A New Proposal for the Action of Vasopressin, Based on Studies of a Complex Synthetic Membrane

RICHARD M. HAYS

From the Department of Medicine and the Unit for Research in Aging, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT The total osmotic flow of water across cell membranes generally exceeds diffusional flow measured with labeled water. The ratio of osmotic to diffusional flow has been widely used as a basis for the calculation of the radius of pores in the membrane, assuming Poiseuille flow of water through the pores. An important assumption underlying this calculation is that both osmotic and diffusional flow are rate-limited by the same barrier in the membrane. Studies employing a complex synthetic membrane show, however, that osmotic flow can be limited by one barrier (thin, dense barrier), and the rate of diffusion of isotopic water by a second (thick, porous) barrier in series with the first. Calculation of a pore radius is meaningless under these conditions, greatly overestimating the size of the pores determining osmotic flow. On the basis of these results, the estimation of pore radius in biological membranes is reassessed. It is proposed that vasopressin acts by greatly increasing the rate of diffusion of water across an outer barrier of the membrane, with little or no accompanying increase in pore size.

INTRODUCTION

There is considerable experimental support for the view that water movement across biological membranes proceeds by bulk flow through aqueous channels or pores (1–3). This view originates from the observation that the net flow of water across a tissue predicted from the diffusion rate of isotopically labeled water generally falls far short of the flow actually observed. The ratio between total water flow and "diffusional" flow may reach values as high as 100 to 1 in certain tissues; this is true, for example, in the toad bladder in the presence of vasopressin (4). It has been the practice to estimate a mean equivalent pore radius from this ratio, assuming Poiseuille flow across a porous barrier in the cell membrane.

Objections have been made to this method of estimating pore radius. It has not been shown, for example, that aqueous channels in cell membranes

are in the form of right circular cylinders. The use of the bulk viscosity coefficient for water in the Poiseuille equation is not justified in all instances (3), and Dainty and House have shown that values for the diffusion of isotopic water may be somewhat reduced by the presence of an unstirred layer (5). An additional and possibly more serious objection derives from the fact that biological membranes are structurally complex, consisting of a number of permeability barriers arranged in series. While the investigator may wish to estimate the porosity of a component of the cell membrane, he is obliged to use values for tracer flux and water flow obtained from measurements across the entire membrane. The assumption is made that the particular porous barrier of interest is rate-limiting for both tracer flux and water flow; only on this basis can the calculation of pore radius be made. If, in biological membranes, different barriers limit tracer flux and water flow, estimates of pore radius will be erroneous (6).

The experiments to be described show that pore radii calculated from the ratio of total water flow to diffusional flow in a complex synthetic membrane are indeed without meaning. The true rate of tracer diffusion is many times greater across the layer limiting water flow than is apparent in measurements made across the entire membrane. On the basis of these observations, it is proposed that vasopressin may act by increasing the diffusion rate of water across a critical layer in the cell membrane.

MATERIALS AND METHODS

I. *Cellulose Acetate Membranes*

The cellulose acetate membranes used were those developed by Loeb and Manjikian for desalination by the process of reverse osmosis (7). They were prepared by mixing cellulose acetate (Eastman 398-10), formamide, and acetone in proportions of 5:6:9 by weight. The membranes were cast at room temperature on glass plates and the exposed surface was allowed to evaporate for 20 sec. The membrane was placed in a water bath at 2°C for 1 hr, then heated for 4 min in a water bath at 85°C. Membranes prepared in this way have been shown to possess a thin, dense "skin" approximately 0.25 μ in thickness, covering the surface from which evaporation took place. The rest of the membrane (approximately 100 μ in thickness) consists of a highly porous layer (8). The membrane is shown diagrammatically in Fig. 1. In the process of reverse osmosis, the brine solution is filtered through the skin at 40 or more atmospheres pressure. The skin has a low permeability to salt, permitting less than 10% of it to pass, but allowing enough water flow, of the order of 20 gal per sq ft per day, to make the process a practical one for desalination. Membranes prepared for this study gave acceptable values for salt rejection (88–97%) and water flow (0.05 ml hr^{-1} cm^{-2} atm^{-1}) when tested at 700 psi in a desalination cell (9).

II. *Measurement of L_p and ω_T*

In the present studies, measurements of hydraulic permeability (L_p) were carried out in a stainless steel pressure cell attached to a tank of nitrogen. The membranes

were placed on a stainless steel screen with their skin surfaces up, and the flow of distilled water across the membranes was determined at a pressure of 8 atmospheres. The permeability coefficient of the membranes to tritiated water (ω_T) was determined in a Lucite diffusion chamber, with distilled water bathing both sides of the membrane. Tritiated water was added to the solution in one chamber half, and its rate of appearance in the opposite half determined at timed intervals. Vigorous stirring was provided by rotating bar magnets equipped with vertical Lucite fins. Samples were counted in a Tricarb liquid scintillation spectrometer (Packard Instrument Company, Downers Grove, Ill.). Calculation of ω_T and correction for back diffusion of the isotope were done by a method previously described (4). Membrane thickness was measured with a vernier micrometer. The permeability of the membranes to salt was determined by conductance measurements made with a Radiometer type CDM 2 conductivity meter (Radiometer, Copenhagen, Denmark).

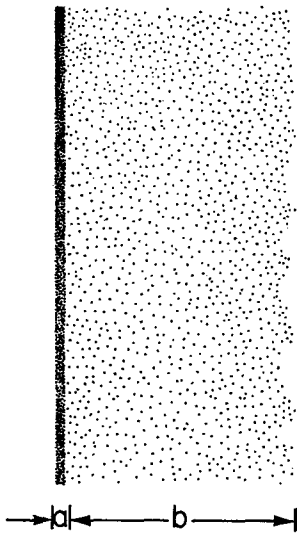

FIGURE 1. Schematic cross-section of cellulose acetate desalination membrane. A thin skin (*a*) overlies, and is in series with a thick, highly porous layer (*b*).

CALCULATIONS

The calculation of the mean equivalent pore radius has been discussed in previous publications (10, 11). Briefly stated, two types of flow across a membrane may be distinguished: The first, L_p, is the hydraulic water flow in the presence of a hydrostatic or osmotic driving force:

$$L_p = \frac{J_v}{\Delta_p} \tag{1}$$

or (provided the reflection coefficient is 1):

$$L_p = \frac{J_v}{\Delta\pi} \tag{2}$$

where J_v is the volume flow in $ml \cdot cm^{-2} \cdot sec^{-1}$, Δ_p is the difference in hy-

drostatic pressure, and $\Delta\pi$ the difference in osmotic pressure across the membrane.

The second flow, ω_T, is the diffusion of isotopically labeled water across the membrane in the absence of net water flow:

$$\omega_T = \frac{J_T}{RT\Delta c_T} \tag{3}$$

where J_T is the flow of tagged water (THO) in $mol \cdot cm^{-2} \cdot sec^{-1}$ and Δc_T is the difference in isotope concentration across the membrane.

The ratio of hydraulic or osmotic flow to the flow of isotopically labeled water may be indicated by the ratio g:

$$g = \frac{L_p}{\bar{V}_w \omega_T} \tag{4}$$

where $\bar{V}$ is the molar volume of water.

In a simple porous membrane of thickness Δx, in which the pores run through its entire thickness, one may express g in terms of the length and area of the pores; η, the viscosity coefficient of water, and (assuming that the isotopically labeled water moves through the membrane by a process of self-diffusion), D, the self-diffusion coefficient of water in water:

$$g = \frac{L_p}{\bar{V}_w \omega_T} = \frac{\frac{\pi r^4}{8\eta \Delta x}}{\frac{\pi r^2 D \bar{V}_w}{RT\Delta x}} \tag{5}$$

Here, L_p is expressed in terms of the Poiseuille equation. Since the area (πr^2) open to flow and diffusion is the same, and since Δx is the same for flow and diffusion, these terms may be cancelled, yielding the expression

$$g = \frac{r^2 RT}{8\eta \bar{V}_w D} \tag{6}$$

From this relationship r, the mean equivalent pore radius, may be calculated.

When one deals with more complex membranes, where two or more barriers exist in series, the above analysis does not necessarily apply. The estimated porosity of the entire membrane may not reflect the true porosity of the barrier controlling water flow, since L_p and ω_T must of necessity be measured across the entire thickness of the membrane. If one wishes to obtain information about the contribution of each barrier individually, it is necessary to employ an expression for g which takes into account the existence of barriers in series. This may be done by writing the coefficients for hydraulic and isotopic flow in the form of resistances in series (12, 13):

$$\frac{1}{L_p} = \frac{1}{L_{pa}} + \frac{1}{L_{pb}} \tag{7}$$

and

$$\frac{1}{\omega_T} = \frac{1}{\omega_{Ta}} + \frac{1}{\omega_{Tb}} \tag{8}$$

where a and b refer to two barriers in series. The expression for g then becomes:

$$\bar{V}_g = \frac{L_p}{\omega_T} = \frac{\frac{1}{\omega_{Ta}} + \frac{1}{\omega_{Tb}}}{\frac{1}{L_{pa}} + \frac{1}{L_{pb}}} \tag{9}$$

or:

$$g = \frac{RT}{8\eta D\bar{V}_w} \frac{\frac{\Delta x_a}{n_a r_a^2} + \frac{\Delta x_b}{n_b r_b^2}}{\frac{\Delta x_a}{n_a r_a^4} + \frac{\Delta x_b}{n_b r_b^4}} \tag{10}$$

where Δx is the thickness of the barrier, n the number of pores, r the pore radius, D the self-diffusion coefficient, and η the viscosity coefficient of water. The relative contribution of the Δx and r terms of each separate barrier to the over-all ratio g of the membrane can be seen in equation 10. If, for example, barrier a has the characteristics of a diffusion barrier (r approaching molecular dimensions), and barrier b is a thin, highly porous layer, the values for $\frac{1}{\omega_{Tb}}$ and $\frac{1}{L_{Pb}}$ would be small relative to $\frac{1}{\omega_{Ta}}$ and $\frac{1}{L_{pa}}$. Barrier a would control both hydraulic flow and isotopic diffusion, and the ratio g would be determined by this barrier alone. The importance of barrier b in determining ω_T for the membrane would increase, on the other hand, as the ratio $\Delta x_b/\Delta x_a$ increased. One can introduce values for Δx_a and Δx_b, for example, which would result in hydraulic flow being controlled by barrier a, and diffusional flow of water by barrier b. This case is of particular interest for the studies that follow.

RESULTS

I. L_p and ω_T of Cellulose Acetate Membranes

L_p and ω_T for three reverse osmosis membranes are shown in Table I. The membranes were prepared in the standard fashion; they averaged 110 μ in thickness. L_p and ω_T are shown in columns 2 and 3, and their ratio, g, in column 4. The mean equivalent pore radii calculated from these data are relatively large, with a mean value of 25 A.

II. L_p and ω_T in Altered Cellulose Acetate Membranes

If one assumes that there are only two barriers to water flow across the membrane (skin and porous layer), and if L_p and ω_T of the porous layer are known, L_p and ω_T of the skin can be calculated from equations 7 and 8. We were able to obtain these coefficients for the porous layer in two ways: first by casting membranes in which the formation of a skin was prevented, and, second, by physically removing the skin.

TABLE I

COEFFICIENTS OF STANDARD MEMBRANES

Membrane	$L_p/\bar{V}_w$ $\times 10^{14}$	ω_T $\times 10^{14}$	$g = \frac{L_p}{\omega_T \bar{V}_w}$
	$mol \cdot dyn^{-1} \cdot sec^{-1}$	$mol \cdot dyn^{-1} \cdot sec^{-1}$	
1	76.2	1.48	51.5
2	84.9	1.79	47.4
3	70.7	1.71	41.3

In the first experiments, membranes were cast at 4°C rather than at room temperature, and placed immediately in ice water. After an hour, the membranes were stored at room temperature in distilled water; no heat curing was carried out. The mean thickness of these membranes was 122 μ. Mem-

TABLE II

COEFFICIENTS OF "SKINLESS" MEMBRANES

Membrane	$L_p/\bar{V}_w$ $\times 10^{14}$	ω_T $\times 10^{14}$
	$mol \cdot dyn^{-1} \cdot sec^{-1}$	$mol \cdot dyn^{-1} \cdot sec^{-1}$
1	1,693	1.78
2	1,456	1.76

branes of this type were 40 times more permeable to salt than standard membranes. Data obtained with two of these membranes are shown in columns 2 and 3 of Table II. There was a striking increase in L_p, which is approximately 20 times that of the standard membranes (Table I). On the other hand, there was little if any change in ω_T. L_p is clearly determined by the skin, but ω_T is determined by the porous phase.

Although this qualitative experiment did reveal the rate-limiting barriers for flow and diffusion, there was enough variation between individual membranes to prevent the calculation of L_p and ω_T of the skin. Therefore, the coefficients for a given membrane were determined before and after the physical removal of the skin. The experiments were carried out on membranes which were cast at a greater thickness than the standard membranes, in

order to eliminate the possibility of perforating the membranes during removal of the skin. These membranes averaged 200 μ in thickness. The coefficients for the intact membranes are shown in Table III (columns 2 and 3). The skin layer was then removed; in membranes 1 and 2, this was done by carefully passing a glass microtome knife, of the type used in preparing sections for electron microscopy, across the skin. In membranes 3–8, the skin was lightly sandpapered in an ice water bath with No. 280 Wetordry silicon carbide paper (Minnesota Mining and Manufacturing Co., St. Paul, Minn.). Columns 4 and 5 show the coefficients of these membranes following the procedures. There was striking increase in L_p with only a small increase in

TABLE III
EFFECT OF REMOVAL OF SKIN AND CALCULATION OF SKIN COEFFICIENTS

Membrane	Intact		Porous layer		Skin		g
	$L_p/\bar{V}_w$ × 10^{14}	ω_T	$L_p/\bar{V}_w$ × 10^{14}	ω_T	$L_p/\bar{V}_w$ × 10^{14}	ω_T	
	$mol \cdot dyn^{-1} \cdot sec^{-1}$		$mol \cdot dyn^{-1} \cdot sec^{-1}$		$mol \cdot dyn^{-1} \cdot sec^{-1}$		
1	51.5	1.07	488	1.27	57.8	6.79	8.5
2	45.2	1.09	865	1.22	47.8	10.23	4.7
3	61.1	1.13	1,193	1.40	64.1	5.86	10.9
4	58.7	1.13	1,034	1.28	62.5	9.63	6.5
5	94.9	1.36	3,580	1.43	97.5	27.20	3.6
6	73.5	1.14	2,810	1.34	77.5	7.63	10.2
7	77.8	1.25	3,580	1.45	79.6	9.09	8.8
8	71.6	1.31	813	1.41	78.1	18.48	4.2
Mean values	66.8	1.19	1,795	1.35	70.6	11.86	7.18

ω_T . In columns 6 and 7, L_p and ω_T of the skin are calculated from equations 7 and 8, and in column 8, g for the skin is shown. The pore radius calculated from g is 9 A, considerably less than that calculated for the whole membrane.

III. *Effect of Removal of Skin on K_{trans}NaCl*

Removal of the skin by sandpapering had a profound effect on the permeability of the membrane to salt. The K_{trans} NaCl of a representative intact membrane, determined conductimetrically, was 65 × 10^{-7} cm·sec^{-1}. Following removal of the skin, the K_{trans} was 3,300 × 10^{-7} cm·sec^{-1}, a 50-fold increase.

DISCUSSION

The results obtained show that in a complex membrane, where permeability barriers exist in series, the rate of hydraulic flow of water may be determined

by one barrier, and the rate of water diffusion by another. The dense skin of the cellulose acetate membrane offers a high resistance to flow, but the skin is so thin that its resistance to the diffusion of tritiated water is small compared to that of the thick porous layer. Durbin (14) has also pointed out that a thick layer of this type can determine ω_T .

The thickness of the porous layer is not the only factor determining its relatively higher resistance to diffusion. Despite the high porosity of this layer, its excluded volume and tortuosity are considerable, and only a fraction of this layer is open to diffusion. Taking Δx of the porous layer (thick membranes, Table III) as 200×10^{-4} cm, and the measured permeability of this layer to tritiated water as 2.8×10^{-4} cm·sec^{-1}, one can calculate a permeability coefficient for the porous layer of 0.56×10^{-5} cm^2·sec^{-1}. This may be compared to Wang, Robinson, and Edelman's value of 2.4×10^{-5} cm·sec^{-1} (15) for the self-diffusion of water; approximately one-fourth of the area of the porous layer is open to diffusion.

Under these circumstances, a calculation of the "porosity" of a complex membrane based on conventional isotope and flow measurements gives a value which does not apply to either layer, but represents an intermediate value between the skin and the porous layer.

Although the pore radii calculated for the skin are relatively small, they still exceed the values one would expect for a membrane capable of a high degree of salt exclusion. If one regards the skin as simply a thin layer of dense cellulose acetate, the present value for g is three times higher than that obtained by Thau et al. for such a dense membrane (16). Part of the discrepancy can be explained by the fact that the sandpapering or microtome procedure removed more than just the skin, as shown by measurements with a micrometer; approximately 20μ of the total thickness of 200 μ were removed in a typical experiment. Thus, the portion removed contained both skin and some of the porous layer, and must itself be considered a complex membrane. While the procedure was crude, and probably led to an overestimation of the porosity of the skin, it serves to illustrate the point of the present study regarding the hazards of estimating pore size in a complex membrane.

I. *Application to Biological Membranes*

Biological tissues are far more complex in structure than the cellulose acetate membrane discussed, and contain a number of permeability barriers. Some offer a high resistance, others a low resistance to water flow or diffusion. In epithelial structures such as frog skin, gut, or toad bladder, in which one or more layers of epithelial cells are attached to a supporting layer of muscle or connective tissue, it is probable that most of the barriers offer some resistance to flow and diffusion. It has been assumed that the cytoplasm and the supporting layer offer no more resistance than an equally thick layer of water

(4), and that the main resistance barrier is the cell membrane. Although this assumption is a reasonable one, it is nevertheless true that tracer flux will be falsely low, and the calculated porosity of the cell membrane falsely high, to the extent that these other barriers influence diffusion without influencing flow.

However, it is probably within the cell membrane itself that the influence of series barriers is most important. The membrane, with its bimolecular leaflet and associated protein layers, may reasonably be regarded as having

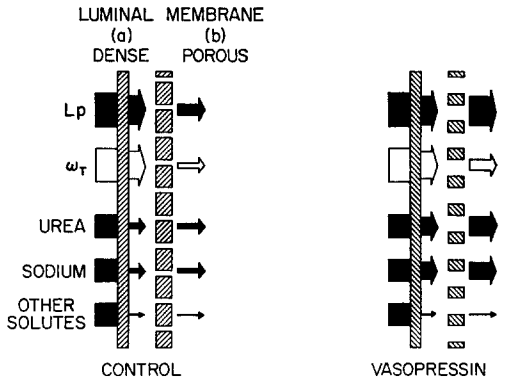

FIGURE 2. Schematic representation of the pore enlargement hypothesis of vasopressin action. Hydraulic flow of water (L_p) is shown by solid arrows, and diffusional flow (ω_T) by open arrows. L_p and ω_T are determined by the underlying barrier, both in the control state and in the presence of vasopressin. Vasopressin increases the porosity of the underlying barrier, resulting in bulk flow of water, and a relatively small increase in ω_T. It has been suggested (20) that vasopressin also increases the permeability of the dense diffusion barrier to urea and sodium, but not to other solutes.

more than one permeability barrier; in addition, electron microscopic studies of a variety of epithelial membranes show extraneous superficial layers, whose function is still poorly understood (17). Further, the cell membrane appears to control cellular permeability; in the toad bladder, for example, the luminal membrane of the epithelial cell determines the rate of water flow and diffusion into the cell, both in the presence and absence of vasopressin. This conclusion is supported by studies on the labeling of intracellular water by tritiated water (4), and on the swelling of the epithelial cells following vasopressin (18). It has been proposed (4) that the hormone acts by enlarging pores in a permeability barrier at the luminal surface of the cell, with an increase in the estimated mean pore radius from 8 to 41 A. This calculation is based on the finding that vasopressin produces a 40-fold increase in L_p, but only a 70% increase in ω_T; the ratio L_p/ω_T rises from 6 to over 100 following the hormone, and this marked increase in nondiffusional flow could best be accounted for by the formation of large pores. Since there was

no difference in the permeability of the bladder to most solutes tested before and after the hormone (19), it was necessary to place a fine diffusion barrier over the porous barrier; this diffusion barrier was originally believed to be unresponsive to vasopressin but rate-limiting to most solutes. Subsequent studies by Lichtenstein and Leaf (20), employing amphotericin B have suggested that the fine diffusion barrier itself might be responsive to vasopressin in the case of urea and sodium; Orloff and Handler, however (21), have questioned the validity of experiments of this type. The "pore enlargement" hypothesis is shown schematically in Fig. 2.

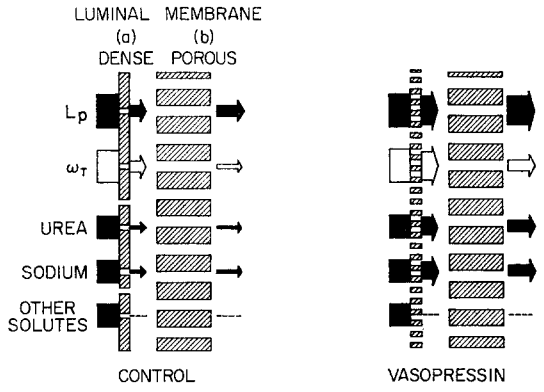

FIGURE 3. Present hypothesis of the mechanism of action of vasopressin. L_p is determined both in the absence and presence of the hormone by the dense (*a*) barrier. Vasopressin produces a marked increase in the number of small pores in the dense barrier, resulting in a large increase in L_p . ω_T increases as well, but its over-all rate following vasopressin is limited by the thick (*b*) barrier. Both urea and sodium penetrate the dense barrier more rapidly after the hormone, and their rate of penetration continues to be limited by this barrier. The movement of other solutes through the pores opened by vasopressin is regarded as exceedingly low or nonexistent.

An alternative to this hypothesis now appears possible. It is based on the finding with the cellulose acetate membrane that osmotic flow can be limited by one barrier (thin, dense barrier), and the rate of diffusion of isotopic water by a second (thick, porous) barrier in series with the first. It is proposed that the luminal membrane includes a thin superficial barrier (*a*) which is rate-limiting with respect to L_p, both in the presence and absence of hormone. Beneath this barrier is a thick, porous barrier (*b*), whose structure is not altered by vasopressin (Fig. 3). In the absence of hormone, this barrier provides a resistance to ω_T approximately equal to barrier *a*, so that ω_T for the luminal membrane is determined by both the membrane layers. Vasopressin could act in a membrane of this type by increasing the number, but not the size, of small pores in barrier *a*. There would be a 40-fold rise in

ω_T , and, consequently, a 40-fold rise in L_p . Barrier *b* would now be completely rate-limiting for ω_T , and, as a result, the large increase in ω_T would not be experimentally apparent. Values for ω_T under these conditions have been calculated, using equation 8 and Hays and Leaf's data for the permeability of the luminal cell membrane to tritiated water (4). The results are shown in Table IV. It is apparent that despite the large increase in ω_T across barrier *a*, the increase in ω_T across the entire membrane is small.

The case shown is one in which ω_T increases 40-fold, proportional to the increase in L_p . It is of interest that there is one report by Grantham and Burg (22) of such a proportional increase in ω_T and L_p following vasopressin in the isolated rabbit collecting tubule.

If vasopressin produced a less than 40-fold increase in ω_T , some increase in pore size would be required to account for the increase in L_p . If ω_T increased 20-fold, for example, a 30% increase in pore radius would be required. Whittembury (6) has obtained evidence in studies on the osmotic behavior of toad skin epithelial cells that vasopressin increases the equivalent pore radius from approximately 4.5 to 6.5 A. Pore radii obtained by this method were smaller than those estimated by Andersen and Ussing (2) from studies of osmotic flow and isotope diffusion, and a model membrane, composed of narrow and wide pores in series, was proposed to account for this discrepancy. Significantly smaller pores appear to be present in the toad bladder, both in the presence and absence of vasopressin; the passage of small molecules such as thiourea across the bladder is not increased by vasopressin (19), while thiourea movement across the toad skin increases after the hormone (2). Further, the reflection coefficient for urea in the vasopressin-treated toad bladder is 0.79 (19), compared to 0.6 in the toad skin (6). The available data, then, would support the view that the principal effect of the hormone is on the number, rather than the size of aqueous channels.

TABLE IV

ω_T ACROSS INDIVIDUAL BARRIERS OF LUMINAL MEMBRANE

	$\omega_T \times 10^{14}$	
	Control	Vasopressin
	$mol \cdot dyn^{-1} \cdot sec^{-1}$	
Barrier *a*	1.04	41.6
Barrier *b*	1.24	1.24
Entire membrane*	0.56	1.20

* Data for entire membrane from Hays and Leaf (4).

The effect of vasopressin on the movement of sodium, as well as urea, across the membrane is also attributed to the change in barrier *a* (lower half of Fig. 3). These solutes penetrate the barrier at low rates in the absence of the hormone, and although their rate of penetration after the hormone is significantly increased, it is still below their rate of movement through barrier *b*. Therefore, barrier *a* is rate-limiting for urea and sodium both in the absence and presence of vasopressin. The situation is comparable to that in the cellulose acetate membrane, where the skin proved to be highly permeable to water, but rate-limiting to sodium chloride. The rate of movement of other solutes across the toad bladder is exceedingly low, and may be by pathways other than those shown in barrier *a*. At any rate, due to the small size of the pores opened up by vasopressin, there is no change in their rate of movement.

Modifications of this model are possible; for example, part of the effective thickness of layer *b* may be an unstirred layer in the cytoplasm of the cell. Also, changes in the thickness or the solubility properties of barrier *a* could contribute to the increase in ω_T . The important feature of the present proposal, however, it that different barriers in the membrane control diffusion and flow.

This hypothesis changes our picture of the luminal membrane of the toad bladder in several respects. First, the radius of pores controlling L_p in the absence of the hormone would be considerably less than the 8.4 A originally calculated (4). The pore radius would approach more closely the dimensions of the water molecule, and the properties of the *a* layer would approach those of a diffusion barrier. Second, if vasopressin induces the formation of a large number of pores, with mean radii identical to or only slightly larger than those of the original pores, most solutes would continue to be excluded from the membrane, and the specificity of the hormone effect would be preserved. Urea and other amides, sodium, and certain alcohols would cross the membrane more rapidly by virtue of specific interactions with the small pores; in this way, the problem of specificity of hormone action would be met without necessitating a barrier in series with the vasopressin-sensitive layer (Fig. 2). Third, so long as aqueous channels were present in some phase of the membrane (*b* layer), and this layer contributed significantly to the solute resistance, the solvent drag effect on such solutes as urea (4) would still be observed in the over-all structure. Under these conditions, the *a* layer could retain the properties of a diffusion barrier. Fourth, the ultimate effect of vasopressin is placed at the cell surface, where such processes as secretion of surface-coating material could play a significant part in the action of the hormone.

I am greatly indebted to Dr. Ora Kedem for her advice and encouragement during my sabbatical year at the Weizmann Institute of Science, Rehovoth, Israel. I wish also to thank Drs. Aharon Katchalsky and Sidney Loeb for their helpful comments, and Miss Dorit Caliph for her expert technical assistance.

This investigation was supported in part by United States Public Health Service Grants AM-03858, HD-00674, and GM-09432 (to Dr. O. Kedem).
Dr. Hays is a Career Investigator of the Health Research Council of the City of New York.
Received for publication 9 August 1967.

REFERENCES

1. KOEFOED-JOHNSEN, V., and H. H. USSING. 1953. The contributions of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28**:60.
2. ANDERSEN, B., and H. H. USSING. 1957. Solvent drag on non-electrolytes during osmotic flow through isolated toad skin and its response to antidiuretic hormone. *Acta Physiol. Scand.* **39**:228.
3. HAYS, R. M., and A. LEAF. 1962. The state of water in the isolated toad bladder in the presence and absence of vasopressin. *J. Gen. Physiol.* **45**:933.
4. HAYS, R. M., and A. LEAF. 1962. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45**: 905.
5. DAINTY, J., and C. R. HOUSE. 1966. An examination of the evidence for membrane pores in frog skin. *J. Physiol.*, (*London*). **185**:172.
6. WHITTEMBURY, G. 1962. Action of antidiuretic hormone on the equivalent pore radius at both surfaces of the epithelium of the isolated toad skin. *J. Gen. Physiol.* **46**:117.
7. LOEB, S. 1966. A composite tubular assembly for reverse osmosis desalination. *Desalination.* **1**:35.
8. RILEY, R. L., U. MERTEN, and J. O. GARDNER. 1966. Replication electron microscopy of cellulose acetate osmotic membranes. *Desalination.* **1**:30.
9. JAGUR-GRODZINSKI, J., and O. KEDEM. 1966. Transport coefficients and salt rejection in uncharged hyperfiltration membranes. *Desalination.* **1**:327.
10. PAPPENHEIMER, J. R. 1953. Passage of molecules through capillary walls. *Physiol. Rev.* **33**:387.
11. ROBBINS, E., and A. MAURO. 1960. Experimental study of the independence of diffusion and hydrodynamic permeability coefficients in collodion membranes. *J. Gen. Physiol.* **43**:523.
12. LEAF, A. 1959. The mechanism for the asymmetrical distribution of endogenous lactate about the isolated toad bladder. *J. Cellular Comp. Physiol.* **54**:103.
13. KATCHALSKY, A., and O. KEDEM. 1962. Thermodynamics of flow processes in biological systems. *Biophys. J.* **2**:53.
14. DURBIN, R. P. 1960. Osmotic flow of water across permeable cellulose membranes. *J. Gen. Physiol.* **44**:315.
15. WANG, J. W., C. V. ROBINSON, and I. S. EDELMAN. 1953. Self-diffusion and structure of liquid water. III. Measurement of self-diffusion of liquid water with H^2, H^3, and O^{18} as tracers. *J. Am. Chem. Soc.* **75**:466.
16. THAU, G., R. BLOCH, and O. KEDEM. 1966. Water transport in porous and nonporous membranes. *Desalination.* **1**:129.
17. BRANDT, P. W. 1962. A consideration of the extraneous coats of the plasma membrane. *Circulation.* **26**:1075.

18. PEACHEY, L. D., and H. RASMUSSEN. 1961. Structure of the toad's urinary bladder as related to its physiology. *J. Biophys. Biochem. Cytol.* **10**:529.
19. LEAF, A., and R. M. HAYS. 1962. Permeability of the isolated toad bladder to solutes and its modification by vasopressin. *J. Gen. Physiol.* **45**:921.
20. LICHTENSTEIN, N. S., and A. LEAF. 1965. Effect of amphotericin B on the permeability of the toad bladder. *J. Clin. Invest.* **39**:630.
21. MENDOZA, S. A., J. S. HANDLER, and J. ORLOFF. 1967. Effect of amphotericin B on permeability and short-circuit current in toad bladder. *Am. J. Physiol.* **213**:1263.
22. GRANTHAM, J. J., and M. B. BURG. 1966. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Am. J. Physiol.* **211**:255.