisher (1976). In the rabbit, the medullary thick ascending limb epithelium is composed of a single cell type with shallow tight junctions devoid of gap junctions and desmosomes, and exhibits extensive basal infolding filled with many large rod-shaped mitochondria (*see* Fig. 1, cell type-*a*). In the cortical ascending limb, the lining epithelial cell thickness diminishes considerably, and the cells possess fewer and less extensive basal infoldings containing fewer mitochondria (*see* Fig. 1, cell type-*b*). Welling, Welling and Hill (1978) have demonstrated that the cells of the cortical segment have the shape of a truncated and pleated horn; and due to a lack of apical micro-

villi, the cells have an apical/lateral membrane ratio of 0.1 (only one-tenth of that in the proximal tubule). The thick ascending limbs are covered by a basement membrane which provides strong and elastic support but offers little resistance to the flow of water and solutes (Welling & Welling, 1978). The cortical segments of these limbs may continue for a variable distance beyond the macula densa; the length can be considerable in the rabbit (Morel *et al.*, 1976; Kaissling & Kriz, 1979) but is generally short in the rat (Kaissling et al., 1974; Allen & Tisher, 1976).

The ultrastructural differences between medullary and cortical ascending thick limbs in the rabbit are paralleled by differences in pump $(Na^+ + K^+)$ATPase activity. The medullary segment has a very high $(Na^+ + K^+)$ATPase activity, but the latter is significantly reduced in the cortical segment (Schmidt & Dubach, 1969; Katz et al., 1979). Rat and mouse, however, have equal $(Na^+ + K^+)$ATPase activities in both segments (Doucet et al., 1979; Katz et al., 1979). Thus medullary and cortical thick ascending limbs of rat and mouse contain the cytologic machinery for high magnitudes of active salt transport (Jørgensen, 1977), while in the rabbit cortical segment this capability is less apparent.

In addition, the adenylate-cyclase sensitivity to ADH is quite different in these two segments of the thick ascending limb: the medullary segment contains considerable ADH-induced activity while the cortical segment has no activity (Chabardès et al., 1977). This type of response to ADH, however, shows considerable species variability, being prominent in the mouse medullary thick ascending limb but variable in the rabbit (Chabardès et al., 1977). Imbert-Teboul et al. (1978) also demonstrated, in the rat medullary thick ascending limb, an adenylate cyclase activity which was sensitive to ADH. And Edwards et al. (1980) have shown that cAMP increases protein kinase activity in the medullary thick limb.

Similar studies (Chabardès et al., 1980) have been performed recently in the human thick ascending limbs obtained from kidneys perfused *in vitro* for variable periods of time. No significant ADH-induced adenylate cyclase activity was demonstrated; however, loss of responsiveness due to storage of these kidneys could not be totally excluded (Chabardès et al., 1980).

The long-looped nephrons of both mouse and rabbit contain a thin ascending segment in the inner medulla or inner zone (*IM* in Fig. 1) which attaches to the thick ascending segment at the border between the inner and outer medullary zones. The thick ascending limbs in the mouse are exclusively medullary (Kriz & Koepsell, 1974); both the $(Na^+ + K^+)$ATPase activity, and responsiveness of adenyl cyclase activity to ADH, of the medullary and cortical segments of the thick ascending limbs of long-looped nephrons appear to be similar to corresponding segments in the short-looped nephrons. The ultrastructural organization of the thin descending limb is quite different, however, from that in the short-looped nephrons (Dieterich et al., 1975; Kaissling & Kriz, 1979).

The Connecting Tubule and Collecting Tubule

The anatomy and histology of the rabbit distal convoluted tubule, connecting tubule and collecting tubule have been extensively studied by Kaissling and Kriz (1979). In the rabbit, the cortical thick ascending limb is connected to a short, slightly-coiled distal convoluted segment which proceeds to the cortical collecting tubule via a connecting tubule; the latter segment forms an arcade as it receives multiple tributaries (*see* Fig. 1). The cortical collecting tubule descends through the cortex to join the medullary collecting tubule, each accepting, on average, flow from six nephrons. The collecting tubule epithelium consists of two cell types: the principal cell, found only in collecting tubules, and the intercalated cell, found also in the connecting tubule.

This epithelium also shows gradual ultrastructural alterations along its course from the cortical surface to the papillary tip. The ratio of the principal cell to the intercalated cell decreases (approximately 2:1 in the outer cortex) toward the inner cortex, after which only principal cells occur. In addition, the diameter of the medullary segment increases toward the papillary tip, and is associated with an increase in principal cell height from 5–7 μm in the cortex to 90–120 μm at the papillary tip. The principal cell (*see* Fig. 1, cell type-*c*, *d*) is characterized by extensive basal in-foldings which decrease in complexity and number toward the inner medulla.

The existence of any ADH-responsive cells in either the distal convoluted tubule or connecting tubule has been a controversial issue. Recently, Morel and co-workers have helped to clarify this issue (Morel et al., 1976; Chabardès et al., 1977). Only the most distal segments of the distal convoluted tubule, which connect to the cortical collecting tubule in the outer cortex, appear to have any ADH-responsive adenylate cyclase. Histologically, this segment is composed of the same cells as the cortical collecting tubule, that is, principal and intercalated cells.

Therefore ADH sensitivity begins at the transition of the connecting tubule to the cortical collecting tubule, precisely where the principal cell begins (Imbert et al., 1975*a*, *b*; Kaissling & Kriz, 1979). In

addition, the medullary collecting tubule, which in the inner zone is formed only by principal cells, contains an ADH-sensitive aenylate cyclase (Chabardès et al., 1977) and responds to ADH with an increase in water permeability (Rocha & Kokko, 1974) characterized by distinctive morphological changes (Bonventre et al., 1978). Thus the most probable candidate for ADH sensitivity is the principal cell. This conclusion, however, should be viewed with some caution since Ganote et al. (1968) and Grantham et al. (1969) noted, on transmission electron micrographs of the rabbit cortical collecting tubule, an increase in cell height and intracellular vacuolation of both principal cells and intercalated cells in the presence of ADH.

The intercalated cell is present in the connecting tubule, the cortical collecting tubule and the outer medullary collecting tubule. This cell type is characterized by a "dark" staining cytoplasm in the cortical segment which gradually decreased in staining within the outer medulla. The function of the intercalated cell has not been established, but it appears not to be required for the ADH response, since it is present in the connecting tubule, where an ADH response is absent, and absent in the inner zone medullary collecting duct, where an ADH response is present.

Measurement of Permeability Changes Produced in the Nephron by ADH

The effects of ADH on water permeability in the nephron have been assessed quantitatively in terms of two coefficients: the permeability to water diffusion at zero volume flow, P_{D_w} (μm/sec), and the osmotic water permeability coeffcient P_f (μm/sec). The diffusional water permeability is usually measured by adding tritium-labeled water (THO) to one side of the epithelium and measuring the rate of THO appearance on the other side, generally under conditions of zero transepithelial volume flow. For example, in the isolated perfused cortical collecting tubule, THO is generally added to the perfusate, and P_{D_w} is calculated from the lumen-to-bath tracer flux $J^*(\text{cpm sec}^{-1}\,\text{cm}^{-2})$ as:

$$P_{D_w} = J^*/\bar{C}^*, \tag{1}$$

where $\bar{C}^*$ is the integrated average luminal tracer concentration (Grantham & Burg, 1966; Schafer & Andreoli, 1972*a*).

Alternatively, water permeability may be measured by imposing a transepithelial osmolality difference ($\Delta\pi$, atm) and measuring the resulting net volume flow J_v (cm/sec). The hydraulic conductivity of the epithelium (Lp) is given by $J_v/\Delta\pi$, and, for comparison to P_{D_w}, can be expressed as:

$$P_f = RTLp/\bar{V}_w, \tag{2}$$

where R, T and $\bar{V}_w$ have their usual meanings.

The water permeability response of nephron segments to ADH has been assessed by *in vivo* micropuncture, *in vivo* microcatherization, and *in vitro* perfusion. The only ADH-responsive nephron segments which are accessible *in vivo* to direct examination are the portions of the distal convoluted tubule which reach the cortical surface and, in certain species such as the rat and the golden hamster, the papillary collecting duct. Using free-flow micropuncture samples from rat distal convoluted tubule, Wirz (1956) and Gottschalk and Mylle (1959) showed that in antidiuresis distal tubular fluid approaches isotonicity but remains dilute in water diuresis. In contrast, in the dog (Clapp & Robinson, 1966) and monkey (Bennett et al., 1967), distal tubular fluid remains dilute even during hydropenia with maximal urine osmolalities. Ullrich et al. (1964) and Sonnenberg (1974) used microcatheterization *in vivo* to show that, in the hydropenic state, the collecting duct fluid osmolality increased progressively from the outer medulla to the papillary tip. These observations confirm the early observations of Wirz et al. (1951) who used cryoscopic measurements on slices of the medullary parenchyma to show the same progressive increase in urine osmolality along the medullary collecting duct in hydropenia. Attempts have also been made to estimate the P_f and P_{D_w} responses to ADH in the collecting duct using *in vivo* micropuncture (Ullrich et al., 1964; Persson, 1970); however, the technique is limited by the heterogeneity of the distal convoluted tubule as discussed above. Morgan and Berliner (1968) and Morgan et al. (1968) used microperfusion techniques to study segments of the collecting duct *in situ* in *in vitro* medullary slices. They observed that in the absence of ADH, P_f and P_{D_w} were, respectively, 5.3 and 39 μm/sec. ADH increased both P_f and P_{D_w}, respectively, to 9.5 and 194 μm/sec.

The most widely used method for the study of the response of nephron permeability to ADH has been to utilize *in vitro* microperfusion of isolated tubule segments. Grantham and Burg (1966) observed that freshly dissected rabbit cortical collecting tubule segments had a high initial water permeability which declined temporally, reaching a minimum approximately 180 min after sacrifice of the animal; when ADH was introduced into the bathing solution, the water permeability rose rapidly to its initial high value. These observations have been confirmed sub-

Table 1. Effect of ADH on water and solute permeation in cortical collecting tubules

Bath [ADH] ($\mu U/ml^{-1}$)	P_f	P_{D_w} ($cm\,sec^{-1} \times 10^4$)	P_{D_i}	
			Urea	Thiourea
0	20±4.0	4.7±0.5	0.03	0.03
250	186±8.9	14.2±0.6	0.02	0.02

The results are expressed as mean values ±SEM (standard error of the mean). From Schafer and Andreoli (1972*a*, *b*) and Schafer, Troutman and Andreoli (1974).

sequently in detail (Grantham & Orloff, 1968; Schafer & Andreoli, 1972*a*). Table 1 lists P_f and P_{D_w} values measured in this nephron segment at 23 °C. P_f is more than fourfold higher than P_{D_w} in the absence of ADH, and more than 13-fold higher in the presence of the hormone (Grantham & Burg, 1966; Grantham & Orloff, 1968; Schafer & Andreoli, 1972*a*).

In spite of the dramatic increase in both the diffusional and osmotic water permeability coefficients observed with ADH in the cortical collecting tubule, this epithelium remains virtually impermeable to even the smallest hydrophilic nonelectrolytes (Table 1). The permeabilities of urea, thiourea and acetamide were measured from the fluxes of ^{14}C-labeled nonelectrolytes from luminal solutions to bath in the presence or absence of ADH. The measured lumen-to-bath fluxes at zero volume flow were within detectable limits, but unaltered by ADH (Grantham & Burg, 1966; Burg et al., 1970; Schafer & Andreoli, 1972*b*; Schafer et al., 1974).

The same conclusion, namely that cortical collecting tubules remain virtually impermeable to small hydrophilic solutes either in the presence or absence of ADH, also obtains from solute reflection coefficient measurements in this nephron segment. Thus we (Schafer & Andreoli, 1972*b*) observed that, either in the presence or absence of ADH, urea, NaCl and sucrose all had unity reflection coefficients in cortical collecting tubules. Stated in another way, these results indicate that, for cortical collecting tubules, the antidiuretic response involves a profound increase in the water permeability of an epithelium which can discriminate more than 10^3-fold between water and hydrophilic solutes, e.g. urea, having effective radii less than twice as large as that of a water molecule.

Site of the Change in Water Permeability with ADH

Morphological observations of the response of the collecting tubule to ADH have confirmed that the final site of ADH action on water permeability, via cAMP, is at the apical epithelial surface. The photomicrographs in Fig. 2 show the effects of ADH on the morphology of isolated cortical collecting tubules perfused with a hypotonic solution (125 mOsmol/liter Krebs-Ringer's phosphate buffer) and bathed with an isotonic one (290 mOsmol/liter Krebs-Ringer's). During the initial period of high water permeability, the apical surfaces of tubular cells bulge into the lumen and the cobblestone pattern of the cell margins is visible. After 180 min of perfusion, i.e. when tubular water permeability is at a minimum, the cells become flattened and the cell margins indistinct (middle micrograph, Fig. 2). Furthermore, within 10 min after adding ADH to the bathing solution, the cells swell and bulge apically, and the cell margins become sharply outlined (Schafer & Andreoli, 1972*a*).

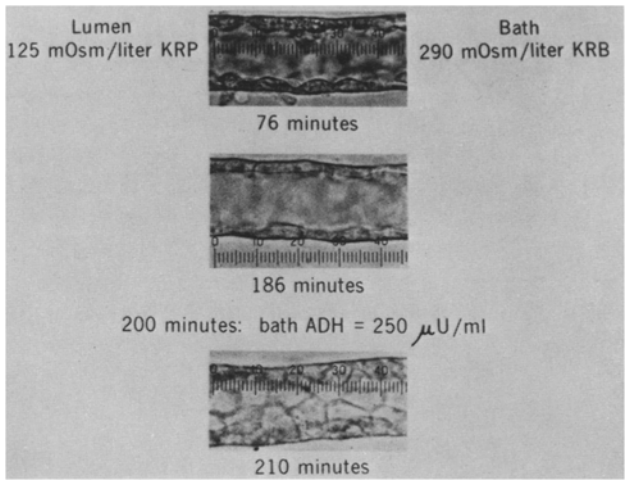

Fig. 2. Rate-limiting site for water permeation. Segment of cortical collecting tubule was perfused with 125 mOsm Krebs-Ringer's-phosphate (KRP) buffer and bathed in 290 mOsm Krebs-Ringer's-bicarbonate (KRB) buffer at 25° C. Time shown below each photomicrograph is that elapsed after rabbit was sacrificed. At 200 min ADH was added to the bath. Interval between small divisions in photomicrograph scale is 2.13 μm. (Adapted from Andreoli and Schafer, 1977)

Ganote et al. (1968) and Grantham et al. (1969), using transmission electron micrographs of tubules fixed at various stages in the ADH response, showed that, in the presence of lumen-to-bath osmotic flow, there was an increase in the apex-to-base cell height, intracellular vacuolation, and distension of lateral intercellular spaces. In contrast, these epithelial cells swelled dramatically with bathing solution hypotonicity either in the presence or absence of ADH. Thus these observations indicate clearly that the apical epithelial surface is rate-limiting to lumen-to-bath osmosis and responds to the ADH signal with an increase in water permeability, (Grantham & Burg,

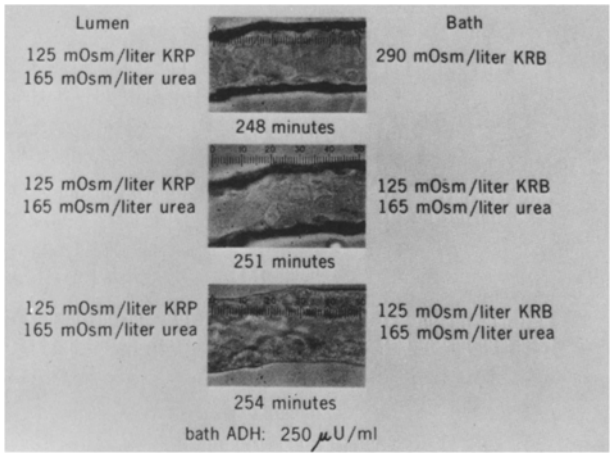

Fig. 3. Rate-limiting site for urea permeation in cortical collecting tubule. Perfusing solution was 125 mOsm KRP buffer plus 165 mOsm urea throughout the experiment. Bath was initially 290 mOsm KRB buffer, but was changed to 125 mOsm KRB plus 165 mOsm urea at 250 min after rabbit was sacrificed. Bath contained 250 μU ml^{-1} ADH throughout. Interval between small divisions in photomicrograph scale is 2.13 μm. (Adapted from Andreoli and Schafer, 1977)

1966; Ganote et al. 1968; Grantham et al., 1969; Schafer & Andreoli, 1972*a*).

The apical epithelial surface is also the primary resistance to transepithelial permeation of small hydrophilic nonelectrolytes. Fig. 3 shows photomicrographs from an experiment in which a cortical collecting tubule was perfused and bathed intitially with an isotonic Krebs-Ringer's phosphate buffer in the presence of 250 μU/ml ADH. Subsequent isosmotic substitution of urea for 165 mOsmol/liter of the NaCl in the pefusate caused no gross morphological changes in the cell layer, and produced no net volume flow. However, when urea was substituted for NaCl in the bathing solution, there was rapid, marked cell swelling while transepithelial water flux remained zero (Schafer & Andreoli, 1972*b*). These results indicate that, even in the presence of ADH, the apical surfaces of cortical collecting tubules constitute the primary resistance locus for transepithelial urea permeation. Clearly then, under normal physiologic circumstances, i.e. when luminal fluids have the same or lower osmolalities than peritubular media, both the apical membranes and junctional complexes of cortical collecting tubules are tight to urea.

While the observations shown in Fig. 2 indicate that the apical surfaces of cortical collecting tubules are the rate-limiting barrier for water flow, and that these surfaces respond to ADH by increasing water permeability, the data do not indicate whether the hormone alters the water permeability of apical plasma membranes or of junctional complexes. Grantham et al. (1969) addressed this problem in part by showing that, when cells were swollen in hypotonic bathing solutions, a return to the isotonic bathing solution caused dilation of lateral intercellular spaces and shrinkage of the cells. Thus these workers argued that the primary route for transepithelial water flow was across apical plasma membranes, then via lateral plasma membranes and intercellular spaces to peritubular media. The issue has also been addressed on theoretical grounds for toad (Civan, 1970) and frog (Jard et al., 1971) urinary bladders, with the conclusion that the majority of transepithelial water flow must be transcellular.

We (Schafer et al., 1974) have addressed this problem experimentally by comparing ADH-independent osmotic water permeability and urea permeability for situations in which the permeability properties of junctional complexes were either unmodified, i.e. the control circumstance, or were altered by exposure to luminal hypertonicity. It is relevant to note in this regard that, in electrically tight epithelia (Frömter & Diamond, 1972) such as the toad bladder and frog skin, hypertonicity of the apical solution induces marked decreases in transepithelial electrical resistances, and an increase in junctional complex permeability to hydrophilic solutes (Lindley et al., 1964; Ussing & Windhager, 1964; Ussing, 1966; DiBona & Civan, 1973; de Bermudez & Windhager, 1975). Associated with these changes are marked alterations, presumed to represent opening, in the structure of junctional complexes, observed either by differential interference contrast microscopy *in vitro* (Di Bona, 1978) or by transmission electron microscopy of fixed specimens (Ussing & Windhager, 1964; Erlij & Martinez-Palomo, 1972). Of importance in the present context, Ussing (1966, 1969) reported that when apical solutions bathing toad skins were hypertonic while serosal solutions were isotonic, there was net water flow from serosa to mucosa. However, in contrast to the expected solvent drag effect, mucosa-to-serosa flux of the probe solute sucrose, which was present in equal concentrations in both solutions, was increased and exceeded the oppositely directed flux.

Two classes of explanations have been proposed for this "anomalous solvent drag." Ussing (1969) has argued that junctional complexes are opened by apical hypertonicity, thus allowing solute to enter lateral intercellular spaces from the hypertonic apical solution via the junctions. The resulting hypertonicity of lateral spaces causes a reverse osmotic volume flow toward the open basilar ends of the spaces together with sucrose flow. Alternatively, Biber and Curran (1968), Franz et al. (1968), and Galey and van Bruggen (1970) have proposed that serosa-to-mucosa

Table 2. Effect of luminal hypertonicity on ADH-independent water and urea permeation in cortical collecting tubules

Coefficient	Lumen (mOsm/l^{-1})	Bath (mOsm/l^{-1})	P_i ($\mu m\ sec^{-1} \times 10^4$)	
			Lumen→bath	Bath→lumen
P_f	125 KRP	290 KRB	20 ± 4 ($n=34$)	–
	290 KRP+ 200 urea	290 KRB	–	83 ± 15 ($n=5$)
$P_{D_{urea}}$	125 KRP	290 KRB	0.04 ± 0.01	–
	290 KRP+ 200 urea	290 KRB	0.22 ± 0.02 ($n=4$)	0.033 ± 0.01 ($n=4$)

Adapted from Schafer, Troutman and Andreoli (1974).

osmotic flow opens junctional complexes and allows a solute-solute coupled flow, such that the mucosa-to-serosa flow of the solute producing apical hypertonicity drives a parallel net sucrose flow in the same direction.

In terms of either explanation, it is important to note that the solute producing the luminal hypertonicity must penetrate the junctional complexes in order for "anomalous solvent drag" to occur. Thus the following experiments can be interpreted by assuming that, in the presence of luminal hypertonicity, acceleration of solute flux in a direction opposite to solvent flow indicates that the probe solute has penetrated junctional complexes.

Table 2 shows the results of experiments designed to evaluate the effects of luminal hypertonicity on ADH-independent water and urea flows in isolated cortical collecting tubules (Schafer et al., 1974). Table 2 shows clearly that, for these circumstances, there occurred striking rectification of ADH-independent osmosis; the value of P_f during bath-to-lumen flow was more than fourfold higher than that for the opposite flow. And importantly, with the hypertonic perfusate, the urea flux coefficient measured from the lumen-to-bath ^{14}C-urea flux was more than six times greater than the flux coefficient for urea in the bath-to-lumen direction, and higher than that for urea movement in either direction with isotonic luminal fluids, either with or without ADH (Schafer et al., 1974). In other words, luminal hypertonicity produced by urea resulted in so-called "reverse solvent drag," or more explicitly, acceleration of urea flow in a direction opposite to that for solvent flow. And in accord with the interpretations presented above, this effect may be rationalized most easily by assuming that junctional complexes become more permeable to urea during luminal hypertonicity induced by urea. Yet during the condition of high junctional urea permeability, P_f was 84 μm/sec (Table 2), i.e. less than one-half of the P_f value observed in the presence of ADH when urea permeability remains low (Table 1).

These observations are consistent with the view that water flow through junctional complexes which have distinctly high urea permeabilities is inadequate to account for the rate of lumen-to-bath (antidiuretic) water flow in the presence of ADH, when junctional complexes are rather impermeable to urea. Consequently, it is reasonable to argue that lumen-to-bath water flow, in the presence or absence of ADH, proceeds primarily through apical plasma membranes rather than through junctional complexes, and that the water permeability change induced by ADH occurs in the apical membranes of the collecting duct (Ganote et al., 1968; Grantham et al., 1969; Schafer et al., 1974), as it may in other hormone-sensitive epithelia (Civan, 1970; Jard et al., 1971).

Evaluation of the P_f/P_{D_w} Ratio: The Narrow Channel Hypothesis

Large Pores Do Not Explain P_f/P_{D_w} and $P_f/P_{D_{urea}}$ Ratios

One of the classical ways of accounting for P_f/P_{D_w} ratios in excess of unity is to assume that a membrane contains pores sufficiently large to permit laminar or quasilaminar flow during osmosis (Koefoed-Johnson & Ussing, 1953; Pappenheimer, 1953). Thus the P_f/P_{D_w} ratio exceeds unity because net volume flow varies with r^4 (r = pore radius) while zero volume flow THO exchange varies with r^2; and by combining Fick's first law with Poiseuille's law, one may derive an expression relating the P_f/P_{D_w} ratio to the pore radius r (Robbins & Mauro, 1960). It is interesting to note in this context that such expressions, which utilize macroscopic rate coefficients, are adequate to describe transport processes in planar bilayer membranes containing amphotericin B-cholesterol pores, which have effective radii of approximately 4 Å (Holz & Finkelstein, 1970; Andreoli, 1973).

In the case of cortical collecting tubules, the ADH-dependent P_f/P_{D_w} ratio of approximately 13 (Table 1) requires, in terms of large pore theory, an effective pore radius of ~13 Å (Schafer & Andreoli, 1972*a*). Yet as indicated above (Table 1), cortical collecting tubules are virtually impermeable to small hydrophilic solutes such as urea (effective hydrodynamic radius ≃2.2 Å), both in the presence and absence of ADH. Likewise, both with and without hormone, urea, NaCl and sucrose (effective hydrodynamic radius ≃5.2 Å) all have unity reflection coefficients in

these tubules (Schafer & Andreoli, 1972*b*). In short, while the P_f/P_{D_w} ratio rises in the presence of ADH, these tubules show no evidence for the kind of solute sieving predicted from large pore theory for the measured range of P_f/P_{D_w} ratios. Thus we conclude that the effect of ADH on the P_f/P_{D_w} ratios in these tubules depends on water transport through permeation pathways too small to permit laminar or quasilaminar osmotic flow.

Post-Apical Membrane Diffusion Resistances Explain Part of the P_f/P_{D_w} Disparity

A second class of explanations accounting for disparities between P_f and P_{D_w} assumes that a membrane might be homogeneous and that the mode of osmotic water transport across a membrane is diffusional, but that unstirred layers in series with the membrane impede THO diffusion at zero volume flow but not net volume flow during osmosis (Dainty, 1963; Cass & Finkelstein, 1967; Andreoli & Troutman, 1971). According to this view, the relation between P_f and P_{D_w} is:

$$\frac{1}{P_{D_w}}=\frac{1}{P_f}+R_{uns}, \tag{3}$$

where R_{uns} (sec cm^{-1}) is the diffusion resistance of the unstirred layer. Both the equality between P_f and P_{D_w}, when R_{uns} approaches zero, and the validity of Eq. (3), for $R_{uns}>0$, have been established in unmodified homogeneous planar lipid bilayer membranes (Cass & Finkelstein, 1967; Andreoli & Troutman, 1971).

In the case of the cortical collecting tubule, the issue is more complex. For the ADH-dependent values of P_f and P_{D_w} listed in Table 1, Eq. (3) requires an R_{uns} value of 704 sec cm^{-1}. Now R_{uns} may be expressed as (Schafer & Andreoli, 1972*a*):

$$R_{uns}=\frac{\beta \Delta x}{D_w^o} \tag{4}$$

where D_w^o is the free diffusion coefficient for water [2.36×10^{-5} cm^2 sec^{-1} (Wang et al., 1953)] and Δx is the thickness of the cortical collecting tubule [$\sim$7.0 μm (Schafer & Andreoli, 1972*a*)]. In these terms, the parameter β is the equivalent of a tortuosity factor (Mackay & Meares, 1959) for post-apical membrane diffusion resistances. Evidently, by using the values cited above, Eq. (3) requires a value of approximately 22 to account for the ADH-dependent P_f/P_{D_w} ratio in cortical collecting tubules (Table 1).

Earlier studies involved the use of highly lipophilic probes to compute a value for the β parameter in cortical collecting tubules (Schafer & Andreoli, 1972*a*). The argument depended on the fact that such solutes have remarkably high permeability coefficients in bilayer membranes, on the order of 1 cm sec^{-1} (Holz & Finkelstein, 1970; Schafer & Andreoli, 1972*a*) and zero reflection coefficients in cortical collecting tubules (Schafer & Andreoli, 1972*a*). Thus it was reasoned that $1/P_{D_i}$ for such solutes would reflect primarily the value of R_{uns} and thus permit calculation of the β parameter.

Table 3. Highly lipophilic probes as an index to post-apical membrane diffusion constraints in cortical collecting tubules

Lipophile	P_{D_i} (cm sec$^{-1}\times10^4$)	β (Eq. 2)
n-Butanol	12.2	12
Pyridine	12.6	12
Phenol	16.4	8
Ethyl acetate	13.7	11
5-Hydroxyindole	4.1	24

From Schafer and Andreoli (1972*a*) and Hebert and Andreoli (1980).

Table 3 lists the results of a series of such P_{D_i} measurements in cortical collecting tubules and the values of β computed from Eq. (2) from the P_{D_i} data. It is evident that, with the exception of 5-hydroxyindole (a solute having a lower oil:water partition coefficient than the other solutes listed in Table 3), one obtains a β value of $\sim$10 for cortical collecting tubules from P_{D_i} measurements. Now by inserting this latter value into Eqs. (3) and (4), the observed ADH-dependent P_f/P_{D_w} ratio of 13 in these tubules may be reduced to 7.5, which is clearly greater than unity. Thus one may conclude that a portion of the ADH-dependent P_f/P_{D_w} ratio in cortical collecting tubules depends on post-apical membrane diffusion constraints (i.e., $\beta\simeq10$), but that other mechanisms are also operative (Schafer & Andreoli, 1972*a*; Hebert & Andreoli, 1980), as will be described below.

We have no certain way of defining explicitly the nature of the post-apical membrane diffusion resistance, $\beta\Delta x/D_w^o$, but in principle it could be due either to diffusion constraints in the cell and/or bulk solutions. Clearly cellular constraints to diffusion may be either frictional or geometric; but since the epithelial cells of the cortical collecting tubule are isotonic to the peritubular medium, frictional constraints imposed by an increase in cytoplasmic viscosity would be due necessarily to macromolecules. In this circumstance these large molecules would be expected to retard volume flow according to Poiseuille's law while having little effect on tracer water diffusion (Wang et al., 1954). Consequently geometric factors, more specifically a reduction in the area available for

cellular water transport, could account for a cellular component to the post-apical membrane diffusion resistance (Schafer et al., 1974). In addition, bulk phase unstirred layer effects might account for or contribute to the observed diffusion resistance, but would be restricted necessarily to the bath (peritubular media) because of the geometry of the luminal space. Thus it is likely that the post apical membrane diffusion resistance in the cortical collecting tubule is referable either to a reduction in the area available for cellular water transport and/or bathing media unstirred layer effects. At present we have no basis for determining the relative importance of these two factors.

The Narrow Channel Hypothesis: Single-File Diffusion Through Small Aqueous Channels

From the preceding analysis, if the post-apical membrane diffusion constraints encountered by water are accurately measured by the highly lipophilic probes, i.e. if the value of β for these tubules is approximately 10, there still remains a "corrected" P_f/P_{D_w} ratio of ~ 7.5. The latter cannot be due to a large pore permitting convection during osmosis, given the water:urea selectivity characteristics of these tubules. Rather, we (Al-Zahid et al., 1977; Hebert & Andreoli, 1980) have argued that these latter selectivity characteristics, as well as the "corrected" P_f/P_{D_w} ratio, can be accounted for jointly by a mechanism for water transport through narrow aqueous channels in apical plasma membranes.

Following the proposal of Lea (1963) and others (Dick, 1966; Levitt, 1974), if apical plasma membranes contain aqueous channels sufficiently narrow to preclude side-by-side passage of water molecules, water transport will follow single-file kinetics and the relation

$$\frac{P_f}{P_{D_w}} = n_w \tag{5}$$

will obtain, where n_w is the number of water molecules in a channel. The situation is somewhat more complex for an epithelial plasma membrane which contains narrow pores, and is in series with post-apical membrane diffusion constraints which impede tracer water diffusion at zero volume flow but not transepithelial osmosis. In this circumstance, combination of Eqs. (3) and (4) with Eq. (5) yields:

$$\frac{1}{P_{D_w}} = \frac{n_w}{P_f} + \frac{\beta \Delta x}{D_w^o}, \tag{6}$$

which expresses the combined effects of post-apical membrane diffusion resistances and the single-file effect on a P_f/P_{D_w} ratio (Hebert & Andreoli, 1980).

We (Hebert & Andreoli, 1980) have recently evaluated these relations in cortical collecting tubules by assessing the temperature-dependence of P_f and P_{D_w} in the presence and absence of ADH. The line of argument is as follows. Wang et al. (1953) found that, in the range 0–55 °C, free diffusion of water obeys the relation:

$$\frac{D_w^o \eta}{T} = \phi, \tag{7}$$

where the constant ϕ has a value of $7.29 \pm 0.15 \times 10^{-10}$ cm^2 poise °K^{-1} sec^{-1}. By substituting the above relation in Eq. (6) we obtain:

$$\frac{1}{P_{D_w}} = \frac{n_w}{P_f} + \frac{\beta \Delta x \eta}{\phi T}, \tag{8}$$

and by re-arranging:

$$\frac{T}{\eta} \cdot \frac{1}{P_{D_w}} = \frac{T}{\eta} \cdot \frac{n_w}{P_f} + \frac{\beta \Delta x}{\phi}. \tag{9}$$

Eq. (9) indicates that P_f and P_{D_w} measurements made at varying temperatures form a linear relation which permits calculation of n_w from the slope and β from the intercept, if we assign bulk aqueous viscosity values corresponding to the appropriate temperature. Moreover, it is apparent that analysis of the n_w and β parameters in terms of Eq. (9) depends exclusively on permeability data rather than on extrapolation from activation energy measurements. We stress this aspect of the argument since, in our view, activation energy (E_A, kcal mole^{-1}) measurements in epithelia are subject, at a minimum, to at least two kinds of artifacts: those relating to the effects of multiple diffusion resistances in series; and those relating to the effect of temperature-dependent variations in the number of water permeation sites.

Each of these latter issues, and their effect on E_A measurements, is considered in a subsequent section (*cf. below*). In the present context, it is relevant to note that Eq. (9) obviates either kind of difficulty. First, it is evident that the $\beta \Delta x$ term in Eq. (9) considers explicitly the contribution of series diffusion resistances to P_{D_w} measurements. Second, the formulation described by Eq. (9) is independent of any temperature-dependent change in channel number. So long as P_f and P_{D_w} values are measured at the same given temperature, over a range of temperatures, Eq. (9) provides for a straight line relation between P_f and P_{D_w} regardless of temperature-dependent changes in channel number. Any change in channel number will alter the absolute value of P_f

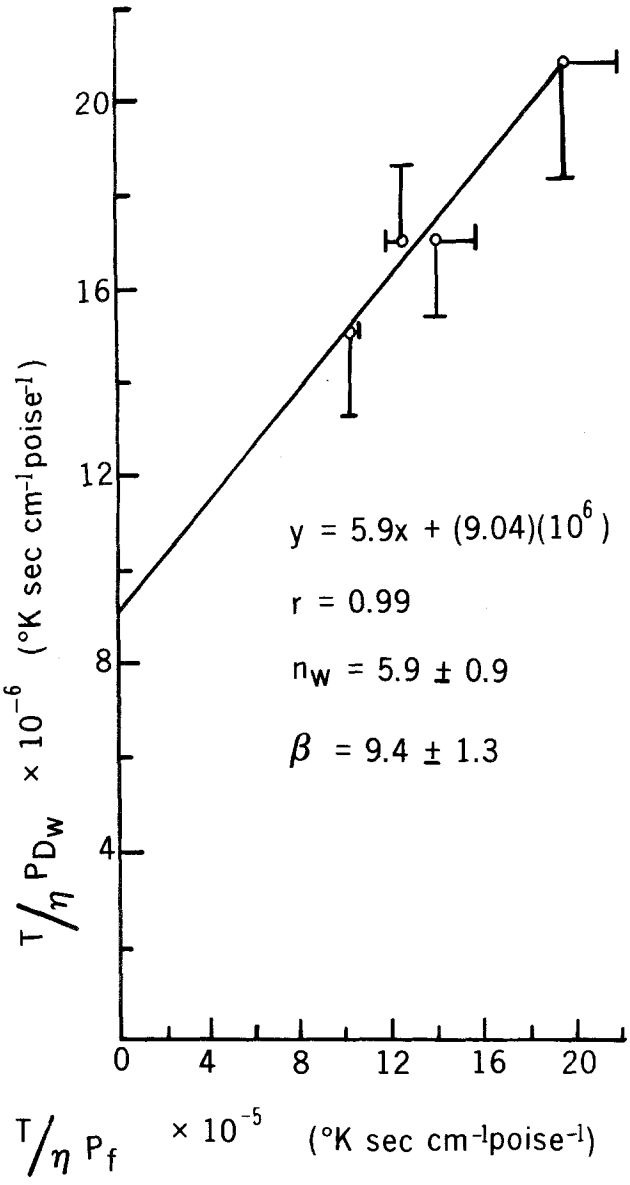

Fig. 4. A plot of experimental values ±SEM listed in Table 1 according to Eq. (9). The values of η used at the various temperatures were free solution viscosity values. (Adapted from Hebert and Andreoli, 1980)

and P_{D_w} but not the ratio of the two variables, i.e. their relation to one another. Consequently, the slope and intercept in Eq. (9), i.e. the n_w and β parameters, will remain constant.

The experimental approach (Hebert & Andreoli, 1980) was predicated on these considerations. P_f and P_{D_w} were measured over the temperature range 6–38 °C in cortical collecting tubules exposed to ADH, and the results were plotted according to Eq. (9). These data are shown in Fig. 4; and evidently, one obtains a close linear relation among the data, in conformity with the expectation of Eq. (9).

Table 4. Estimate of n_w from ADH-independent P_f and P_{D_w} measurements

T (°C)	P_f	P_{D_w}	n_w
	(μm sec^{-1})		
25	20	3.92	4.5
37	66	7.60	7.2

Adapted from Hebert and Andreoli (1980).

Moreover, the β value of 9.4 ± 1.3 computed from the zero intercept of this relation (Fig. 4) coincides nicely with the β value of 10–12 deduced from P_{D_i} measurements with highly lipophilic solutes (Table 3); thus three independent sets of measurements – P_f, P_{D_w}, and P_{D_i} for highly lipophilic solutes – converge on a β value of approximately 10. Finally, the slope of the relation shown in Fig. 4 yields an n_w value of 5.9 ± 0.9, which is similar to the n_w value of 5.3 computed from the P_f/P_{D_w} ratio for gramicidin A channels in lipid bilayers by Rosenberg and Finkelstein (1978*a*). A similar value for n_w in gramicidin A channels was also calculated by these workers from electrokinetic measurements (Rosenberg & Finkelstein, 1978*b*).

ADH Increases the Number of Narrow Aqueous Channels in Apical Plasma Membranes

We have argued (Al-Zahid et al., 1977) that ADH increases the water permeability of apical plasma membranes by increasing the number of narrow aqueous channels within the latter. If this argument is correct, then ADH-independent values of P_f and P_{D_w}, together with the β value of 9.4 listed in Fig. 2, should yield an n_w value comparable to that shown in Fig. 4. Table 4 lists, for two different temperatures, mean ADH-independent values of P_f and P_{D_w} obtained previously (Hebert & Andreoli, 1980). By inserting these values into Eq. (9) together with the β value of 9.4 from Fig. 4, we obtain, for the ADH-independent case, n_w values of 4.5 and 7.2 for the 25 °C and 37 °C cases, respectively. Thus with or without ADH, and at varying temperatures, one obtains closely comparable values for the n_w parameter.

Taken together, these observations are consistent with two general conclusions. First, a β value of approximately 10 is adequate to account for a portion of the discrepancy between P_f and P_{D_w} measurements, either in the presence or absence of ADH. Second, it is reasonable to argue that water transport through apical plasma membranes follows single-file

Table 5. Contributions of narrow aqueous channels (n_w) and post-apical membrane diffusion resistance (β) to the P_f/P_{D_w} ratio

ADH	T (°C)	P_f (cm sec^{-1} × 10^4)	P_{D_w}	n_w	$\frac{\beta \Delta x/D_w^o}{1/P_f}$	P_f/P_{D_w}
−	25	20	3.9	4.5	0.5	5.1
−	37	66	7.6	7.2	1.3	8.7
+	23	242	18.8	5.9	6.8	12.9
+	38	446	30.6	5.9	8.8	14.6

The values of P_f, P_{D_w} and n_w are from Fig. 2 and Table 4. The P_f/P_{D_w} ratio was computed from Eq. (8).

kinetics typical of narrow channels, both in the presence and absence of ADH. Thus these results are in agreement with the hypothesis (Al-Zahid et al., 1977; Andreoli & Schafer, 1977; Hebert & Andreoli, 1980) that the main route for water transport across apical plasma membranes is through narrow aqueous channels, and that ADH increases the number of such channels in apical plasma membranes.

Equally consistent with this view are three other observations. First, both in the presence and absence of ADH, the narrow channels in apical plasma membranes retain remarkable degrees of water:urea selectivity (*cf. above*). Second, as will be indicated below, the apparent E_A for water transport in these tubules, both with and without ADH, is approximately the same, in the range of 8–10 kcal mole. Finally, n_w remains a constant in the presence and absence of ADH, but the P_f/P_{D_w} ratio varies, with or without ADH, because of varying ratios of the $\beta \Delta x/D_w^o$ term in Eq. (6) to P_{D_w}.

This latter argument may be illustrated easily by considering the relations compiled in Table 5. In this regard, Eq. (6) may be rewritten as:

$$P_f/P_{D_w} = n_w + \frac{\beta \Delta x/D_w}{1/P_f}, \tag{10}$$

where the latter term represents the ratio of the post-apical membrane resistance to that of the apical membrane. It is apparent from Table 5 that, in the ADH-dependent case where $\frac{\beta \Delta x/D_w}{1/P_f}$ is large, the P_f/P_{D_w} ratio exceeds n_w; but in the ADH-independent case the P_f/P_{D_w} ratio approximates n_w. Thus in this epithelium, while n_w is static, the P_f/P_{D_w} ratio varies and becomes larger than n_w, depending on the ratio of the post-apical membrane diffusion resistance to the apical membrane diffusion resistance. Temperature affects both resistances, while ADH affects only the apical membrane resistance, $1/P_f$. These data also indicate that the apical membrane is the primary

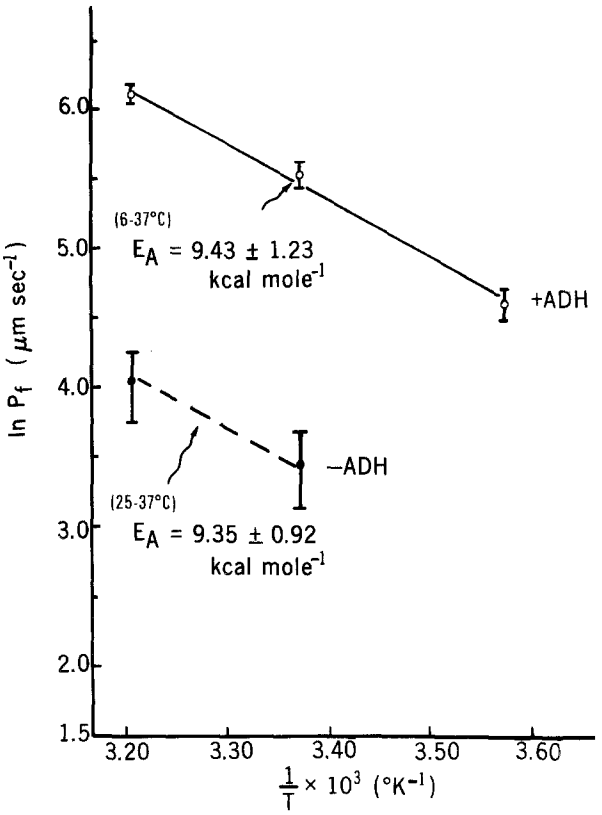

Fig. 5. Arrhenius plots of the relation between ln P_f and $1/T$ over the indicated temperature ranges in cortical collecting tubules in the absence (closed circles, ±SEM) and presence (open circles, ±SEM) of ADH. E_A values were calculated according to Eq. (11). (Adapted from Al-Zahid et al., 1977; Hebert and Andreoli, 1980)

resistance barrier for ADH-independent water diffusion $\left(\frac{\beta \Delta x/D_w}{1/P_f} \leqq 1, \text{ Table 5}\right)$, while the post-apical membrane resistance dominates the ADH-dependent case $\left(\frac{\beta \Delta x/D_w}{1/P_f} \simeq 7-9, \text{ Table 5}\right)$.

The Apparent E_A for Water Transport in Cortical Collecting Tubules

The Raw Data

The measured activation energies obtained recently in this laboratory for osmotic water flow (P_f) and for tracer water diffusion at zero volume flow (P_{D_w}), in cortical collecting tubules with or without ADH, are summarized in Figs. 5 and 6, respectively. Evidently, both the P_f and P_{D_w} seemingly obey the Arrhenius relation. Moreover, while the E_A for P_f determination, either with or without ADH, is constant at 9–10 kcal mole^{-1}, the results in Fig. 6 indicate that the E_A for P_{D_w} measurements is about twice greater in the

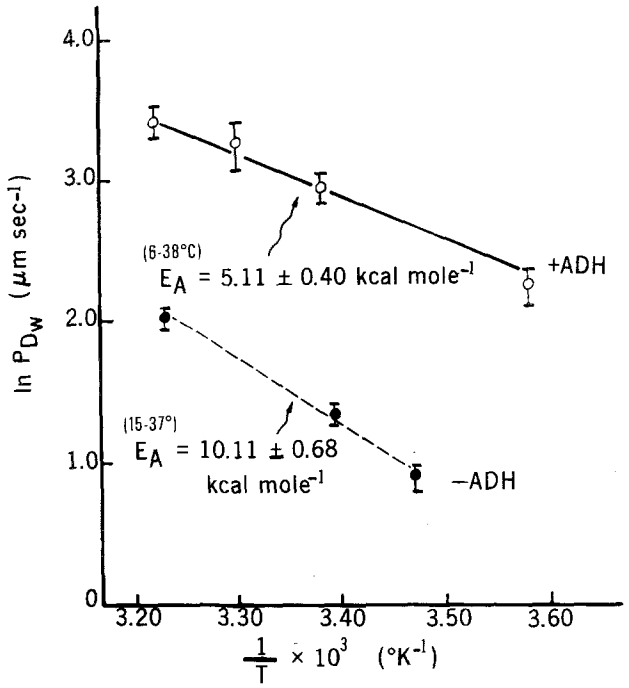

Fig. 6. Arrhenius plots of the relation between ln P_{D_w} and $1/T$ over the indicated temperature ranges in cortical collecting tubules in the absence (closed circles, ±SEM) and presence (open circles, ±SEM) of ADH. The E_A values were calculated according to Eq. (11). (Adapted from Hebert and Andreoli, 1980)

ADH-independent case than in the presence of ADH. Thus at first glance, one might say that these data diverge from the view, set out in the preceding section, that the water permeation channels in apical plasma membranes have the same molecular characteristics in the presence and absence of ADH. However, a different factor may account for this seeming discrepancy: for a β value of approximately 10, a significant fraction of the total resistance to ADH-dependent THO diffusion resides in the diffusion resistance in series with apical plasma membranes.

Correction for Diffusion Constraints in Series with Apical Membranes

To illustrate this view, we now show that when corrections are made for post-apical membrane diffusion constraints, the apparent E_A for ADH-dependent THO diffusion through apical plasma membranes approach that for osmotic volume flow. Consider in this regard a membrane containing narrow channels, when unstirred layers are not present. By inserting Eq. (5) into the Arrhenius relation for the activation energy, we obtain:

$$E_A = R\left(\frac{1}{T_1}-\frac{1}{T_2}\right)^{-1} \ln \frac{^2P_{D_w}}{^1P_{D_w}}$$
$$= R\left(\frac{1}{T_1}-\frac{1}{T_2}\right)^{-1} \ln \frac{^2P_f}{^1P_f}, \quad (11)$$

where the permeability coefficients $^2P_{D_w}$ and ^2P_f are at T_2, and $^1P_{D_w}$ and ^1P_f are at T_1. Eq. (11) indicates that, in the absence of unstirred layer effects, E_A is independent of n_w. This interesting relation bears emphasis: from Eqs. (5) and (6), it is evident that n_w affects the P_f/P_{D_w} ratio; but from Eq. (11), it is equally evident that n_w has no effect on E_A measurements.

In the cortical collecting tubule, observed P_{D_w} values, particularly in the presence of ADH, must be corrected for post-apical membrane diffusion constraints (*cf.* Eq. 4 and Table 5). To do this we re-write Eq. (6) as:

$$P^c_{D_w} = \left(\frac{1}{P_{D_w}} - \frac{\beta \Delta x}{D^o_w}\right)^{-1} = \frac{P_f}{n_w} \quad (12)$$

where $P^c_{D_w}$ is the permeability coefficient for THO diffusion through apical membrane channels corrected for diffusion resistance in series with apical membranes. Thus we may define the corrected apparent activation energy for water diffusion through apical membranes as:

$$E^c_A = R\left(\frac{1}{T_1}-\frac{1}{T_2}\right)^{-1} \ln \frac{^2P^c_{D_w}}{^1P^c_{D_w}}. \quad (13)$$

Table 6 lists the P_{D_w} values determined previously by us (Hebert & Andreoli, 1980), the values of $P^c_{D_w}$ computed according to Eq. (12) using a β value of 9.4 (Fig. 4), and the values of E^c_A computed from Eq. (13). It is evident that, without ADH, P_{D_w} is nearly the same as $P^c_{D_w}$ since $\beta \Delta x/D^o_w$ is small with respect to $1/P_{D_w}$; likewise, the calculated values of E^c_A are nearly the same as the measured E_A value. These results indicate that, without ADH, apical membranes are the dominant resistance locus for THO diffusion.

With ADH, the calculated values of $P^c_{D_w}$ are about twice as great as the measured values of P_{D_w} since $\beta \Delta x/D^o_w$ is approximately half of $1/P_{D_w}$. The ADH-dependent values of E^c_A are closely comparable to the measured E_A values for ADH-independent THO diffusion and to the measured values of E_A for P_f determinations, with or without ADH. These observations indicate that, with or without ADH, the energetic requirements for zero volume flow water diffusion or net water flow through apical plasma membrane channels remain constant. Put differently, these data are in accord with the view that the molecular characteristic of narrow aqueous channels in apical membranes of these tubules are the same with or without hormone.

Table 6. Corrected apparent activation energy for water diffusion through apical plasma membranes

ADH	T (°C)	$\beta \Delta x/D^o_w$ (sec μm$^{-1} \times 10^4$)	$P^{obs}_{D_w}$ (μm sec^{-1})	$P^c_{D_w}$	E^{obs}_A (kcal mole^{-1})	E^c_A (kcal mole^{-1})
−	15	360	2.41	2.64	8.3	8.6 (15–25 °C)
−	25	270	3.92	4.38	10.0	10.8 (25–37 °C)
−	37	201	7.54	8.89		
+	6	477	9.16	16.25	5.5	8.5 (6–23 °C)
+	23	279	18.77	39.37	4.9	8.2 (23–38 °C)
+	38	197	30.64	77.30		

The values of $P^c_{D_w}$ and E^c_A were calculated from Eqs. (10) and (11) using the data in Figs. 4, 5 and 6. Adapted from Hebert and Andreoli (1980).

Table 7. Corrected activation energy for *n*-butanol diffusion through apical plasma membranes

T (°C)	P_{D_b} (μm sec^{-1})	$1/P_{D_b}$ (sec μm$^{-1} \times 10^4$)	$\beta \Delta x/D^o_w$ (sec μm$^{-1} \times 10^4$)	$P^c_{D_b}$ (μm sec^{-1})	E^c_A (kcal mole^{-1})
30	11.61	861	597	37.8	15.3 (23–30 °C)
23	8.39	1192	711	20.8	15.7 (15–23 °C)
15	5.25	1905	894	9.89	12.1 (6–15 °C)
6	3.14	3185	1190	5.01	

Adapted from Hebert and Andreoli (1980).

The True E_A for Water Transport

The results in the preceding section indicate that, when corrections are made for post-apical membrane diffusion constraints, the apparent E_A for water transport, either zero volume flow diffusion or net volume flow, is approximately 9–10 kcal mole^{-1}; and that the value remains constant in the presence or absence of ADH. However, for water permeation through narrow channels, P_f is the product of two terms:

$$P_f = m P'_f \tag{14}$$

where m is the number of apical membrane channels, and P'_f is the hydraulic conductance per channel. Thus Eq. (11) may be expressed as:

$$E_A = \left[R\left(\frac{1}{T_1} - \frac{1}{T_2}\right)^{-1}\right]\left[\ln \frac{{}^2P'_f}{{}^1P'_f} + \ln \frac{{}^2m}{{}^1m}\right] \tag{15}$$

where ${}^2m$ and ${}^1m$ are the channel numbers at T_2 and T_1, respectively.

Eq. (15) indicates that the measured E_A for P_f determinations provides a direct estimate of the E_A for the individual channels only if the channel number in apical membranes is independent of temperature. But Kachadorian et al. (1979) have argued that, in the ADH-treated toad urinary bladder, the number of water permeation sites may diminish with decreasing temperature. If the latter argument were applicable to the cortical collecting tubule, the apparent E_A of 9–10 kcal mole^{-1} for osmotic volume flow or for zero volume flow water diffusion (Figs. 5, 6; Table 6) might be greater than the true E_A for water permeation through individual channels.

Thus the present data are in accord with three general conclusions:

1) Water transport through apical plasma membranes, with or without ADH, occurs by single-file diffusion through narrow aqueous channels containing approximately $6 H_2O$ molecules per channel; and ADH increases osmotic volume flow by increasing the number of such channels in apical membranes.

2) In the absence of ADH, apical plasma membranes constitute the primary barrier to zero volume flow THO diffusion and osmosis. The P_f to P_{D_w} ratio is therefore accounted for by n_w, the average number of water molecules per channel. In the presence of ADH, post-opical membrane diffusion constraints constitute the primary barrier to zero volume flow THO diffusion while apical plasma membranes are the primary barrier to osmosis. Thus the P_f to P_{D_w} disparity is greater than n_w and reflects a significant contribution by post-apical membrane diffusion constraints (i.e., the β parameter).

3) The apparent E_A for either zero volume flow

or osmotic water transport through apical plasma membranes, either with or without ADH, is approximately 9 kcal mole^{-1} when appropriate corrections are made for post-apical membrane diffusion resistances (Figs. 5, 6; Table 7).

Finally, when appropriate corrections for unstirred layer effects are made, the ADH-dependent values for P_f, P_{D_w}, the P_f/P_{D_w} ratio and the apparent E_A for water diffusion in toad urinary bladder epithelial cells (Hays & Franki, 1970; Hays et al., 1971) are virtually the same as the P_f (Table 1), $P^c_{D_w}$ and E^c_A values observed in the mammalian collecting tubule. And in toad urinary bladder, the water permeation pathway may be easily dissociated from the pathways for urea or Na^+ permeation (Andreoli & Schafer, 1977). Thus it may be that, in both mammalian and amphibian species, the water-conserving effects of ADH on hormone-sensitive epithelia may be mediated by comparable mechanisms, i.e., insertion of narrow aqueous channels for single-file water diffusion through apical plasma membranes.

Pseudo-"Breaks" in E_A Measurements

It is commonly believed that a "break" in the Arrhenius relation between permeability and temperature provides *prima facie* evidence for a membrane phase transition. In this section, we illustrate how varying the ratio of two series diffusion resistances can result in an apparent break in an Arrhenius relation without necessarily producing a membrane phase transition.

At 23 °C, the highly lipophilic solute *n*-butanol has a zero reflection coefficient in apical plasma membranes of these tubules (Schafer & Andreoli, 1972*b*) and the diffusion resistance for that solute is determined primarily by post-apical membrane diffusion resistances (Table 1). Fig. 7 shows the apparent Arrhenius relation for *n*-butanol diffusion: in the range 23–38 °C, the apparent E_A is 4–5 kcal mole^{-1}, a value which is virtually the same as the E_A for *n*-butanol diffusion in free solution; while below 23 °C, the apparent E_A for *n*-butanol diffusion is about twofold greater. The following considerations indicate that these results depend on the fact that transepithelial *n*-butanol diffusion involves two series resistance loci which have differing E_A values for *n*-butanol diffusion.

In analogy with Eq. (12), the observed transepithelial permeability coefficient for butanol (P_{D_b}) may be corrected for post-apical diffusion constraints by the expression:

$$P^c_{D_b} = \left(\frac{1}{P_{D_b}} - \frac{\beta \Delta x}{D^o_b}\right)^{-1} \tag{16}$$

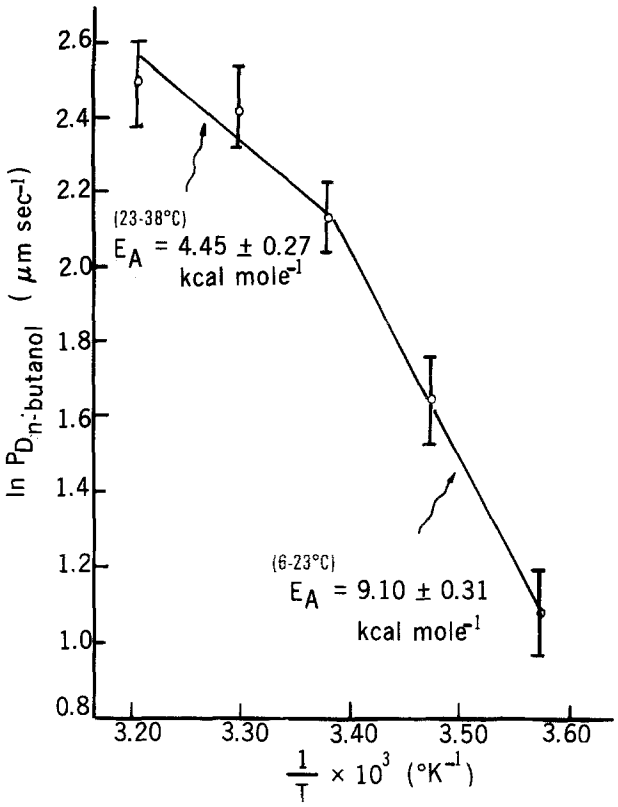

Fig. 7. Arrhenius plot of the relation between ln $P_{D_{n\text{-butanol}}}$ and $1/T$ between 6–38 °C, in cortical collecting tubules. The open circles are mean experimental values with the indicated SEM. E_A values were calculated according to Eq. (11). (Adapted from Hebert and Andreoli, 1980)

where $P^c_{D_b}$ is the corrected permeability coefficient for butanol diffusion through apical plasma membranes and D^o_b is the free diffusion coefficient for butanol diffusion. Thus the corrected activation energy E^c_A for *n*-butanol diffusion through apical plasma membranes may be computed from Eq. (13).

Table 7 lists experimentally measured values of P_{D_b}, values of $P^c_{D_b}$ computed according to Eqs. (13) and (16) using a β value of 9.4 (*cf.* Fig. 4). The results show clearly that, for the temperature range 6–30 °C, E^c_A is relatively constant and in the range 12–15 kcal mole^{-1}. Thus the apparent break in the Arrhenius plot for *n*-butanol diffusion (Fig. 7) is not referable to a phase transition of the hydrophobic core of apical membranes, but rather to a temperature-dependent shift in the locus of the primary resistance to *n*-butanol diffusion. Below 23 °C, post-apical membrane diffusion constraints constitute a progressively smaller fraction of the total transepithelial diffusion resistance. Consequently, the measured E_A for *n*-butanol diffusion rises to approximately 9 kcal mole^{-1} (*cf.* Fig. 7), thereby reflecting a proportionately greater contribution of apical plasma membranes to the measured activation energy.

Table 8. Comparison of the permeability properties of gramicidin A-treated lipid bilayers with the proposed ADH-induced water channel in cortical collecting tubules

	Gramicidin channel	Cortical collecting tubules
P_f	$\left\{\begin{array}{l} 342 \times 10^{-4} \text{ cm sec}^{-1} \text{ mho cm}^{-2} \\ 9.58 \times 10^{-15} \text{ cm}^3 \text{ sec}^{-1} \text{ channel}^{-1} \end{array}\right\}$	186×10^{-4} cm sec^{-1}
P_{D_w}	$\left\{\begin{array}{l} 65 \times 10^{-4} \text{ cm sec}^{-1} \text{ mho cm}^{-2} \\ 1.82 \times 10^{-15} \text{ cm}^3 \text{ sec}^{-1} \text{ channel}^{-1} \end{array}\right\}$	14.2×10^{-4} cm sec^{-1}
$P_f/P_{D_{urea}}$	$>10^3$	$\simeq 10^4$
G_{Na}	2.8×10^{-12} mho channel^{-1}	0.4×10^{-3} mho cm^{-1}
n_w	5–7	$\simeq 6$

No. of Gramicidin channels to account for the P_f in cortical collecting tubules $\simeq 2 \times 10^{12}$ channels cm^{-2}. G_{Na} per channel $\simeq 2 \times 10^{-16}$ mho channel^{-1}.

Sources:

Gramicidin A: $P_f/P_{D_{urea}}$ (Finkelstein, 1974); water permeability (Rosenberg & Finkelstein, 1978*a*, *b*); G_{Na} (Finkelstein, 1974); n_w (Rosenberg & Finkelstein, 1978*a*, *b*).

Cortical collecting tubules: P_f, P_{D_w}, $P_{D_{urea}}$ (Table 1); G_{Na} (O'Neil & Boulpaep, 1979); n_w (Fig. 4; Table 4).

Comparison of ADH-Dependent Apical Membrane Water Channels with Gramicidin a Channels

In lipid bilayers, gramicidin A dimers form aqueous channels having radii of approximately 2 Å (Urry et al., 1971; Hayden & Hladky, 1972), which admit water but virtually exclude urea, resulting in water/urea selectivity ratios ($P_f/P_{D_{urea}}$) in excess of 10^3 (Finkelstein, 1974; *see* Table 8). Thus a 2 Å radius channel in luminal membranes of the cortical collecting tubule, similar to the gramicidin A dimer in bilayer membranes, might account for the high ADH-dependent P_f/P_{D_i} ratios for urea, thiourea, and acetamide in this epithelium (Tables 1, 8).

In addition, as discussed above, water movement through both the gramicidin A channel and the apical membrane channel in the cortical collecting tubule is governed by single-file diffusion kinetics. And as shown in Table 6, the number of water molecules n_w, which occupy, on the average, a single channel, is remarkably similar for these channels: n_w is 5–7 for gramicidin A as determined by Rosenberg and Finkelstein (1978*a*, *b*) both from the P_f/P_{D_w} ratios and from electrokinetic data; and n_w is approximately 6 in the cortical collecting tubule (Fig. 4; Table 4).

Although such similarities are striking, there are also major differences in the sodium conductances, G_{Na}, of the two channels. Using the water permeability of the gramicidin A channel determined by Rosenberg and Finkelstein (1978*a*, *b*) of 9.58×10^{-15} cm^3 sec^{-1} channel^{-1}, one requires approximately 2×10^{12} channels cm^{-2} of luminal surface area in the cortical collecting tubule to account for a P_f of 186×10^{-4} cm sec^{-1} (Table 6). Given the Na^+ conductance of the cortical collecting tubule determined by O'Neil and Boulpaep (1979), 0.4×10^{-3} mho cm^{-2}, the maximal Na^+ conductance of each apical membrane water channel would be 2×10^{-16} mho channel^{-1}, which is four orders of magnitude less than the Na^+ conductance of gramicidin channels (Table 6). Thus although the gramicidin A channel serves as a good model for single-file water diffusion through a narrow channel, the ion conducting characteristics of the ADH-dependent water channel in apical membranes of cortical collecting tubules must be quite different from those of gramicidin A channels.

Table 9. Effect of ADH on moderately lipophilic solutes in cortical collecting tubules

Solute	T (°C)	ADH	P_{D_i} (cm sec^{-1} $\times 10^4$)	Apparent E_A (kcal mole^{-1})	
n-Butyramide	25	–	0.18 ± 0.03		
		+	0.36 ± 0.05	19.4 ± 1.6	
	37	–	0.67 ± 0.07		16.6 ± 1.6
		+	1.19 ± 0.11		
Isobutyramide	25	+	0.16 ± 0.02		
	37	+	0.46 ± 0.05	15.8 ± 1.2	
Antipyrine	25	+	0.42 ± 0.06		
	37	+	1.54 ± 0.24	19.6 ± 1.8	

Adapted from Al-Zahid et al., 1977.

Parallel Paths for Water and Solute Permeation

While it seems likely that ADH promotes antidiuresis by increasing the number of water-conducting channels in apical plasma membranes, the hormone also affects, by as yet unspecified means, the permeability characteristics of the hydrophobic core of apical plasma membranes of collecting ducts. Thus Table 9 (Al-Zahid et al., 1977) illustrates that the hormone produced slight but consistent increases in the transepithelial permeability coefficients of three moderately lipophilic solutes each of which has an oil/water partition coefficient $\geqq 0.0008$. However, at least three lines of argument indicate that these moderately lipophilic solutes cross apical plasma membranes by a pathway in parallel with the water permeation route.

First, a comparison (*cf.* Al-Zahid et al., 1977) of the P_f/P_{D_i} ratios for hydrophilic (Table 1) or moderately lipophilic (Table 9) solutes indicates that, either with or without ADH, apical plasma membranes have water/solute discrimination ratios which are 10- to 1,000-fold greater than in simple lipid bilayer membranes. This occurs because the latter are both less permeable to water, and more permeable to moderately lipophilic solutes, than apical plasma membranes of the collecting tubule. It is difficult to envision ADH-mediated effects on the hydrophobic regions of luminal membranes which permit, simultaneously, higher rates of water diffusion but appreciably lower permeation rates of nonelectrolytes (having β_{oil} values $\geqq 0.0008$) than in synthetic bilayers. Rather, ADH-mediated water and nonelectrolyte diffusion across luminal membranes may involve parallel pathways: narrow channels for water and a hydrophobic region for moderately lipophilic solutes.

Second, support for this view also obtains by comparing in cortical collecting tubules, the E_A values for moderately lipophilic solutes (Table 9) with that for water (Fig. 5, Table 6). In this regard, the activation energy for water or solute permeation in lipid bilayers (termed E_A^w for water or E_A^s for solutes) may be expressed as the sum of two terms: the energy required to break any hydrogen bonds with neighboring water molecules (E_A^{Hw} or E_A^{Hs}); and the energy needed for diffusion in the bilayer (E_A^{Dw} or E_A^{Ds}) (Price & Thompson, 1969; de Gier et al., 1971; Cohen, 1975; Andreoli & Schafer, 1977). Cohen (1975) computed 1.8 kcal mole^{-1} to be the activation energy per hydrogen bond in aqueous solution for both water and solutes, and found that for liposomes of a given composition at the same temperature, E_A^{Dw} and E_A^{Ds} were the same.

In the cortical collecting tubule, the ADH-dependent E_A values for water and moderately lipophilic solute permeation are clearly different, respectively, 9–10 (Fig. 5, Table 6) and 16.6–19.6 kcal mole^{-1} (Table 9). And taking four, three, and two as the number of aqueous hydrogen bonds for water, isobutyramide or *n*-butyramide, and antipyrine, respectively, these E_A^w and E_A^s data yield apparent values of 1.4 and 10.6–15.6 kcal mole^{-1}, respectively, E_A^{Dw} and E_A^{Ds} for apical membranes of cortical collecting tubules. Thus the permeation pathway for water appears to be through small aqueous channels in the apical membranes of these tubules, while moderately lipophilic solutes traverse the hydrophobic regions of apical membranes.

Finally, it should be noted that, in amphibian epithelia, changes in the fluidity of hydrocarbon regions of apical membranes occur (Pietras & Wright, 1974, 1975; Levine et al., 1976). However, these changes seem to be insufficient to account for ADH-dependent changes in water permeability. Thus Masters et al. (1978) measured changes in membrane fluidity in suspensions of single mucosal cells isolated from the toad urinary bladder by polarized fluorescence emission spectroscopy utilizing the hydrophobic fluorescent probe perylene. Their data indicate that while there was approximately a 7% decrease in the microviscosity of the cellular membrane, this would produce only a 6.5% increase in water permeability, well below the change in water permeability which ADH produces on this tissue.

ADH-Induced Neutral Sodium Chloride Transport in the Thick Ascending Limb

In amphibian epithelia, ADH contributes to osmotic homeostasis not only by increasing the water permeability of the apical cell membranes of these epithelia (presumably by a mechanism similar to that in the mammalian collecting duct [*cf. above*; Koefoed-Johnsen & Ussing, 1953; Hays & Franki, 1970; Hays et al., 1971; Hays, 1972, 1976]) but also by increasing net sodium chloride absorption, measured either as isotopic Na^+ transport or, identically, as the zero-voltage short-circuit current (Ussing & Zerhan, 1951; Frazier et al., 1962; Leaf & Hays, 1962). In these anuran epithelia, both basal transepithelial sodium transport and the ADH-induced increase in Na^+ transport appear to depend on an electrogenic transport process, presumably Na^+-selective channels in the apical cell membrane which are blocked by amiloride (Bentley, 1968; Cuthbert & Shum, 1975, 1976; Benos et al., 1976; Sudou & Hoshi, 1977). The ADH-induced increases in sodium transport and water permeability are dissociable, suggesting that these transport pathways are in parallel (Bentley, 1968; Taylor et al., 1973; DeSousa et al., 1974).

In contrast, in cortical collecting tubules, ADH has no effect on transepithelial electrical resistance and produces only minor changes in open-circuit transepithelial voltage or net lumen-to-bath Na^+ transport (Helman et al., 1971; Frindt & Burg, 1972). Thus although we cannot totally discard the possibility that ADH affects sodium transport in the mammalian collecting duct, the effect, if any, appears negligible.

However, the finding of an ADH-induced adenyl cyclase (Imbert et al., 1975*a*, *b*; Morel et al., 1976; Chabardès et al., 1977, 1980; Imbert-Teboul et al., 1978) and protein kinase (Edwards et al., 1980) in the medullary thick ascending limb of Henle in some mammalian species (*cf. above*) suggests that ADH

may have a major effect on NaCl transport in this segment and not the collecting tubule. And the recent demonstration of an ADH-induced increase in transepithelial voltage (Hall, 1979) and net rates of NaCl absorption (Hall & Varney, 1979; Hebert et al., 1980*b*) in the isolated mouse medullary thick ascending limb supports such a contention. This effect is restricted to the medullary segment, and does not occur in cortical thick ascending limbs (Hebert et al., 1980*b*), a result which coincides exactly with the presence of an ADH-induced adenyl cyclase in this segment.

The mechanism of the basal and ADH-induced NaCl transport, and the generation of transepithelial voltages, appear to be quite different from that in amphibian epithelia. Both the basal and the ADH-mediated increases in net salt efflux in the mouse isolated perfused medullary thick ascending limb are unaltered by 10^{-3} M luminal amiloride, but are obliterated by 10^{-4} M luminal furosemide (Hebert et al., 1980*b*), a drug which inhibits electrically neutral or coupled NaCl transport (Eveloff et al., 1978; Frizzell et al., 1979). Moreover, the NaCl absorptive mechanism and the transepithelial voltage are absolutely abolished by substituion for either of these ions (Hebert et al., 1980*b*). And the medullary thick limb is 2–3 times more permeable to Na^+ than to Cl^- (Hebert et al., 1980*b*).

We (Hebert et al., 1980*b*) have suggested that the ADH-induced salt efflux from the medullary segment, as well as basal NaCl absorption rates, can be accounted for by a model in which sodium chloride crosses luminal membranes as a neutral species, results in the development of hypertonic interspaces, and generates a lumen positive voltage, with the latter being due to two extracellular dilution voltages in series, one a Na^+ dilution voltage between interspace and lumen and the other a Cl^- dilution voltage between interspace and peritubular fluid. In this model, cellular chloride entry depends on a secondary active transport phenomenon, where the energy is provided by the transcellular sodium gradient, which in turn is maintained by the ($Na^+ + K^+$)ATPase. Thus our model does not require an active chloride transport mechanism (i.e., electrogenic chloride pump or Cl^--activated ATPase) suggested by others to account for "active" chloride transport across the thick ascending limb (Burg & Green, 1973; Rocha & Kokko, 1973; Burg, 1976; Stoner, 1977), and is similar to the model of NaCl transport suggested for the gall bladder, ileum, colon and shark rectal gland (Nellans et al., 1973, 1974; Frizzell et al., 1975; Schultz, 1977; Silva et al., 1977; Frizzell et al., 1979).

Thus the ADH-induced increase in NaCl absorption in the medullary thick ascending limb is referable to an increase in electroneutral NaCl transport from the lumen through cells into lateral intercellular spaces (i.e. an increase in the NaCl "pump" rate) and not to a change in the passive permeability properties of the epithelium; and the net NaCl absorption rate depends upon both the NaCl pump rate and the rate of NaCl backleak from peritubular (interstitial) media to luminal fluid. In the *in vitro* condition with isotonic peritubular media, the ADH-induced NaCl absorption rate in the medullary thick ascending limb (Hall & Varney, 1979; Hebert et al., 1980*b*) is comparable to that of the NaCl absorptive rates by proximal convoluted or straight tubules in most mammalian species. Yet conventional wisdom argues that, *in vivo*, ADH appears to have little or no direct effect on external NaCl balance (Leaf et al., 1953; Peters & Roch-Ramel, 1970; Fejes-Tóth et al., 1977). How can these two seemingly contradictory findings be rationalized?

The answer may be deduced from two additional *in vivo* findings, namely, that in the presence (antidiuresis) or absence (water diuresis) of ADH urine in the late thick ascending limb is equally dilute, at least for certain species (Clapp & Robinson, 1966; Bennett et al., 1967); and that in going from the water diuretic to the antidiuretic state the NaCl and urea concentrations in the outer medullary interstitium are dramatically increased (Ullrich & Jarausch, 1956; Ruiz-Guiñzaú et al., 1964). Since ADH does not alter the permeability properties of the medullary thick ascending limb (Hebert et al., 1980*b*) the increase in medullary salt concentration during antidiuresis will necessarily increase dramatically NaCl backleak from the interstitium to tubular fluid, particularly since the medullary thick limb is, in electrical terms, remarkably leaky (Burg & Green, 1973; Hebert et al., 1980*a*, *b*).

There may exist an additional mechanism for modulating the effect of ADH on net NaCl absorption by the medullary thick ascending limb. Thus as noted by Dousa (1972), increases in peritubular osmolality inhibit the activation of adenylate cyclase by ADH in slices of bovine renal medulla. In this regard, we (Hebert et al., 1980*a*) have recently demonstrated that increasing peritubular media osmolality with either the nonelectrolytes urea and mannitol or with NaCl results in a reduction in the ADH- and cAMP-stimulated NaCl pump rate. This decrease is noncompetitive since supramaximal concentrations of either ADH or cAMP failed to reverse this peritubular media osmolality-induced reduction in NaCl pump rate. Moreover, the noncompetitive down-regulation is apparently the direct result of increased peritubular media osmolality, rather than changes in epithelial cell volume (Hebert et al., 1980*a*). Therefore, in the *in vivo* condition, the ADH-mediated increase in

NaCl absorption in the medullary thick ascending limb may be offset both by increased NaCl backleak, when peritubular (i.e. interstitial) NaCl concentrations rise, and by a noncompetitive negative feedback reduction in the ADH-stimulated NaCl pump rate produced by increasing peritubular osmolality.

In summary the effects of ADH on nephron function can be integrated into the following scheme:

1) ADH, via cAMP, enhances the water but not the urea permeability of the cortical and outer medullary collecting ducts by increasing the number of small aqueous channels, which exclude urea, in apical plasma membranes of these tubule segments. Thus for any steady-state interstitial concentrations of urea and NaCl, ADH increases the concentrating power of the cortical and outer medullary collecting ducts.

2) In addition ADH, via cAMP, increases the NaCl pump rate by the medullary thick ascending limb resulting in an increase in outer medullary interstitial NaCl concentration, and thereby enhancing the concentrating power of the outer medullary collecting duct.

3) The ADH-mediated increase in the steady-state outer medullary osmolality results in both increased NaCl backleak and a noncompetitive down regulation of the ADH-stimulated NaCl pump rate in the medullary thick ascending limb. This negative feedback system accounts for the maintenance of medullary hypertonicity and the diluting power of the medullary thick ascending limb without affecting external salt balance.

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