Studies on the Movement of Water through the Isolated Toad Bladder and Its Modification by Vasopressin

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ABSTRACT Measurements of diffusion permeability and of net transfer of water have been made across the isolated urinary bladder of the toad, *Bufo marinus*, and the effects thereon of mammalian neurohypophyseal hormone have been examined. In the absence of a transmembrane osmotic gradient, vasopressin increases the unidirectional flux of water from a mean of 340 to a mean of 570 μl per cm^2 per hour but the net water movement remains essentially zero. In the presence of an osmotic gradient but without hormone net transfer of water remains very small. On addition of hormone large net fluxes of water occur; the magnitude of which is linearly proportional to the osmotic gradient. The action of the hormone on movement of water is not dependent on the presence of sodium or on active transport of sodium. Comparison of the net transport of water and of unidirectional diffusion permeability of the membrane to water indicates that non-diffusional transport must predominate as the means by which net movement occurs in the presence of an osmotic gradient. An action of the hormone on the mucosal surface of the bladder wall is demonstrated. The effects of the hormone on water movement are most simply explained as an action to increase the permeability and porosity of the mucosal surface of the membrane.

INTRODUCTION

The studies of Koefoed-Johnson and Ussing (1) and of Andersen and Ussing (2) on the isolated toad skin have done much to clarify the mode of passage of water through living membranes and the means by which such passage may be modified by neurohypophyseal hormones. The present study was undertaken to examine water movement through another anuran tissue, the toad bladder, which has been known to serve as a reservoir from which water can be reabsorbed in response to dehydration or administration of neurohypophyseal hormones (3–5). Bentley (6, 7) has recently reported his studies

on water transport through this tissue which is transparently thin and readily studied *in vitro* (8).

The present paper, in accord with Ussing's studies, indicates that very little net water movement occurs across the bladder except in the presence of neurohypophyseal hormones and that net transport of water is predominantly non-diffusional resulting from some form of bulk flow in the bladder, presumably similar to that observed in inert porous membranes (9, 10). The accompanying papers will deal with the movements of small solute molecules through this tissue and examine the validity of applying the viscous and diffusive properties of bulk water to water crossing this living tissue.

METHODS AND MATERIALS

The urinary bladder of the toad, *Bufo marinus*, was utilized in these studies. Previous publications from this laboratory (8, 11–13) have outlined the general techniques employed and described the appearance and several of the properties of this tissue.

Unidirectional flux of water through the bladder was measured with deuterium or tritium oxides. Tracer amounts of these isotopes of water were used to measure conventional transepithelial permeability coefficients (12). The tagged water was added to the medium bathing one surface of the bladder mounted between two halves of a lucite chamber. The medium on both sides of the bladder was continuously stirred by a bubbling device. From the rate of appearance of the isotopic water in the medium bathing the opposite surface and its concentration in the medium on the side to which it was added the permeability coefficient, K_{trans}, is readily calculated according to the familiar Fick equation.

$$K_{trans} = \frac{Qw}{(C_1 - C_2)A}$$

in which Qw is the net increase of isotopic water per unit time on the side opposite to that to which it was initially added and C_1 and C_2 are the mean concentrations of isotope on the two sides during the 30 minute periods of measurement. A is the cross-sectional area of the chamber. The diffusion permeability of the bladder to labeled water is so high that even in the course of a 1 hour experiment the concentration of isotopic water in the medium on the side to which it was added falls appreciably and the concentration on the opposite side increases sufficiently to give rise to a significant back flux of labeled water. However, when the values of K_{trans} obtained by the simple Fick calculation were compared with those obtained from the more rigorous Northrop-Anson equation (9) agreement within 2 per cent was obtained.

Net water movement was measured gravimetrically from the change in weight of medium in the two half-chambers during the course of an experiment. A known weight of medium was added by calibrated pipets to each weighed half-chamber. At the conclusion of an experiment the medium from each half-chamber was carefully aspirated into weighed syringes and placed in weighed covered flasks. From the difference in weight of the half-chambers, syringes, and flasks (corrected for the

weights of any solutions added or subtracted from either half-chamber during an experiment) the net water movement through the bladder was obtained.

In order to facilitate comparison in the same units of the unidirectional flux of water across the bladder measured isotopically with the net transfers of water measured gravimetrically, the value of K_{trans} has been converted to a flux. K_{trans} multiplied by the molar concentration of water in the bathing medium (approximately 55.3 mM per cm^3 of medium) times the partial molal volume of water (approximately 18 microliters per millimole) gives the unidirectional flux of water across the isolated bladder which will be expressed as microliters per cm^2 per hour ($\mu l/cm^2/hr.$). The unidirectional flux of water was measured under conditions of zero net water transfer except as specifically indicated and then the measurements are reported in the mucosal to serosal direction, with the net water movement, for purposes of uniformity.

The deuterium oxide was reduced over hot zinc in an evacuated system and the DH formed was measured in a mass spectrometer (Model 21-201, Consolidated Electrodynamics Corporation, Pasadena, California). The majority of measurements of diffusion permeability utilized tritium oxide which was counted with a tricarb liquid scintillation spectrometer (Packard Instruments Company, La Grange, Illinois) using a vehicle containing 0.05 gm *p-bis* [2-(5-phenyloxazolyl)] benzene, 7 gm 2,5-diphenyloxazole, and 50 gm naphthalene made up to a liter with *p*-dioxane. The final water content was 1.0 ml per 15 ml of this scintillation mixture (14). The C^{14}-carboxy inulin was similarly counted.

By tissue labeling with isotopic water is simply meant the specific activity of tritium in the tissue water divided by its specific activity in the labeled medium expressed as per cent. The inulin space was similarly measured allowing 2 hours for equilibration with the tissue of the C^{14}-carboxy inulin (New England Nuclear Corporation) added to the serosal medium. The tritium oxide was eluted from a trichloracetic acid homogenate of the tissue, as described (15), after the wet weight of the tissue had been obtained. The C^{14}-carboxy inulin was eluted from the dried tissue with 3 ml of 0.05 N nitric acid after wet and dry weights of tissue were obtained and an aliquot of this volume was counted in the liquid scintillation spectrometer. The water content of the toad bladder after careful blotting on Whatman No. 54 filter paper was considered to equal the loss in weight after drying at 95°C for 24 hours and averaged 80.8 ± 1 per cent (SD) in 28 measurements. Hence a figure of 81 per cent was used to calculate the concentration of tritium in total tissue water when this value could not be directly determined.

The basic Ringer's solution contained Na, 113.5; K, 3.5; HCO_3, 2.38 meq per liter; and Ca, 0.89 mM. Its total solute concentration was 218 mOsM per kg water and its pH, 7.8–8.0, when equilibrated with air. The results of the permeability measurements were the same when determined in a Ringer's solution with pH adjusted to 7.4. The diluted Ringer's solution used in contact with the mucosal surface to produce a transmembrane osmotic gradient was prepared by diluting the Ringer's solution with a solution of identical composition except for the omission of all the sodium chloride. All osmotic activities of the media were measured at the start and end of each experiment with a Fiske osmometer. The choline Ringer's solution was made up with choline replacing all the sodium; osmotic activity was adjusted to 218 mOsM per kg water with a concentrated choline stock solution.

RESULTS

Permeability to Water in Absence of an Osmotic Gradient

Table I shows that in ten control experiments the unidirectional flux of water measured through the isolated toad bladder with isotopic water, deuterium, or tritium oxides, is high and remains on the average quite constant during three consecutive 30 minute periods. In contrast, when vasopressin was added to the serosal bathing medium at the end of the first period in an additional thirteen experiments a definite increase in the unidirectional flux was observed. The average increase for the two periods following hormone

TABLE I

EFFECT OF VASOPRESSIN ON DIFFUSION PERMEABILITY (UNIDIRECTIONAL WATER FLUX) OF ISOLATED TOAD BLADDER TO WATER MEASURED WITH DHO OR THO IN ABSENCE OF OSMOTIC GRADIENT

	Periods (30 min.)			Mean difference (Period 2 − 1)	SE mean difference	*P*
	1	2	3			
	μl/cm²/hr.					
Control	343	338	339	−5	±9	>0.5
With hormone*	338	543	599	+205	±35	<0.001

* Hormone added at end of first period (2 units commercial vasopressin to medium bathing serosal surface).

Control includes ten experiments; seven measured from mucosal to serosal surface and three in opposite direction.

Hormone-treated group includes thirteen experiments; nine were mucosal to serosal fluxes and four were measured in reverse direction.

15 ml frog Ringer's solution bathing each surface; 3.14 cm² area of chambers.

was 70 per cent of the initial value. Since neurohypophyseal hormones added to the medium bathing the mucosal surface of the bladder are without effect on this tissue (8), the hormone in these experiments was always added to the serosal bathing medium.

The equality of permeability to water in the two directions across the bladder is documented by the results shown in Table II. In these experiments net movement of water was measured gravimetrically and one unidirectional flux was determined with THO. Within the limits of experimental error, the results indicate no net movement of water and hence an equal permeability to water in the two directions.

The effect of vasopressin is to increase the unidirectional flux of water across the bladder but in the absence of an osmotic gradient across the tissue net movement of water remains essentially zero (Table II). The small net positive movement of water (mucosal to serosal) with hormone may be secondary to the stimulation of sodium transport by the hormone (13).

However, the effect is very small and probably within the limits of error of the method used and was therefore not further pursued. The last line of Table II probably constitutes the most rigorous validation of the method

TABLE II

NET AND UNIDIRECTIONAL FLUXES OF WATER IN ABSENCE OF AN OSMOTIC GRADIENT WITHOUT AND WITH VASOPRESSIN

No. of experiments	Sodium in medium	vasopressin	Net flux	Unidirectional flux
			μl/cm²/hr.	
5	Present	Absent	−1.9 ± 1.5	394 ± 64
4	Present	Present	+3.2 ± 0.4	526 ± 65
4	Absent	Present	+0.2 ± 1.2	642 ± 84

All values of unidirectional flux were measured with THO from mucosal to serosal sides.
Net flux was measured gravimetrically; (+) indicates mucosal to serosal net movement and (−) the reverse.
Duration of experiments 60 to 90 minutes.
20 ml isotonic Ringer's solution bathing each surface of the bladder; chamber area 7.07 cm².
When vasopressin was used 1.0 unit added to each side.

TABLE III

NET AND UNDIRECTIONAL FLUXES OF WATER ACROSS THE ISOLATED TOAD BLADDER WITH AN OSMOTIC GRADIENT AND WITHOUT AND WITH VASOPRESSIN

No. of experiments	Vasopressin	Osmotic gradient	Net flux	Unidirectional flux	Tissue dry weight
		mOsM/kg water	*μl/cm²/hr.*		*per cent*
6	Absent	160	5 ± 1.3	312 ± 32	20.2 ± 0.39
6	Present	60	77 ± 7	572 ± 36	
8	Present	150	186 ± 9	717 ± 24	17.4 ± 0.24
5	Present	170	208 ± 21	766 ± 34	

In all experiments serosal medium was Ringer's solution and mucosal medium was appropriately diluted.
20 ml medium bathing each side; chamber area 7.07 cm².
1.0 unit vasopressin added to each side.
Net flux measured gravimetrically and unidirectional flux measured with THO in mucosal to serosal direction.
Duration of experiments 60 to 90 minutes; unidirectional flux is mean of two 30 minute periods.
Results are averages plus or minus the standard error.

used to measure net movements of water as no transport of sodium was occurring to affect a possible movement of water.

Permeability to Water in Presence of an Osmotic Gradient

Table III shows the unidirectional and net fluxes of water from mucosal to serosal surfaces in the presence of an osmotic gradient established by bathing

the serosal surface with Ringer's solution and the mucosal surface with diluted Ringer's solution to attain the appropriate transmembrane concentration gradient. The initial and final total solute concentrations of the two bathing media were separately averaged and their difference gives the mean osmotic gradient present during the experiment. The value actually determined for

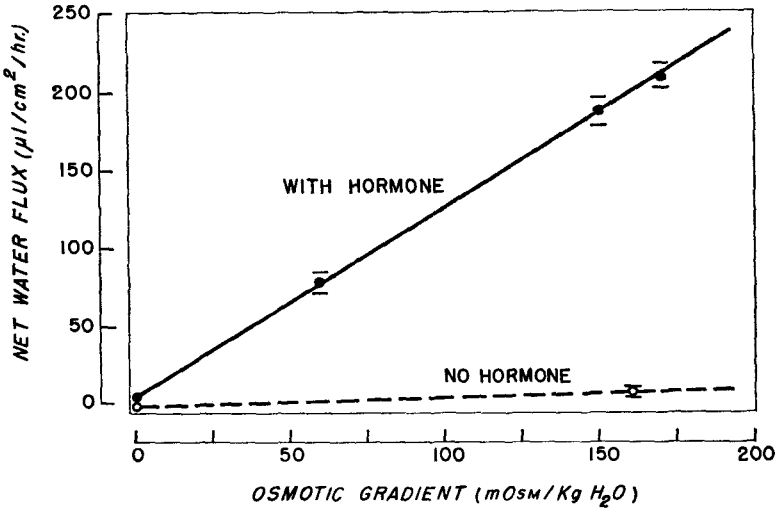

FIGURE 1. Dependence of net water flux on osmotic gradient in presence or absence of neurohypophyseal hormone. (Reprinted from Membrane Transport and Metabolism, (A. Kleinzeller and A. Kotyk, editors), Prague, The Publishing House of Czechoslovakia, 1961, 252). Data from Tables II and III together with additional experiments, in which net fluxes were determined without simultaneous unidirectional fluxes, are plotted. Thus, with hormone the points, at osmotic gradients of 59, 150, and 170 represent six, eight, and fifteen experiments, respectively, and their mean values and SE of means are 77.2 ± 6.9, 186 ± 8.8, and 209 ± 7.9 μl per cm² per hour, respectively. The regression equation relating water movement to osmotic gradient is $y = 1.22\ x + 4.22$. Twelve experiments without hormone but with an osmotic gradient of 160 mOsM per kg water gave a mean net flux of +4.3 ± 1.3 μl per cm² per hour.

each experiment varied only 2 or 3 mOsM per kg water from the figure recorded for the group.

In the absence of hormone the net movement of water remains small despite the osmotic gradient. Following the addition of hormone, however, the net movement of water increases strikingly. Although there is considerable variation from experiment to experiment the net movement is linearly proportional to the concentration gradient as seen in Fig. 1. The mean net flux at each concentration gradient studied is plotted with ± one standard error of the mean. The small net movement in the absence of hormone contrasts with the large net movement with vasopressin. With the largest osmotic gradient studied the net movement of water attains a value over

one-fourth that of the unidirectional flux measured simultaneously in the same direction.

The Effect of Sodium-Free Ringer's Solution and Potassium-Free Ringer's Solution on Permeability to Water

Because a function of this membrane is to transport sodium actively from the mucosal to serosal bathing medium, in the same direction as the net movement

TABLE IV

NET AND UNIDIRECTIONAL FLUXES OF WATER ACROSS ISOLATED TOAD BLADDER WITH VASOPRESSIN IN SODIUM-FREE OR POTASSIUM-FREE RINGER'S SOLUTION

	No. of experiments	Net flux Δ_w	Unidirectional flux ϕ_w
		μl per cm² per hr.	
A. Sodium-free choline Ringer's and Ringer's solution compared			
Sodium-free	6	91 ± 13	503
With sodium	6	133 ± 16	516
Mean difference		42	
SE mean difference		±23	
B. Potassium-free Ringer's solution and Ringer's solution compared			
Potassium-free	5	170 ± 21	573
With potassium	5	194 ± 11	672

Experiments in A and B done on paired bladder halves.
Osmotic gradient 150 mOsM per kg water in all experiments with mucosal side hypotonic.
20 ml solution bathing each side of membrane; chamber area 7.07 cm²; 0.5 or 1.0 unit vasopressin added to serosal medium in each experiment.
Analysis of medium at end of experiment showed <0.4 meq per liter of sodium in two experiments with sodium-free medium. Mean potassium concentration was 0.072 meq per liter at end of experiments in potassium-free medium while control half-bladders were exposed to 3.5 meq of potassium in the medium.
Unidirectional flux measured with THO and net flux obtained gravimetrically both from mucosal to serosal surfaces.
Values are averages plus or minus standard errors.

of water, it became important to determine whether the large net movements shown in Table III were in some way dependent upon the active transport of sodium. This was tested in two ways: (*a*) by replacing all the sodium in the medium with a choline-Ringer's solution so that no sodium was present to be transported and (*b*) by using a potassium-free Ringer's solution which stops all active sodium transport even in the presence of sodium (16).

In the sodium-free, choline Ringer's solution, just as in the sodium Ringer's solution, little net water transport occurs with an osmotic gradient in the absence of added hormone. Thus in four experiments in which the osmotic

gradient was 150 mOsM per kg water, net movements of +8, −1, +4, and −7 μl per cm² per hour were obtained. When the hormone was added, however, a large net movement of water occurred as shown in Table IV A in which the fluxes in choline and sodium Ringer's solution on paired half-bladders are recorded. Unidirectional water fluxes were the same but the net water movement was depressed, but not significantly, in the choline Ringer's solution. In five experiments in the potassium-free Ringer's solution again only a slight decrease was noted as compared to the paired controls, as shown in Table IV B. In an additional seven unpaired experiments in potassium-free Ringer's solution the net water movement averaged 166 ± 14 as compared with another 8 unpaired measurements in regular Ringer's solution which averaged 186 ± 9 μl/cm²/hr. Thus there appears to be a slight (but not statistically significant) reduction in net transfers of water in the absence of potassium. However, the reduction by our technique does not appear to be as large as has been reported (7).

Site of Action of the Hormone in the Bladder

We have previously described a simple method for determining at which surface of the toad bladder the hormone has its action with respect to the permeability changes it induces in the bladder (15, 17). This method is based on a determination of the permeability of the two opposite surfaces of the tissue to the lactate ion (12). It was found that the mucosal surface was only about one-fifteenth as permeable to lactate as was the serosal surface. Because of the simple histology of this bladder and the fact that the mucosal surface directly contacts the mucosal bathing medium we have tentatively identified the two diffusion barriers with the opposite surfaces of the single layer of mucosal cells. If the resistance of the bladder to diffusion can be subdivided into two such barriers, then it is clear that the increased diffusion of water all the way across the bladder induced by hormone could result from an action of the hormone to increase the permeability of either one or both of the diffusion barriers. If the tagged water, THO, is added to the mucosal bathing medium an action of the hormone on the mucosal diffusion barrier will result in an increased rate of entry of the tagged water into the bladder and, hence, to a rise in its concentration within the tissue. On the other hand, if the hormone acts only to increase the permeability of the serosal diffusion barrier, then the tagged water molecules will leave the bladder more rapidly across that surface and the concentration of tagged water within the cell will fall.

Table V shows the results of experiments done simultaneously on paired half-bladders, one treated with hormone and the other serving as control. The time of exposure to the THO added to the mucosal bathing medium was kept exactly the same for both half-bladders. In eight out of the ten

paired experiments, the concentration of THO in the tissue water was higher in the hormone-treated bladder half and the difference for the entire group is significant at the 2 per cent level. Although the difference between control and hormone-treated pairs is not as striking as was the case for urea (15) (with which the per cent increase in transmembrane penetration after hormone is much greater than for water), nevertheless, a definite action of the hormone on a barrier to water diffusion, located at or near the mucosal surface of the bladder, is demonstrated. The results do not permit us to say whether there was no effect or a lesser effect of the hormone on the serosal

TABLE V

EFFECT OF NEUROHYPOPHYSEAL HORMONE ON LABELING OF TISSUE WATER BY THO FROM MUCOSAL SIDE

Experiment	Time elapsed after THO added	Per cent labeling Vasopressin	
		Absent	Present
	min.		
1	10	21	32
2	10	23	29
3	10	23	19
4	10	25	29
5	20	12	32
6	20	21	27
7	20	20	30
8	20	22	17
9	20	22	33
10	20	19	24
Mean		20.6	27.2
$\Delta \pm$ SE mean		6.6 ± 2.3	
P		<0.02	

side as well. The finding of an action of the hormone again at or near the mucosal surface is especially striking as the hormone is effective only when applied to the serosal bathing medium (8).

The same conclusion regarding the site of action of the hormone may be drawn from an entirely different approach. In Table III the dry weight of tissue at the conclusion of each experiment expressed as per cent of wet tissue weight is given for the two groups of experiments with comparable osmotic gradients, of which only one was exposed to hormone. The increase in water content of the hormone-treated bladders of 2.79 ± 0.45 per cent is significant even when this figure is reduced to correct for the slightly lower osmotic activity of the serosal bathing medium in the experiments with hormone added. This increased water content of the tissue suggests cell swelling in the

presence of hormone. But the changes in per cent dry weight apparently do not reflect the degree of cell swelling which occurs. When, in addition to the dry weight, the inulin space of the tissue was measured from the serosal side, this value was markedly reduced, as shown in Table VI. As the net movement of water from mucosal to serosal surface during the measurement would be expected to depress the size of the serosal inulin space by only some 2 to 15 per cent, these data constitute strong evidence of swelling of the cells.

TABLE VI

EFFECT OF NEUROHYPOPHYSEAL HORMONE ON TISSUE SOLIDS AND SEROSAL INULIN SPACE

Experiment	Inulin space Vasopressin		Tissue dry weight Vasopressin	
	Absent*	Present*	Absent*	Present*
A. Without an osmotic gradient				
1	35	34	19.7	18.8
2	42	34	18.7	17.8
B. With an osmotic gradient‡				
3	38	14	19.1	17.2
4	40	12	20.0	17.8
5	34	9	17.7	16.0
6	44	10	18.4	16.6

Duration experiments 120 minutes; 2 units vasopressin added to serosal bathing medium after 60 minutes.

Inulin space expressed as per cent of total tissue water; dry weight is per cent of wet tissue weight.

* Experiments done on paired bladder halves.

‡ Ringer's solution on serosal side of membrane undiluted. Ringer's solution on mucosal surface diluted to yield transmembrane osmotic gradient of 170 mOsM.kg water; 30 ml of medium on each side of 7.07 cm^2 membrane.

Under the conditions of the experiments shown in Table VI the non-inulin space increased with hormone from 61 to 89 per cent of tissue water, indicating nearly a 50 per cent increase in cell volume. Such an increase in cell volume, in association with an increased net movement of water across the bladder, can only result from an action of the hormone to increase the permeability of the mucosal surface of the membrane. Histological confirmation by electron microscopy of such swelling of the mucosal layer of cells has been obtained in this laboratory by Keller (18) and by Peachey and Rasmussen (19).

DISCUSSION

Although the permeability of the isolated toad bladder to water is high relative to other substances tested (20), nevertheless, the bladder constitutes a con-

siderable impediment to the movement of water. The self-diffusion coefficient of water in water at 25°C has been determined by Wang, Robinson and Edelman (21) to be 2.4×10^{-5} cm² per second. Accepting an average permeability coefficient (K_{trans}) of 1000×10^{-7} cm per second from Table I and a representative bladder thickness of 50 microns (estimated from a usual tissue wet weight of about 5 mg per cm² and an assumed density close to 1.0) the diffusion constant of water in toad bladder would be approximately 5×10^{-7} cm² per second or about 0.02 of its self-diffusion coefficient. Neurohypophyseal hormones approximately double the diffusion permeability of the bladder to water.

This reduction in diffusion permeability of water through the bladder could result either from the presence of a uniform surface in the bladder which has 0.02 to 0.04 of the permeability of free water or the presence of a restricted area of 0.02 to 0.04 of the bladder surface which has the permeability of free water. The latter picture has generally been assumed and a fractional area of the membrane surface which is permeable to water has been calculated. The validity of such calculations, however, depends upon the assumption that the water penetrates the rate-limiting diffusion barrier through channels having the properties of bulk water. This assumption will be considered in a later paper (22).

The finding that net movement of water is proportional to the transmembrane osmotic gradient and that no movement of water occurs in the absence of a gradient (Fig. 1) indicates that water moves passively across the bladder. The active transport of sodium contributes very little directly to net movement of water as seen by comparison of the net movement of water without an osmotic gradient in Table II but with or without sodium transport. Furthermore, a large net water movement occurs even in the complete absence of sodium (Table IV A) in response to hormone under conditions in which the hormone has no detectable effect on the energy metabolism of the tissue (23). Of course, the osmotic gradient *in vivo* must arise from the active reabsorption of sodium and this, therefore, is the energy-requiring step in water reabsorption.

The contribution of net diffusion flux to the total observed net movement of water across the bladder may be directly assessed (1, 9, 10, 24). The results of such calculations for the data shown in Table III are presented in Table VII. It is seen that only one-sixth of the small net flux occurring in the absence of vasopressin could have occurred from diffusion alone. After hormone less than 1 per cent of the larger net fluxes could be attributed to diffusion. Clearly non-diffusional transport predominates in the net movement of water across this tissue in response to hormone and an osmotic gradient.

As Pappenheimer (25), Ussing (1), and others (9, 10, 24, 26) have indicated, if one assumes that the non-diffusional component of water transfer is

laminar flow through pores which follows Poiseuille's law, one may calculate the equivalent mean pore radius of such channels assuming them to be right circular cylinders positioned perpendicularly to the surface. Table VII shows the results of such a calculation made according to the method of Robbins and Mauro (9) from the ratio of the total net flux, assumed to result largely from Poiseuille flow, and the net diffusion flux. This calculation assumes that all the area in the tissue available to penetration of water by diffusion is comprised of pores having the mean radius as indicated. According to this calculation the mechanism of action of neurohypophyseal hormone is to render more porous the permeability barrier in the membrane facilitating thereby the movement of water by bulk flow through the bladder wall. This

TABLE VII
RELATION OF TOTAL NET AND DIFFUSION NET FLUXES OF WATER THROUGH ISOLATED TOAD BLADDER WITHOUT AND WITH VASOPRESSIN

Hormone	ΔC	Total observed net flux	Diffusion net flux	Diffusion flux / Total flux	Mean pore radius
	mOsM/kg water	$\frac{moles}{sec.}$ *per* $\frac{dyne}{cm^2}$			*A*
		$\times 10^{-15}$	$\times 10^{-15}$		
None	160	0.20	3.5	1/6	8.4
Present	60	8.0	6.0	1/134	41
Present	150	7.7	7.0	1/110	37
Present	170	7.6	7.4	1/102	36

has been the view proposed by Ussing and associates (1, 2) from their studies with the toad skin.

The net transfer of water induced by hormone is considerably larger with bladder for a given osmotic gradient than with toad skin. In fact, even with the increase in diffusion permeability of the bladder to water in the presence of vasopressin, the calculated dimensions of such "pores" are much larger than the values estimated for toad skin (1). These calculations, however, utilize measured permeabilities across the entire thickness of the tissue as though it were a homogeneous structure. The bladder, morphologically and functionally, is clearly non-homogeneous, and we have indicated how the contribution of each of the series diffusion barriers to the total transmembrane permeability coefficient may be assessed (12). The general equation is

$$K_{trans} = \left(\frac{1}{k_1} + \frac{1}{k_2} + \cdots \frac{1}{k_n}\right)^{-1}$$

which for the simplest case of two permeability barriers at opposite surfaces of the bladder, for which evidence has been deduced, can be evaluated from data presented in this study. As indicated elsewhere (15) the mucosal, k_m, and serosal, k_s, permeability coefficients can be evaluated from the transmembrane diffusion permeability and the tissue labeling. From Table I a mean diffusion permeability of 340 μl per cm^2 per hour without hormone and of 570 μl per cm^2 per hour with hormone are obtained. We have found that by 20 minutes the water of toad bladder immersed in Ringer's solution has come into diffusion equilibrium with the medium. Therefore, from Table V we may

TABLE VIII

EVALUATION OF THE DIFFUSION PERMEABILITY TO THO OF THE OPPOSITE SURFACE OF THE TOTAL BLADDER

Vasopressin	Tissue labeling	Uncorrected			Corrected for 38 per cent inulin space	
		K_{trans}	k_m	k_s	k_m	k_s
	per cent			$\mu l/cm^2/hr.$		
Absent	18.6	340	418	1828	485	1133
Present	29.2	570	805	1952	1075	1212

k_m and k_s are permeability coefficients of mucosal and serosal surface of membrane, respectively, and K_{trans} is the transmembrane permeability coefficient from Table I.
The value of 38 per cent for the inulin space measured from the serosal surface is the average of the measurements of Table VII made without vasopressin.
The values of tissue labeling with THO are the average of five paired measurements made at 20 minutes from Table VI when diffusion equilibrium of tissue and medium water was known to have occurred.

obtain the steady state tissue labeling from the five paired values obtained at 20 minutes (omitting Experiment 8) with the isotopic water added initially to the mucosal bathing medium. An average of 18.6 per cent without and 29.2 per cent in the presence of vasopressin was obtained.

Since,

$$k_s = \frac{K_{trans}}{\text{Per cent labeling}} \times 100$$

and,

$$k_m = \frac{K_{trans}}{\text{100 minus Per cent labeling}} \times 100$$

these values may be calculated and the results are shown in Table VIII. Although the cytoplasm between the two surface diffusion barriers could have

been separately evaluated the diffusion of water through cytoplasm must approach its free diffusion rate and hence would be so rapid as to constitute an insignificant barrier to diffusion compared with the two surfaces. The small contribution of the cytoplasm to the K_{trans} has therefore been ignored.

In order now to evaluate the porosity of the two surface diffusion barriers we need only to know the appropriate driving force or osmotic gradient across each face in an actual experiment. As indicated in Table VI, addition of hormone in the presence of an osmotic gradient of 170 mOsM per kg water resulted in an increase in non-inulin space from 61 to 89 per cent of tissue water. With a serosal medium of 220 mOsM per kg of water and a dilute mucosal medium of 50 mOsM per kg of water the mean concentration in the cell (assuming no change in its solute content) would be 146 mOsM per kg of water. Repeating the calculations of pore radius using these concentration gradients and the diffusion permeability coefficients of Table VIII, mean values of 38 and 40 A for mucosal and serosal surfaces, respectively, are obtained. In the presence of the hormone, therefore, these calculations suggest that the opposite surfaces of the membrane attain essentially equal permeability and porosity to water.

In so far as they apply to movements of water, these findings and interpretations support the earlier studies of Koefoed-Johnsen and Ussing (1) on water transfer through another anuran tissue.

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