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Effect of Antidiuretic Hormone on Water and Solute Permeation, and the Activation Energies for these Processes, in Mammalian Cortical Collecting Tubules

Evidence for Parallel ADH-Sensitive Pathways for Water and Solute Diffusion in Luminal Plasma Membranes

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Summary. The present studies were designed to assess the ways in which antidiuretic hormone (ADH) alters water and solute permeation across isolated, rabbit cortical collecting tubules. In earlier work, it was observed: that ADH produced a tenfold increment in P_f (cm per sec), the osmotic water permeability coefficient, and a fourfold increment in P_{D_w} (cm per sec), the diffusional water permeability coefficient; that small hydrophilic solutes such as urea, thiourea and acetamide (each having oil/water partition coefficients ≤ 0.0008) had vanishingly low permeation coefficients and unity reflection coefficients, even in the presence of ADH; that lumen to bath osmosis involved a transcellular route; and, that the disparity between P_f and P_{D_w}, either with or without ADH, could be rationalized in terms of cellular diffusion constraints, i.e., that water transport across luminal membranes was diffusional.

The present experiments evaluated the effects of ADH on diffusion of moderately lipophilic solutes (e.g., butyramide, isobutyramide, and antipyrine, each solute having an oil/water partition ≥ 0.0008) across luminal membranes of rabbit cortical collecting tubules, and the effects of ADH on the apparent activation energies (E_A, kcal per mole) for water and solute permeation across these tubules. Three major results were obtained: (1) ADH produced a 60–100% increase in the permeation rates for these solutes. (2) The ADH-dependent apparent E_A for water permeation was 9.35 ± 0.92 kcal per mole, and the ADH-dependent apparent E_A for permeation of moderately lipophilic solutes was in the range 15.8–19.6 kcal per mole. (3) The ADH-independent E_A values for these transport processes were statistically indistinguishable from the ADH-dependent E_A values.

When viewed in the context of transport mechanisms for water and solute permeation across synthetic lipid bilayer membrane systems, these results are consistent with the possibility that diffusion of water and moderately lipophilic solutes across mammalian collecting tubules may involve parallel sites in luminal plasma membranes: routes for water diffusion

which are either aqueous and/or disorganized, particularly with respect to synthetic lipid bilayer lamellae; and, discrete hydrophobic regions for diffusion of moderately lipophilic solutes. ADH may act by increasing the number of both types of sites within luminal plasma membranes.

The purpose of this paper is to examine further the ways in which antidiuretic hormone (ADH) increases the rate of water and solute permeation across cortical collecting tubules isolated from the rabbit. Earlier observations from this and other laboratories indicated that, in this nephron segment, ADH increased P_f (cm per sec), the transepithelial osmotic water permeability coefficient, from approximately 20×10^{-4} to 200×10^{-4} cm per sec, and P_{D_w} (cm per sec), the transepithelial permeability coefficient for water diffusion, from 4.5×10^{-4} to 14.4×10^{-4} cm per sec [15, 41, 44]; these hormone-dependent changes in P_f and P_{D_w} were due to increases in the water permeability of luminal membranes [13, 15, 41, 44]. ADH did not increase detectably the diffusional permeation coefficients (P_{D_i}, cm per sec, for the ith solute) for hydrophilic solutes such as urea, thiourea and acetamide [6, 42], and, both in the presence or absence of ADH, the reflection coefficients of luminal surfaces for urea, NaCl and sucrose, i.e., solutes having hydrodynamic radii in the range 1.8–5.2 Å, were unity [42].

The diffusion resistance of the cell layer (R_c, sec per cm) of cortical collecting tubules, exclusive of luminal membranes, was estimated from the permeation coefficients of highly lipophilic species having zero reflection coefficients across luminal membranes, both in the presence and absence of ADH [42]. R_c was 15–25 times greater than predicted for a layer of free solution having the same thickness as collecting tubule epithelium ($6.5–7.0 \times 10^{-4}$ cm), and sufficient to account quantitatively for the P_f/P_{D_w} discrepancy, with or without ADH [44]. Stated in another way, the cell layer in series with luminal membranes operated as an intraepithelial unstirred layer which hindered tracer water exchange diffusion at zero volume flow, but not transepithelial net volume flow in the lumen to bath direction [41, 43, 44].

A quantitative analysis of the osmotic transient phenomenon [43], i.e., the time course of osmotic volume flow across membranes in series with unstirred layers, indicated that, in cortical collecting tubules: cellular constraints to water diffusion at zero net volume flow were referable to geometric factors, specifically a 25-fold reduction in the area available for water transport within the epithelial cell layer; but, these cellular diffusion constraints were not sufficient to produce significant errors in

P_f determinations. The analysis of the osmotic transient phenomenon [43], coupled with an evaluation of the effects of luminal hypertonicity on water and solute permeation through junctional complexes [44], provided evidence in support of the view, expressed previously by Grantham *et al.* [16], that a major fraction of lumen to bath osmotic volume flow in these tubules, with or without ADH, involved a transcellular rather than a paracellular route. Based on these considerations, we have proposed that, in mammalian cortical collecting tubules, the mode of water transport across luminal plasma membranes is diffusional [3, 41, 42, 44].

Similar considerations may apply to the action of ADH on water transport across apical membranes of anuran epithelia. The ADH-dependent value of P_f in toad urinary bladder is approximately $200\text{–}240 \times 10^{-4}$ cm per sec [21, 23], and Hays and Franki [21] have found that the ADH-dependent value of P_{D_w} for the epithelial cell layer of that tissue, exclusive of supporting stroma, is 11×10^{-4} cm per sec. Clearly, the latter data are in close accord with the ADH-dependent values of P_f and P_{D_w} in rabbit cortical collecting tubules. Moreover, Parisi and Piccinni [36] have provided direct evidence for significant hindrances to water diffusion within the epithelial cell layer of toad urinary bladder. Thus it could be argued that, in anuran epithelia as well as in mammalian cortical collecting tubules, ADH-mediated water transport in luminal (or apical) plasma membranes is diffusional [21].

ADH also increases the rate of solubility-diffusion for other molecular species in apical plasma membranes. Recently, Pietras and Wright [34, 35] and Levine *et al.* [27] observed that, in toad urinary bladder, ADH produced small increments in the diffusional permeability coefficients for moderately lipophilic species. Based on these observations, both groups of workers concluded that ADH altered the "fluidity" of apical plasma membranes, and proposed that such a fluidity change accompanied hormone-mediated increments in the permeation rates for water and moderately lipophilic solutes [27, 34, 35].

The present experiments were designed to evaluate the effects of ADH on the diffusion of moderately lipophilic species across isolated rabbit cortical collecting tubules, with particular regard to the relationship between these events and the effect of the hormone on water transport. The experimental data indicate clearly, in accord with earlier results in toad urinary bladder [27, 34, 35] and with our preliminary observations in cortical collecting tubules [1], that ADH produced slight increases in P_{D_i} for moderately lipophilic solutes, that the apparent activation energies

(E_A, kcal per mole) for diffusion of these solutes were appreciably greater than for P_f, and, that ADH had no effect on the magnitude of E_A for either P_{D_i} or P_f.

When viewed in the context of transport mechanisms for water and solute permeation across synthetic lipid bilayer membrane systems, these results are consistent with the possibility that diffusion of water and moderately lipophilic solutes across mammalian collecting tubules may involve parallel sites in luminal plasma membranes: routes for water diffusion which are either aqueous and/or disorganized, particularly with respect to synthetic lipid bilayer lamellae; and, discrete hydrophobic regions for diffusion of moderately lipophilic solutes. ADH may act by increasing the number of both types of sites within luminal plasma membranes.

Materials and Methods

The methods used in this laboratory for microperfusing cortical collecting tubule segments isolated from rabbit kidney have been described in detail previously [41–44], and are quite similar to those set forth originally by Burg *et al.* [5, 15]. Stated briefly, female New Zealand white rabbits, 1.5–3.0 kg in weight, were sacrificed by rapid decapitation with no prior treatment; the time of decapitation was used as the zero time reference for all experiments. Segments of cortical collecting tubules, 2.5 to 4.2 mm in length, were obtained by freehand dissection from kidney slices immersed in cold (0–5 °C) Krebs-Ringer bicarbonate buffer (KRB) containing (in mM): 115 NaCl, 25 $NaHCO_3$, 10 Na acetate, 1.2 NaH_2PO_4/Na_2HPO_4, 5 KCl, 1.0 $CaCl_2$, 1.2 $MgSO_4$, 5.5 glucose and 5.5% calf serum (Microbiological Associates, Bethesda). The KRB buffer was equilibrated with 95% O_2–5% CO_2, and adjusted to pH 7.4, 290 mOsm per liter.

The single tubule segments were transferred to a temperature-regulated (±0.5 °C) chamber, volume approximately 1.2 cm^3, on the stage of an inverted microscope [41, 43]. The tubules were mounted between two sets of concentric pipets as described previously [41–44], and perfused at rates of 8–14 nl per min with Krebs-Ringer phosphate solutions (KRP) adjusted to pH 7.4. In zero volume flow experiments, the KRP perfusates contained 150 mM NaCl, 2.5 mM KH_2PO_4/K_2HPO_4, 1.0 mM $CaCl_2$, and 1.2 mM $MgSO_4$, adjusted to 290 mOsm per liter; in experiments involving lumen to bath osmotic volume flow, the KRP perfusates contained 60 mM NaCl, 2.5 mM KH_2PO_4/K_2HPO_4, 1.0 mM $CaCl_2$ and 1.2 mM $MgSO_4$, adjusted to 125 mOsm per liter.

As in previous experiments [41–44], the volume marker added to the perfusing solutions was ^{3}H-methoxy-inulin (New England Nuclear Corp., Boston, Massachusetts). The ^{3}H-methoxy-inulin supplied by the manufacturer was dialyzed for at least 36 hr against distilled water [45], using Spectrapor membrane tubing with a molecular weight cutoff of approximately 3500 daltons (Spectrum Medical Industries, Los Angeles, California). An appropriate volume of dialyzed ^{3}H-methoxy-inulin was evaporated to dryness under a stream of N_2, and dissolved in KRP perfusate to give a final isotope concentration of 20–40 cpm/nl.

The bathing solutions were identical to the KRB buffer described above. ADH, when present, was added to bathing solutions at a final concentration of 250 μU per ml. We used either aqueous pitressin (Parke Davis and Co., Detroit, Michigan) or synthetic arginine

vasopressin (Sigma Chemical Co., St. Louis, Missouri, lot number 45C-0275); no difference in response between these two agents was noted. Our earlier observations [41, 44] at 23–25 °C indicated that this concentration of hormone was five to ten times greater than that required to provide a maximum increase in P_f and P_{D_w}. In the present experiments, preliminary trials, using either pitressin or synthetic arginine vasopressin, indicated that approximately 50 μU per ml ADH in the bath produced maximum increments in either P_f or P_{D_i}, both at 37 °C and 21 °C; and 500 μU ADH per ml in the bath, using either form of the hormone, had no greater effect on P_f and/or P_{D_i}. In order to minimize evaporative losses, all bathing solutions were uniformly replaced at 10 min intervals, in 37 °C experiments, and at 20 min intervals, in 23–25 °C experiments.

Collections of perfused fluid were made at 8–10 min intervals using constant bore glass capillaries calibrated with perfusing solution before each experiment (volume calibration range: 1.15 to 1.32 nl per mm). Collection rates, perfusion rates, and net volume absorption rates (J_v, nl per min per mm of tubule length) were calculated using methods described previously [41, 43]. As in prior experiments [41], the leakage rate of volume marker into the bath was less than 1 % of the perfusion rate.

Solute permeability coefficients (P_{D_i} cm per sec, for the ith solute) were measured as described previously [41, 42, 44] from the rate of disappearance of the ^{14}C-labeled species from the luminal perfusing solution. At zero volume flow, P_{D_i} was computed from the expression [41]:

$$P_{D_i} = \frac{\dot{V}^{in}}{A} \ln \frac{C_i^{in}}{C_i^{out}}, \tag{1}$$

where $\dot{V}^{in}$ = perfusion rate (cm^3 per sec), A = inner surface area (cm^2) and C_i^{in} and C_i^{out} are the tracer concentrations (cpm per cm^3) of the ith species in, respectively the perfusate and collected fluid. In net volume flow experiments, P_{D_i} was computed from the Eq. (2):

$$P_{D_i} = \frac{\dot{V}^{in} - \dot{V}^{out}}{A} \left[\frac{\ln (C_i^{in}/C_i^{out})}{\ln (\dot{V}^{in}/\dot{V}^{out})} + 1 \right], \tag{2}$$

where $\dot{V}^{out}$ is the collection rate (cm^3 per sec). A derivation of these equations has been presented previously [41].

1-^{14}C-*n*-butyramide (1.07 mC/mmole; lot number 771) and 1-^{14}C-isobutyramide (1.58 mC/mmole; lot number 1172) were obtained from American Radiochemical Corp., Sanford, Florida; and, ^{14}C-*n*-methyl antipyrine (11.1 mC/mmole; lot number 648269B) was obtained from ICN Co., Irvine, California. The labeled compounds were added to the perfusing solutions at a tracer concentration of 20 μC per ml, which gave final concentrations of 18.7 mM, 12.6 mM and 1.8 mM for, respectively, *n*-butyramide, isobutyramide and antipyrine. The osmolalities of the KRP perfusing solutions containing these solutes were then re-adjusted to 290 mOsm per liter.

In our previous experiments [41–44], the transepithelial osmotic water permeability coefficient P_f (cm per sec) was computed from lumen to bath osmotic volume flows according to the expressions:

$$J_v = -L_p \Delta\pi \tag{3}$$

and

$$P_f = L_p \frac{RT}{\bar{V}_w}, \tag{4}$$

where J_v (cm^3 per sec per cm^2) is the net volume flow (a positive sign denoting volume efflux from the lumen), L_p (cm per sec per atm) is the coefficient of hydraulic conductivity, and R, T, and $\bar{V}_w$ have their usual meaning; the osmotic pressure difference $\Delta\pi$ between bathing and perfusing solutions was computed from the difference between the osmolality of the bathing solution and the antilog of the logarithmic mean of the osmolalities of the initial perfusing

solution and the collected fluid [41]. In certain of the present experiments, specifically, lumen to bath osmotic flows at 37 °C in the presence of ADH, the rates of fluid absorption were sufficiently high that the difference between the osmolalities of the perfusate and collected fluid was 60% or more of the difference between the osmolalities of the perfusate and the bathing solution. Under these conditions, approximating the mean luminal osmolality as the antilog of the logarithmic mean of the osmolalities of the initial perfusing solution and the collected fluid would have resulted in a $\geqq 15\%$ error in estimating P_f. Therefore, it was necessary to develop a more rigorous set of equations for computing $\bar{C}$, the average luminal fluid osmolality.

Consider a cortical collecting tubule segment having an overall length L(cm). We take x as distance along the tubule between $x=0$, where perfusion is initiated, and $x=L$, where perfused fluid is collected. At $x=0$, the tubule is perfused at a rate of $\dot{V}_o$ (cm^3 per sec) with a solution having an initial osmolality C_o (mOsm per cm^3); at $x=L$, fluid having an osmolality C_L is collected at a rate of $\dot{V}_L$. In these tubules, the permeability coefficients for solutes such as urea, thiourea, and acetamide are vanishingly small with respect to either P_f or P_{D_w}, both in the presence or absence of ADH [6, 42, 44], and the reflection coefficients for urea, sucrose, and NaCl, with and without ADH, are unity [42]. Accordingly, it is reasonable to assume that the net loss or gain of solute from the lumen of these tubule segments during osmosis is negligibly small with respect to water loss or gain. Thus, conservation of mass may be expressed as:

$$\dot{V}_o C_o = \dot{V}_L C_L = \dot{V}_x C_x = K \tag{5}$$

where $\dot{V}_x$ and C_x are, respectively, the flow rate and luminal fluid osmolality at x, and K is a constant (mOsm per sec). If the rate of change of volume flow occurs almost exclusively by transepithelial gain or loss of water, we have:

$$\frac{d\dot{V}_x}{dx} = 2\pi r P_f \bar{V}_w [C_x - C_b], \tag{6}$$

where r is the internal radius of the tubule segment (cm), and C_b is the osmolality of the bathing solution. Since the bath volume, approximately 1.2 cm^3, was $\simeq 10^5$ times greater than the luminal volume, we take C_b to be constant.

Eqs. (3), (4), and (6) indicate implicitly that the reflection coefficient of the solute driving osmotic flow is unity; in the present experiments, NaCl, whose reflection coefficient with or without ADH is unity in these tubules [42], was used to produce lumen to bath osmotic flow. Eqs. (3) and (6) also neglect a hydrostatic pressure term driving net volume absorption because in the present experiments, transepithelial hydrostatic pressure differences, if any, were negligible with respect to transepithelial osmotic pressure gradients [41].

Rearranging Eq. (5) and differentiating gives:

$$\frac{d\dot{V}_x}{dx} = -\frac{K}{C_x^2}\frac{dC_x}{dx}. \tag{7}$$

By substituting Eq. (7) in Eq. (6) and rearranging, we have:

$$\frac{dC_x}{C_x^2[C_x - C_b]} = -\frac{2\pi r \bar{V}_w P_f}{K} dx. \tag{8}$$

Integration of Eq. (8) between the limits $x=0$ to $x=L$ and rearranging gives the following expression for P_f:

$$P_f = -\frac{\dot{V}_o C_o}{A\bar{V}_w}\left[\frac{C_o - C_L}{C_o C_b C_L} + \frac{1}{(C_b)^2}\ln\frac{(C_L - C_b)C_o}{(C_o - C_b)C_L}\right]. \tag{9}$$

The integrated average luminal osmolality, $\bar{C}$, may be computed from P_f by the relation:

$$\bar{C} = \frac{\dot{V}_L - \dot{V}_o}{AP_f \bar{V}_w} + C_b. \tag{10}$$

Other reagents, isotope counting techniques, and chemical determinations were as described previously [41–44]. Measurements in a given tubule were used to compute a mean value for that tubule; generally, there were 3–4 measurements per tubule for a given set of experimental conditions (e.g., Figs. 3, 5 and 6). The mean values for individual tubules were then used to calculate a mean value ± standard error of the mean (SEM) for the indicated number of tubules. The experimental results were expressed in this manner. When control and experimental observations were made within the same tubule, P values for mean paired differences were computed from the Student t-test by comparing the differences to zero. The P values for differences between means of unpaired observations were computed from the standard Student t-test.

Results

Constraints on Experimental Design

The present experiments were designed to evaluate the effects of ADH on the permeability coefficients for diffusion of moderately lipophilic solutes, and on the apparent activation energies for water and solute diffusion, across luminal membranes of cortical collecting tubules isolated from rabbit kidney. It is relevant in this connection to consider in some detail three sets of constraints on these measurements in isolated, microperfused cortical collecting tubules.

First, it is necessary to distinguish between transport processes occurring in the presence (termed "ADH-dependent") and absence (termed "ADH-independent") of ADH. Fig. 1, which summarizes results from earlier studies [41, 43, 44], shows that P_f, measured from lumen to bath osmotic flows at 25 °C, declined gradually, reaching a value of 20.0 ± 4.0 (SEM) $\times 10^{-4}$ cm per sec at 180 min following decapitation. Although the data are not shown in Fig. 1, we observed (5 tubules), in accord with earlier results [15, 41], that the P_f minima reached at 180 min (e.g., Fig. 1) remained stable for an additional 180 min when ADH was not added to the bathing media.

Fig. 1 also shows that, when 250 μU per ml ADH was added to the bath, P_f rose to $196 \pm 8.0 \times 10^{-4}$ cm per sec; as has been shown previously, this ADH-dependent P_f increment in cortical collecting tubules remains stable for approximately 240 min [6, 15, 41, 42, 44]. Likewise, prior studies from this and other laboratories have shown that the change in P_{D_w}, including both a decline in P_{D_w} for 150–180 min following decapitation,

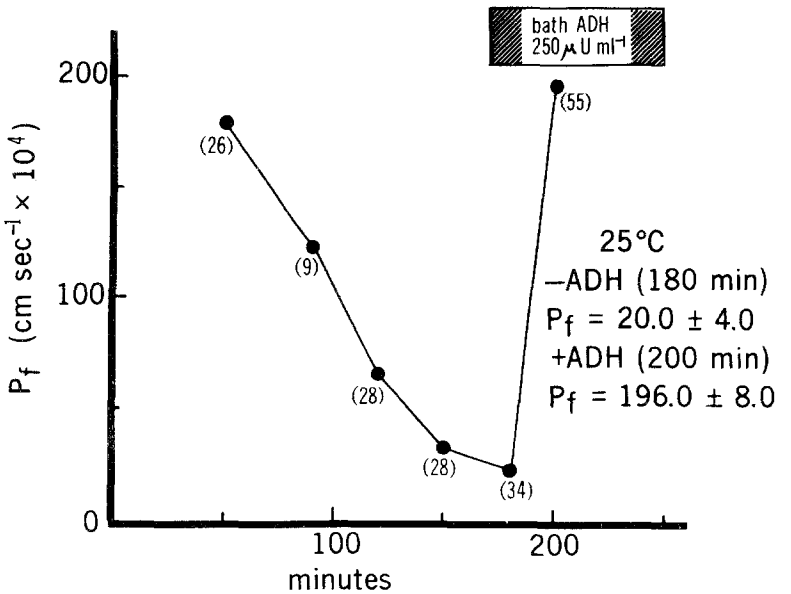

Fig. 1. The effect of ADH on P_f at 25 °C. The figure shows the mean P_f values for the number of tubules listed in parentheses. The values at 180 min (−ADH) and 200 min (+ADH) are the mean values ±SEM. Adapted from references [41, 43, 44]

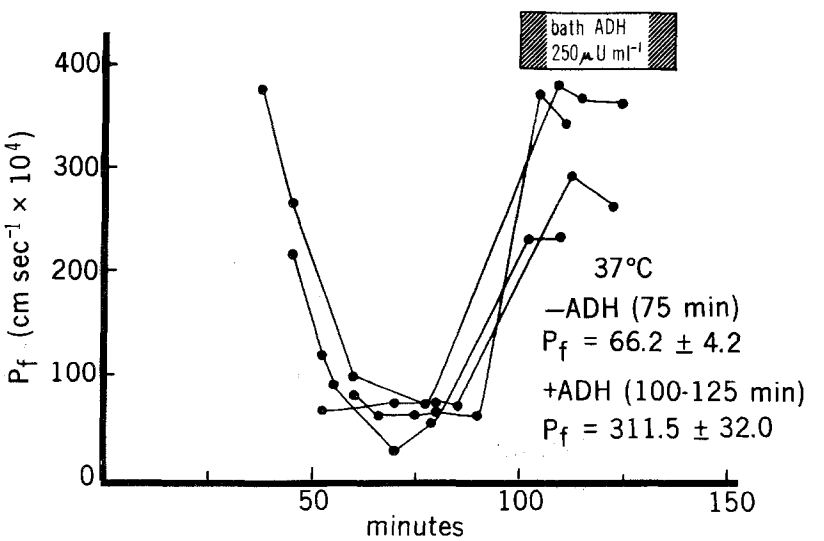

Fig. 2. The effect of ADH on P_f at 37 °C. Each line connects P_f values at varying times in a given tubule. The values at 75 min (−ADH) and 100–125 min (+ADH) are mean values ±SEM for the four tubules shown in the figures

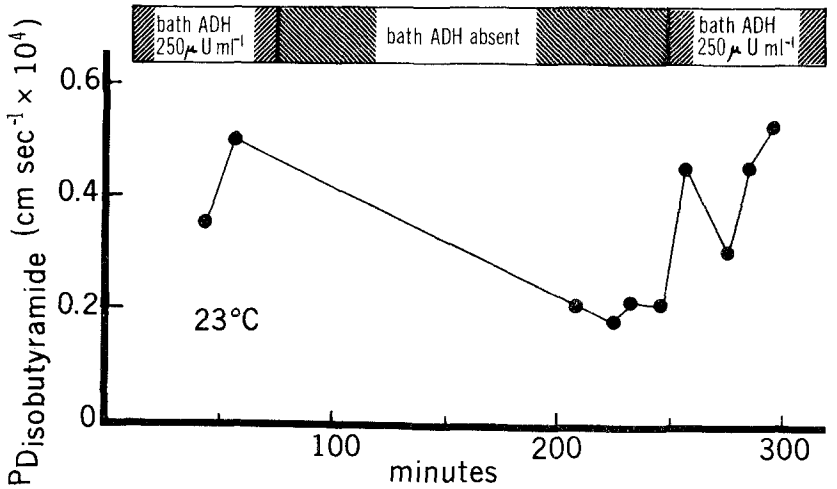

Fig. 3. A representative experiment illustrating the effect of ADH on $P_{D_{isobutyramide}}$ at 23 °C and zero volume flow. Details of the protocol are given in the text

when ADH is omitted from the bathing media, and the rise in P_{D_w} upon subsequent addition of ADH to the bathing media, follows the same temporal sequence as P_f [6, 15, 41, 42, 44].

A comparison of Figs. 1 and 2 indicates that the rate of decline of P_f was greater at 37° than at 25 °C: at 25 °C, the minimum value of P_f occurred 160–180 min after decapitation, while at 37 °C, the minimum P_f values occurred 60–75 min after decapitation. As at 25 °C, the addition of 250 μU per ml ADH to the bath at 37 °C produced a prompt and stable rise in P_f (Fig. 2). Parenthetically, it should be noted that the minimum values of P_f before ADH addition, and the maximum values of P_f after ADH addition, were greater at 37 °C (Fig. 2) than at 25 °C (Fig. 1); this effect has been evaluated more rigorously by paired observations on the same tubules (Figs. 5 and 6; Tables 2 and 3).

To our knowledge, the reasons for the temporal decline in P_f are uncertain. But based on the results in Figs. 1 and 2, as well as prior, comparable observations on P_{D_w} [15, 41], we assumed that P_f and P_{D_i} measurements made either at 160–180 min after decapitation, in the case of 23–25 °C experiments, or at 60–75 min after decapitation, in the case of 37 °C experiments, reflected ADH-independent transport processes. In experiments at 23 °C where ADH was added to the initial bathing media, (e.g., Fig. 3), ADH-independent values of P_{D_i} were taken to be those P_{D_i} values, beginning 160 min after removing ADH from the bath, which remained stable until ADH was again added to the bath.

Second, in the evaluation of activation energies for P_f and P_{D_i}, we made measurements only at two temperatures, rather than over a wide range of temperatures. Two factors dictated this choice of conditions: (a) We wished to evaluate apparent activation energies for P_f and P_{D_i} within the same temperature range. But below 23–25 °C, ADH-independent values of P_f ([15, 41, 44]; Fig. 1) are difficult to distinguish reproducibly from zero, given the current methodology for microperfusing isolated mammalian renal tubules; and, at temperatures greater than 37 °C, preliminary trials indicated that cortical collecting tubules were relatively unstable and tended to deteriorate and/or desquamate rapidly. (b) In the temperature range 23–37 °C, the changes in P_f, particularly without ADH, were sufficiently small that temperature variations of smaller magnitude would not have permitted reproducible detection of significant differences in P_f, even within the same tubule. Because the effects of temperature on P_f and P_{D_i} were evaluated at only two temperatures, we consider the E_A values reported in this paper as apparent activation energies. Thus, the present results provide no information about possible phase transitions within luminal membranes at temperatures below 21 °C, although it should be noted that such phase transitions, at least for temperatures $\leqq 21$ °C, have little bearing on water and solute transport processes in the *in vivo* mammalian nephron.

Finally, the choice of solutes for P_{D_i} measurements was limited. On the one hand, it is difficult with present techniques for microperfusing single renal tubules to measure reproducibly small differences, e.g., from 0.03×10^{-4} to 0.05×10^{-4} cm per sec, among P_{D_i} values in the range $0.02\text{–}0.06 \times 10^{-4}$ cm per sec. Alternatively, in the case of highly lipophilic solutes, our earlier observations indicated that the cell layer, exclusive of luminal membranes, was the major resistance to tracer diffusion [41]. Moreover, it seems probable that differences between the ADH-dependent values of P_f and P_{D_w} (at 25 °C: $P_f = 196 \times 10^{-4}$ cm per sec [41, 42], Fig. 1; $P_{D_w} = 14.4 \times 10^{-4}$ cm per sec [15, 41]) are referable to cellular constraints to diffusion [3, 41, 43, 44]. Thus R_c (sec per cm), the diffusion resistance of the epithelial cell layer, exclusive of luminal membranes, may be expressed as:

$$R_c = \frac{1}{P_{D_w}} - \frac{1}{P_f}. \tag{11}$$

For the ADH-dependent values of P_{D_w} and P_f cited above, R_c from Eq. 11 is approximately 663 sec per cm, and $1/R_c = 15.0 \times 10^{-4}$ cm per sec. In other words, for solutes having P_{D_i} values in excess of $1.5\text{–}2.0 \times 10^{-4}$ cm per sec, R_c contributes an increasingly greater fraction to the total resistance

(i.e., $1/P_{D_i}$) to solute diffusion. Accordingly, in the present experiments, we evaluated solutes having P_{D_i} values in the range of $0.1\text{–}1.0 \times 10^{-4}$ cm per sec, i.e., P_{D_i} values which could be determined – and compared reproducibly and reliably – for solutes where luminal membranes, rather than the epithelial cell layer, might reasonably be construed as constituting the major rate-limiting permeation site.

The Effect of ADH on P_{D_i} for Moderately Lipophilic Species

A representative experiment illustrating the effect of ADH on $P_{D_{\text{isobutyramide}}}$ at zero net volume flow is illustrated in Fig. 3. The perfusing and bathing media were, respectively, 290 mOsm per liter KRP plus ^{14}C-isobutyramide and 290 mOsm per liter KRB; the system was maintained at 23 ± 0.5 °C and J_v was indistinguishable from zero. $P_{D_{\text{isobutyramide}}}$ was measured from the unidirectional flux of ^{14}C-isobutyramide from lumen to bath: first, in the presence of ADH; next, beginning approximately 160 min after removing ADH from the bathing solution; and finally, when ADH was again added to the bathing media. Hormone was added to the bath in the first period to exclude the possibility that the relatively high P_{D_i} values during that period were referable to transport processes other than those mediated by ADH; and, ADH was added to the bath in the final period to exclude the possibility that the lower P_{D_i} values at 200–250 min were due to tubular deterioration, rather than to ADH-independent transport processes. The individual $P_{D_{\text{isobutyramide}}}$ values during each of the three periods were used to calculate the mean tubular $P_{D_{\text{isobutyramide}}}$ value for that period. In the experiment shown in Fig. 3, the mean $P_{D_{\text{isobutyramide}}}$ values were: first ADH-dependent period, 0.43×10^{-4} cm per sec; ADH-independent period, 0.20×10^{-4} cm per sec; final ADH-dependent period, 0.44×10^{-4} cm per sec (the mean $\pm$SEM for the individual ADH-independent P_f values, shown in Fig. 3 differed significantly [$P < 0.001$] from the mean $\pm$SEM of all of individual ADH-dependent values shown in Fig. 3). Thus, ADH resulted in an approximate doubling of the permeation rate for that solute.[1]

1 In 4 tubules, the permeability coefficient for isobutyramide at zero volume flow and 23 °C was also measured in experiments of 250–275 min duration in the absence of ADH. The initial values of P_{D_i} (at 60–75 min) were indistinguishable from the ADH-dependent values listed in Table 1; the values at 150–180 min were indistinguishable from the ADH-independent values listed in Table 1; and, the P_{D_i} values at 150–180 min were stable for an additional 100–125 min. Thus, the decline in isobutyramide permeation rates followed the same temporal sequence as the decline in P_f and P_{D_w} when ADH was absent from the bathing media (*cf.* above).

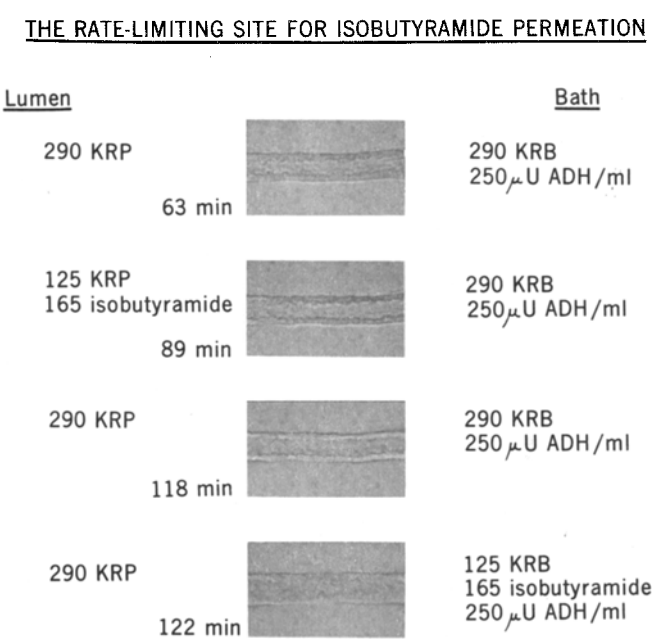

Fig. 4. The rate-limiting site for isobutyramide permeation

In order to evaluate the rate-limiting site for isobutyramide permeation, we assessed the changes in tubular morphology after partial replacement of NaCl in either the luminal or bathing solutions with isobutyramide. The rationale for the technique, utilized previously by us to evaluate the rate-limiting site for urea permeation [42], depends on the consideration that, when isobutyramide replaces NaCl in one of the external media, cellular swelling will occur when the plasma membrane (either apical or baso-lateral) in contact with the isobutyramide-NaCl solution is substantially more permeable to isobutyramide than NaCl. Three such experiments were carried out, one of which is illustrated in Fig. 4. The tubules were initially perfused and bathed with, respectively, 290 mOsm per liter KRP and 290 mOsm per liter KRB; next, the luminal solutions were changed, first to 125 mOsm per liter KRP plus 165 mOsm per liter isobutyramide (89 min), and then to 290 mOsm per liter KRP (118 min); and finally, the bath was changed to 125 mOsm per liter KRB plus 165 mOsm per liter isobutyramide (122 min). ADH (250 μU per ml) was uniformly present in the bathing solution.

Fig. 4 indicates clearly that visible cell swelling occurred when isobutyramide replaced NaCl in the bathing solution (122 min), but not when the nonelectrolyte replaced NaCl in the luminal solution (89 min). Entirely comparable results were obtained in two other experiments

Table 1. The effect of ADH on the diffusional permeability coefficients for moderately lipophilic solutes

Solute	P_{D_i} (cm $sec^{-1} \times 10^4$)			Tubules (number)
	+ADH	−ADH	+ADH	
Isobutyramide	0.23 ± 0.03	0.12 ± 0.02	0.26 ± 0.05	6
Mean paired difference	0.11 ± 0.04 ($P < 0.05$)		0.14 ± 0.04 ($P < 0.01$)	
n-butyramide	0.32 ± 0.04	0.14 ± 0.04	0.28 ± 0.02	6
Mean paired difference	0.18 ± 0.02 ($P < 0.001$)		0.14 ± 0.01 ($P < 0.01$)	
Antipyrine	0.40 ± 0.06	0.25 ± 0.07	0.33 ± 0.08	5
Mean paired difference	0.16 ± 0.03 ($P < 0.01$)		0.08 ± 0.01 ($P < 0.01$)	

The ADH-independent values of P_{D_i} were bracketed by ADH-dependent P_{D_i} measurements following the protocol illustrated in Fig. 3. All experiments were carried out at 23 °C. J_v was indistinguishable from zero in all cases. ADH, when present, was added to the bath at a final concentration of 250 μU per ml. Mean values ±SEM for each of the three periods, and the mean paired differences between these values, were computed as described in *Materials and Methods.*

identical to that shown in Fig. 4. Thus we conclude that the luminal membranes of those tubules were the rate-limiting site for isobutyramide permeation. Likewise, luminal membranes are the rate-limiting site for water, urea, NaCl and sucrose permeation in cortical collecting tubules [6,15, 16, 41, 43], and apical membranes for water and solute permeation in toad urinary bladder [31] and frog skin [30].

Table 1 illustrates, following the protocol shown in Fig. 3, the effects of ADH on P_{D_i} at zero volume flow for three moderately lipophilic species: isobutyramide, *n*-butyramide, and antipyrine. It is evident that ADH produced small (with respect to P_f; Figs. 1, 2; [15, 41–44]) but significant increases in P_{D_i} for each of these solutes. For isobutyramide and *n*-butyramide, P_{D_i} approximately doubled when ADH was added; in the case of antipyrine, the hormone increased P_{D_i} by approximately 60 %[2]. The magni-

2 Collander-Bärlund plots [9, 10] of P_{D_i} in terms of β_{oil}, the oil-water partition coefficient, showed that both with and without hormone, P_{D_i} values were closely correlated ($r \geqq 0.97$) with β_{oil}. Thus, solute permeation in these tubules follows Overton's rule. Alternatively, the P_f values, both with and without ADH, were approximately 10^3-fold greater than those predicted in terms of the relationship between solute permeation and β_{oil}: when the P_f and P_{D_i} values were evaluated together, nonlinear Marquart fitting of the data in terms of varying exponential powers of β_{oil} (or, for that matter, of varying exponential powers of both β_{oil} and molecular weight) yielded no significant correlation between P_f, P_{D_i} and β_{oil}.

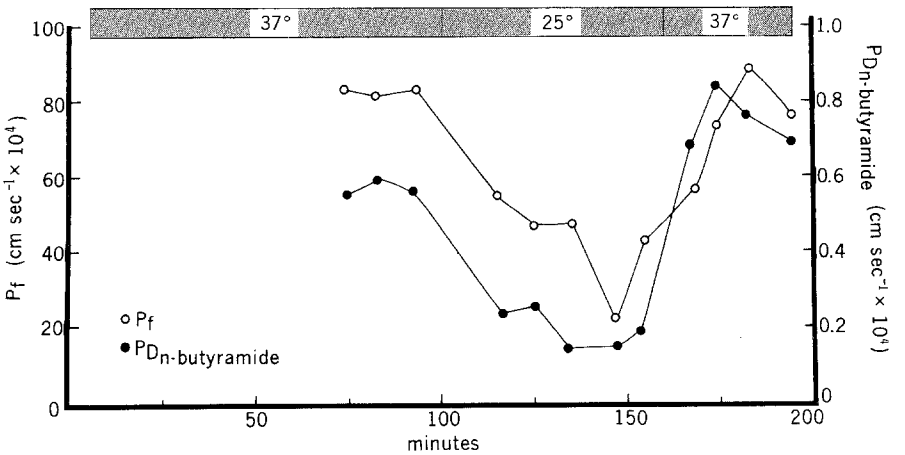

Fig. 5. Experimental protocol for the simultaneous determination of the apparent activation energies for ADH-independent water and *n*-butyramide permeation

tude of the percentage increments (i.e., 60–100%) in P_{D_i} produced by ADH for these three solutes in cortical collecting tubules (Table 1) are in close accord with the magnitudes of the ADH-dependent percentage increments in P_{D_i} observed by Pietras and Wright [34, 35] for these solutes in toad urinary bladder. But the absolute magnitudes of P_{D_i} for these solutes in cortical collecting tubules, both with and without ADH (Table 1), were approximately 10-fold greater than the comparable P_{D_i} values observed in toad urinary bladder by Pietras and Wright [35], even after these workers made corrections for an estimated unstirred layer thickness of 200×10^{-4} cm.

Apparent Activation Energies for Water and Solute Permeation

Figs. 5 and 6 illustrate representative experiments showing the protocols for measuring, respectively, ADH-independent and ADH-dependent activation energies for water and *n*-butyramide permeation simultaneously in the same tubule. In Fig. 5, the tubule was perfused and bathed with, respectively, 125 mOsm per liter KRP plus *n*-butyramide and 290 mOsm per liter KRB. ADH-independent measurements of P_f and $P_{D_{n\text{-butyramide}}}$ at 37 °C were begun approximately 75 min (*cf.* Fig. 2) after decapitation; and, 25 °C measurements were bracketed by 37 °C measurements. The mean values for P_f and $P_{D_{n\text{-butyramide}}}$ for the tubule at 37 °C and 25 °C were computed from the individual flux values during, respectively,

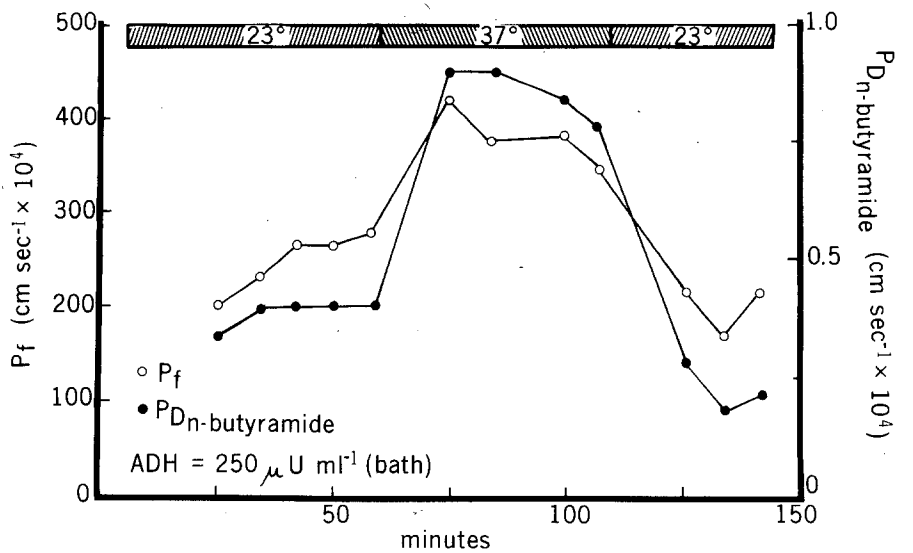

Fig. 6. Experimental protocol for the simultaneous determination of the apparent activation energies for ADH-dependent water and *n*-butyramide permeation

the first plus third periods and the second period. The experiment shown in Fig. 6 was identical except that: measurements at 37 °C were bracketed by 23 °C measurements; and, ADH (250 μU per ml) was uniformly present in the bathing solutions. The apparent activation energies for water and solute permeation were computed from the experimental data according to the Arrhenius relationship:

$$\ln \frac{P_1}{P_2} = -\frac{E_A}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right], \tag{12}$$

where P_1 and P_2 is either P_f or P_{D_i} measured at, respectively, T_1 and T_2, and E_A is the apparent activation energy.

Clearly, usage of the simple Arrhenius relationship in this manner assumes that $\ln P$ varies linearly with $1/T$. This issue was not tested rigorously in the present experiments, since, for the reasons indicated previously, permeation coefficients were measured only at two temperatures. However, a similar assumption has been made by Hays *et al.* [22] in evaluating the effect of ADH on the E_A for water diffusion in toad urinary bladder; and, both in frog skin [18] and in rabbit gall bladder [51], water permeation appears to follow a linear Arrhenius relationship for the temperature range 21–39 °C.

Given this caveat, Tables 2 and 3 summarize the results for simultaneous measurements of the apparent activation energies for water and *n*-butyr-

Table 2. The apparent activation energy for ADH-independent water and *n*-butyramide permeation in paired cortical collecting tubules

	P_f (cm sec^{-1} × 10^4)		Apparent E_A (kcal mole^{-1})
	25 °C	37 °C	
	31.30 ± 8.70	56.12 ± 13.90	9.35 ± 0.92
Mean paired difference	24.3 ± 5.40 ($P < 0.01$) (n = 5)		
	$P_{D_{n\text{-butyramide}}}$ (cm sec^{-1} × 10^4)		
	25 °C	37 °C	
	0.18 ± 0.03	0.68 ± 0.07	19.40 ± 1.60
Mean paired difference	0.50 ± 0.07 ($P < 0.002$) [n = 5]		

The ADH-independent values of P_f and $P_{D_{n\text{-butyramide}}}$ at 25° and 37 °C were measured simultaneously in five separate tubules following the protocol illustrated in Fig. 5. The apparent activation energies (mean ± SEM for all tubules) were computed from the Arrhenius equation as described in the text.

amide permeation. In the absence of ADH (Table 2), E_A was 9.35 ± 0.92 and 19.40 ± 1.6 kcal per mole for, respectively, P_f and *n*-butyramide permeation; the mean paired difference between these two values was 7.7 ± 0.74 ($P < 0.002$). In the presence of ADH (Table 3), E_A was 8.9 ± 1.5 and 16.6 ± 1.6 kcal per mole for, respectively, P_f and *n*-butyramide permeation; the difference between these two values was 10.05 ± 2.14 ($P < 0.002$). Thus, it is clear that, both in the presence and absence of hormone, the apparent activation energy for water permeation was approximately half that for *n*-butyramide transport.

Table 4 compares the apparent activation energies for water and *n*-butyramide permeation in the presence and absence of ADH. It is evident from Table 4 that the E_A values for P_f were virtually the same with and without hormone. In the case of *n*-butyramide permeation, the ADH-independent values for E_A were slightly but not significantly greater ($P > 0.1$) than those in the presence of ADH.

Finally, we evaluated the ADH-dependent activation energies for permeation of these lipophilic solutes at zero volume flow: the experimental conditions were identical to those illustrated in Fig. 5, except that the perfusing solutions contained 290 mOsm per liter KRP plus test solute (either *n*-butyramide, isobutyramide, or antipyrine), and J_v was uniformly

Table 3. The apparent activation energy for ADH-dependent water and *n*-butyramide permeation in paired cortical collecting tubules

	P_f (cm sec^{-1} × 10^4)		Apparent E_A (kcal mole^{-1})
	23° C	37° C	
	227.4 ± 30.6	445.7 ± 19.3	8.9 ± 1.5
Mean paired difference	218.3 ± 28.5 ($P < 0.01$) (n = 4)		
	$P_{D_{n\text{-butyramide}}}$ (cm sec^{-1} × 10^4)		
	23° C	37° C	
	0.36 ± 0.05	1.19 ± 0.11	16.6 ± 1.6
Mean paired difference	0.83 ± 0.07 ($P < 0.01$) [n = 4]		

The ADH-dependent values of P_f and $P_{D_{n\text{-butyramide}}}$ at 23 °C and 37 °C were measured simultaneously in four separate tubules following the protocol illustrated in Fig. 6. The apparent activation energies (mean ± SEM for all tubules) were computed from the Arrhenius equation as described in the text.

Table 4. Comparison of the ADH-dependent and ADH-independent appararent activation energies for water and *n*-butyramide permeation

Coefficient	Apparent E_A (kcal mole^{-1})		P
	−ADH	+ADH	
P_f	9.35 ± 0.92 (n = 5)	8.9 ± 1.5 (n = 4)	> 0.5
$P_{D_{n\text{-butyramide}}}$	19.40 ± 1.6 (n = 5)	16.6 ± 1.6 (n = 4)	> 0.1

The ADH-independent and ADH-dependent values of the apparent activation energies are from, respectively, Tables 2 and 3.

indistinguishable from zero. In all cases, E_A for a given solute was computed from paired measurements of P_{D_i} at 25 °C and at 37 °C in the same tubule. A comparison of Tables 3 and 5 indicates that the apparent activation energy for ADH-dependent *n*-butyramide permeation was approximately the same at zero volume flow as during lumen to bath osmosis: the values of E_A listed for *n*-butyramide permeation in Table 5 (17.50 ± 1.36 kcal per mole) and in Table 3 (16.6 ± 1.6 kcal per mole) did not differ significantly ($P > 0.5$). Thus it seems improbable that the temperature-dependent increment in P_{D_i} for *n*-butyramide shown in Table 3 was the consequence of entrainment of solute and solvent flows.

Table 5. Apparent activation energies for ADH-dependent solute permeation at zero volume flow

Solute	P_{D_i} (cm sec^{-1} × 10^4)		E_A (kcal mole^{-1}) (kcal mole^{-1})	P
	25 °C	37 °C		
n-Butyramide	0.30 ± 0.90	0.91 ± 0.05	17.50 ± 1.36	–
Mean paired difference	0.16 ± 0.03 ($P < 0.001$); [n = 4]			
Isobutyramide	0.16 ± 0.02	0.46 ± 0.05	15.81 ± 1.19	> 0.5
Mean paired difference	0.30 ± 0.05 ($P < 0.002$); [n = 5]			
Antipyrine	0.42 ± 0.06	1.54 ± 0.24	19.61 ± 1.80	> 0.2
Mean paired difference	1.12 ± 0.19 ($P < 0.005$); [n = 6]			

P_{D_i} for each of the three solutes was measured at 25 °C and at 37 °C in paired observations on the number of tubules listed. The protocol was identical to that shown in Fig. 6 except that the perfusing solutions contained 290 mOsm per liter KRP plus test solute; and, J_v was uniformly indistinguishable from zero. The P values in parentheses refer to the mean paired difference between P_{D_i} at 25° and at 37 °C. The P column is for the difference between the apparent activation energies for either isobutyramide or antipyrine permeation with respect to *n*-butyramide permeation.

Table 5 illustrates another noteworthy characteristic. The apparent activation energies for ADH-dependent *n*-butyramide, isobutyramide, and antipyrine permeation were not distinguishable one from the other. However, as indicated in connection with Tables 2 and 3, the activation energies for *n*-butyramide permeation were significantly greater than those for P_f, both with and without hormone ($P < 0.002$ in both cases). Likewise, the activation energies for ADH-dependent isobutyramide and antipyrine permeation listed in Table 5 were both significantly greater ($P < 0.002$) than the E_A values for P_f shown in Table 4.

Discussion

The experiments in this paper were intended to compare the effects of ADH on the transport of water and moderately lipophilic solutes across luminal plasma membranes of mammalian cortical collecting tubules. Within the constraints of experimental design described in *Results*, and the limits of experimental error, the following general conclusions seem warranted.

The present data indicate clearly that, *pari passu* with a tenfold increment in P_f (Fig. 1), ADH produced a distinguishable increment in the diffusional permeability coefficients for *n*-butyramide, isobutyramide, and antipyrine (Table 1), i.e., moderately lipophilic solutes having β_{oil} values in the range of 0.009–0.032 ([9, 10]). And, based on the changes in cellular morphology when *n*-butyramide replaced NaCl in either the perfusing or the bathing solutions (Fig. 4), it seems likely that the luminal membrane of cortical collecting tubule cells was the rate-limiting site for permeation of this solute. Thus we conclude that the ADH-dependent increments in nonelectrolyte permeation in cortical collecting tubules (Table 1) were referable to hormone-mediated changes in transport processes within luminal membranes.

The apparent activation energy for *n*-butyramide permeation was clearly greater than for P_f, both with and without ADH (Tables 2 and 3). A similar consideration applies to the other nonelectrolytes which were examined: the ADH-dependent activation energies for *n*-butyramide, isobutyramide and antipyrine (Table 5) permeation at zero volume flow were in the range of 15.8–19.6 kcal per mole, and clearly greater than 8.9 kcal per mole (Table 3), the ADH-dependent activation energy for water diffusion. Finally, ADH had no significant effect on the activation energies for water and butyramide diffusion (Tables 2–4). Similarly, in toad urinary bladder, Hays *et al.* [22] found that the activation energy for water permeation was 10.6–11.7 kcal per mole, and unaffected by ADH.

In order to evaluate these results, we set three assumptions. First, we consider that P_f estimates the true permeability coefficient for water diffusion across luminal membranes (*cf.* Introduction; [3, 41, 43, 44]). Second, there is no indication of molecular seiving for moderately lipophilic nonelectrolytes: the permeability coefficient for antipyrine, whose molecular weight and molar volume are approximately twice as great as those of *n*-butyramide or isobutyramide, was greater than that for either of the two smaller solutes, both in the presence and absence of ADH (Table 1). Since nonelectrolyte permeation in the presence and absence of hormone was proportional to β_{oil}, we consider that *n*-butyramide, isobutyramide and antipyrine diffused through hydrophobic regions of luminal membranes. Third, we assume that synthetic lipid bilayer membrane systems provide a reasonable frame of reference for analyzing transport processes in hydrophobic bilayer regions of luminal membranes.

A substantial body of evidence [4, 7, 19, 38–40, 49] indicates that water transport across synthetic bilayer membranes is diffusional, and that P_f is the true permeability coefficient for water diffusion for such membranes.

Table 6. A comparison of P_f and P_{D_i} in cortical collecting tubules and synthetic lipid bilayer membranes

System	Solute	Solute β_{oil}	P_{D_i}	P_f	P_f/P_{D_i}	Reference
			(cm sec^{-1} × 10^4)			
Cortical collecting tubules						
−ADH	urea	0.00014	0.03	20	666	[6, 15, 41–43]; Fig. 1
−ADH	acetamide	0.0008	0.034	20	588	[6, 15, 41–43]; Fig. 1
−ADH	thiourea	0.0012	0.03	20	666	[6, 15, 41–43]; Fig. 1
−ADH	butyramide	0.01	0.14	20	142	Fig. 1; Table 5
+ADH	urea	0.00014	0.02	197	9850	[6, 15, 41–43]; Fig. 1
+ADH	acetamide	0.0008	0.036	197	5472	[6, 15, 41–43]; Fig. 1
+ADH	thiourea	0.0012	0.04	197	4925	[6, 15, 41–43]; Fig. 1
+ADH	butyramide	0.01	0.32	197	615	Fig. 1; Table 1
Lipid bilayer membranes						
Sheep red cell lipid/decane (planar)	acetamide	0.0008	0.83	22.9	22.7	[2]
Lecithin/decane (planar)	urea	0.00014	0.036	19	527	[12, 19, 29, 53]
	acetamide	0.0008	1.43	19	13	[12, 19, 29, 53]
	thiourea	0.0012	0.046	19	413	[12, 19, 29, 53]
Lecithin/chloroform-decane (spherical)	acetamide	0.0008	0.24	13	54	[37]
	valeramide	0.023	1.83	13	7.1	[37]

The solute oil/water partition coefficients are from References [9, 10]. The values of P_f and P_{D_i} are for 22–25°C.

Based on the solubility-diffusion characteristics of water in bulk hydrocarbons [46, 47], one may compute a theoretical P_f of approximately 40–50 × 10^{-4} cm per sec at 25° C [7, 38] for lipid bilayers in the liquid crystal state. Since the ADH-dependent and ADH-independent values of P_f at 23–25° C in cortical collecting tubules (Fig. 1; [15, 41, 43]) and toad urinary bladder [21, 23] vary by only fivefold from this theoretical P_f, it might be argued that water and lipophilic solutes are both transported via a common lipid matrix in luminal membranes. However, when viewed in the context of water and nonelectrolyte diffusion through synthetic lipid bilayers, the results in cortical collecting tubules are consistent with the possibility that the ADH-sensitive route for water diffusion differs from that for diffusion of isobutyramide, *n*-butyramide and antipyrine. We now consider this argument in further detail.

Table 6 compares the ADH-dependent and ADH-independent values of P_f and P_{D_i} (at 23–25° C) in cortical collecting tubules with the values of these coefficients in synthetic lipid bilayer membranes. The lipid bilayer

data are for three representative membranes, and are closely comparable with P_f and P_{D_i} values observed in other planar bilayer membranes [7, 38, 39] and in phospholipid vesicles [40]. Table 6 indicates that the ADH-independent P_f in cortical collecting tubules is about the same as that in synthetic bilayer membranes, that the ADH-dependent P_f for cortical collecting tubules is tenfold greater than in bilayer membranes, and that synthetic bilayer membranes are appreciably more permeable to nonelectrolytes having β_{oil} values $\geqq$0,0008, e.g., acetamide, than cortical collecting tubules, even with ADH.

There are three instances where the P_f/P_{D_i} ratio in cortical collecting tubules exceeds by at least tenfold that in synthetic bilayer membranes: (a) For acetamide, both with and without ADH, because P_{D_i} for acetamide is greater in bilayer membranes than in cortical collecting tubules. (b) Sufficient data are not available for bilayer membranes to evalute P_f/P_{D_i} ratios for the solutes listed in Table 1. In spherical bilayer membranes, Poznansky *et al.* [37] obtained a permeability coefficient of 1.83×10^{-4} cm per sec for valeramide, a solute whose β_{oil} and molecular weight are similar to those of butyramide. Table 6 shows that the permeability coefficient for butyramide in cortical collecting tubules, with or without ADH, was appreciably less than that for valeramide in spherical bilayers. Consequently, the P_f/P_{D_i} ratio for butyramide in cortical collecting tubules was 20 (without ADH) to 36 (with ADH) times greater than the P_f/P_{D_i} ratio for valeramide in spherical bilayer membranes. (c) The P_f/P_{D_i} ratios for cortical collecting tubules with ADH are uniformly at least ten times greater than the comparable ratios for bilayer membranes. The latter observation may have wider applicability: the highest P_f value reported for unmodified bilayer membranes, approximately 70×10^{-4} cm per sec at 37°C [17], is about one-fifth of the ADH-dependent P_f in cortical collecting tubules at 37°C (Fig. 2; Table 3).

Thus it is difficult to envision ADH-mediated effects on the fluidity of hydrophobic regions of luminal membranes which permit, simultaneously, tenfold higher rates of water diffusion, but appreciably lower permeation rates for nonelectrolytes having β_{oil} values $\geqq$0.0008, than in synthetic bilayers. Rather, we argue that ADH-mediated water and nonelectrolyte diffusion across luminal membranes may involve parallel pathways: one for water and the other for moderately lipophilic solutes. The water diffusion pathway, although appreciably more permeable to water than synthetic bilayer membranes, excludes small hydrophilic solutes. The regions for nonelectrolyte diffusion admit nonelectrolytes in approximate agreement with Overton's rule, but are less permeable than synthetic bilayer membranes to solutes having β_{oil} values $\geqq$0.0008.

Additional support for this view may be derived by comparing the activation energies for water and nonelectrolyte diffusion through synthetic lipid bilayer membranes with those in cortical collecting tubules. The activation energy for water diffusion (termed E_A^w) across a bilayer membrane may be expressed as the sum of two terms: the energy required for a water molecule to break the four hydrogen bonds formed with neighboring water molecules (termed $E_A^{H_w}$); and, a nonhydrogen bond term (termed $E_A^{D_w}$), representing the energy required for water diffusion within the membrane [8, 38–40]. The activation energy for nonelectrolyte diffusion (termed E_A^s) across a bilayer membrane may be rationalized in terms of the same processes [12, 14], with $E_A^{H_s}$ and $E_A^{D_s}$ representing the components of E_A^s due, respectively, to rupture of hydrogen bonds in solution and diffusion within the membrane. Based on an analysis of water and solute permeation in liposomes, Cohen [8] computed 1.8 kcal per mole as the average activation energy per hydrogen bond in aqueous solution for either water or solutes. And, in liposomes, $E_A^{D_w}$ and $E_A^{D_s}$ vary depending either on liposome composition or whether liposomes of a given composition are above or below their transition temperature [8]: for liposomes of a given composition at the same temperature, $E_A^{D_s}$ and $E_A^{D_w}$ are the same; for liposomes of increasing fluidity, P_{D_i} and P_{D_w} rise while, *pari passu*, E_A^w and E_A^s fall.

In cortical collecting tubules, the ADH-dependent activation energies for water and solute permeation were, respectively, 8.9 and 16.6–19.6 kcal per mole (Tables 3–5). Taking four, three and two as the number of aqueous hydrogen bonds for, respectively, water, isobutyramide or butyramide, and antipyrine [48], these E_A^w and E_A^s data yield, following the analysis applied to synthetic bilayer membranes, apparent values of 0.9 and 10.6–15.6 kcal per mole for, respectively, $E_A^{D_w}$ and $E_A^{D_s}$ for luminal membranes of cortical collecting tubules. Consequently, either $E_A^{D_w}$ and $E_A^{D_s}$ differ within the hydrophobic regions of luminal membranes; or, water traverses a different pathway than moderately lipophilic species in these membranes. In other words, using activation energy measurements in liposomes as a frame of reference, we may infer that, in luminal membranes, water and moderately lipophilic solutes traverse parallel permeation pathways.[3]

We now speculate on the possible nature of the transport pathways in luminal membranes, and the effects of ADH on these pathways. In the present experiments, ADH increased P_{D_i} for butyramide, isobutyramide

3 In red blood cells, $E_A^{D_w}$ is also appreciably less than $E_A^{D_s}$ [52], an observation which has been taken to provide support for the view that water traverses aqueous channels in erythrocyte membranes [52].

and antipyrine (Table 1) but did not affect the apparent activation energy for butyramide permeation (Table 4). Thus it seems improbable that ADH produced a uniform increase in the fluidity of the hydrocarbon matrix in luminal membranes, since, in comparison with synthetic bilayers, such fluidity increases should have been accompanied by a fall in E_A^s due to a reduction in E_A^{Ds} [8]. Rather, we suggest that nonelectrolyte diffusion across cortical collecting tubules may proceed via discrete hydrophobic pathways in luminal membranes, and that ADH increases butyramide permeation, without affecting detectably E_A^s for butyramide, by increasing the number of such pathways. A corollary of this hypothesis is that, in luminal membranes, the hydrophobic region itself may be a mosaic structure containing: pathways for butyramide (and presumably isobutyramide and antipyrine) permeation, whose number may be increased by by ADH; and, more ordered hydrophobic regions unaffected by ADH, which have lower permeation rates and appreciably higher activation energies for transport of moderately lipophilic solutes.

We consider next the pathway for water diffusion in luminal membranes. This process differs in at least two major respects from water diffusion in synthetic bilayers: the ADH-dependent P_f in luminal membranes exceeds reported P_f values in synthetic bilayers (Table 6); and, in luminal membranes with or without ADH, the apparent E_A^{Ds} for nonelectrolytes exceeds the apparent E_A^{Dw} (*cf.* above), while in liposomes of a given composition, E_A^{Dw} equals E_A^{Ds} [8]. Two types of water-conducting pathways might be envisioned to account for these results.

Luminal membranes might contain regions where phospholipids are in a disorganized state, due for example, either to increased hydration [28, 32] of phospholipids and/or to shortening, branching or unsaturation of fatty acid chains [26, 33] in these regions. It is at least conceivable that, for such pathways, E_A^{Hw} is 8 kcal per mole, i.e., diffusing water molecules are fully dehydrated. But because of regional disorganization of phospholipid molecules and attendant increases in membrane fluidity and entropy, the rate of water diffusion is quite high and E_A^{Dw} quite low in these pathways, with respect to the remaining hydrophobic regions of luminal membranes. Clearly, steric constraints within these regions would be needed to exclude urea, thiourea and acetamide, i.e., solutes to which luminal membranes are virtually impermeable, even with ADH (Table 6, [6, 42]); and, butyramide, isobutyramide, and antipyrine, whose molecular weights and molar volumes are greater than those of urea, thiourea, and acetamide.

Alternatively, hydrogen-bonded water molecules might diffuse through

narrow aqueous channels. For example, in bilayer membranes, gramicidin A dimers form aqueous channels having radii of approximately 2 Å [20, 50]. The selectivity ratio for diffusion of water with respect to urea exceeds 10^3 in gramicidin A channels [11]. And, in liposomes formed with gramicidin A, Cohen [8] found that urea, which presumably entered gramicidin A channels, had a lower activation energy (7.2 kcal per mole) for permeation than that (16–20 kcal per mole) for a number of other solutes which were excluded from gramicidin A channels. Thus a 2 Å radius channel in luminal membranes might account for both the high, ADH-dependent P_f/P_{D_i} ratios for urea, acetamide, and thiourea (Table 6), and the lower activation energy for water with respect to nonelectrolyte permeation (Tables 2–5).

However, such a channel would need to have an appreciably lower Na^+ conductance than a gramicidin A channel. Finkelstein [11] found that the water permeability of a gramicidin A channel was 1.12×10^{-14} cm^3 per sec. Thus for an ADH-dependent P_f of 196×10^{-4} cm per sec at 25° C, one requires 1.75×10^{12} gramicidin A channels per cm^2 of luminal membrane; since the Na^+ conductance of a gramicidin A channel is 6×10^{-12} per ohm [11], 1.75×10^{12} gramicidin A channels per cm^2 of luminal membrane would give a luminal membrane resistance of 0.1 ohm cm^2. But the transepithelial resistance of cortical collecting tubules, with or without ADH, is in excess of 800 ohm cm^2 [25]; and, luminal plasma membranes have appreciably higher electrical resistances than either junctional complexes or baso-lateral membranes [24]. Accordingly, if 2 Å radius channels constituted the route for ADH-mediated water diffusion in luminal plasma membranes, the Na^+ conductance of these putative channels would need to be approximately 10^{-4} of the Na^+ conductance of a gramicidin A channel.

The present data are not adequate for evaluating whether the water permeation pathways in luminal membranes involve: disorganized phospholipid regions which exclude urea, thiourea and acetamide by steric hindrance; aqueous channels that exclude urea, thiourea and acetamide, and have Na^+ conductances at least 10^{-4} less than gramicidin A channels; or, other undefined mechanisms. However, since the ADH-dependent increments in the rates of water and butyramide diffusion were not accompanied by significant changes in the activation energies for these processes, we infer that ADH-mediated events increase the number of both the water diffusion routes and the nonelectrolyte permeation pathways in these membranes.

According to this view, luminal membranes are mosaic structures

containing specialized sites for either water or nonelectrolyte permeation which may be in a higher entropy state than the remaining, impermeant regions of luminal membranes; and, ADH increases the number of these specialized pathways without necessarily affecting the ordering of the regions of luminal membranes which are impermeant to water and solutes. Thus the total ADH-dependent change in the entropy of luminal membranes will depend on magnitude of the hormone-mediated increase in the fractional area occupied by the specialized water and solute permeation pathways. Finally, it is interesting to speculate that the routes for nonelectrolyte permeation may be vicinal, and cooperatively articulated with, the routes for water transport. Evidently, additional data are required to evaluate these possibilities.

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