The State of Water in the Isolated Toad Bladder in the Presence and Absence of Vasopressin

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ABSTRACT An attempt has been made to assess the validity of applying the frictional and viscous coefficients of bulk water to the movement of water and solutes through the urinary bladder of the toad. The temperature dependence of diffusion of THO, C^{14}-urea, C^{14}-thiourea, and net water transfer across the bladder was determined in the presence and absence of vasopressin. The activation energy for diffusion of THO was 9.8 kcal per mole in the absence of vasopressin and 4.1 kcal per mole with the hormone present. Activation energies simultaneously determined following vasopressin for diffusion and net transfers of water were similar, and in the same range as known activation energies for diffusion and viscous flow in water. Urea had activation energies for diffusion of 4.1 and 3.9 kcal per mole in the absence and presence of vasopressin, respectively. Thiourea had a high activation energy for diffusion of 6.3 kcal per mole, which was unchanged, 6.6 kcal per mole, following hormone. These findings suggest that in its rate-limiting permeability barrier, water is present in a structured state, offering a high resistance to penetration by water. Vasopressin enlarges the aqueous channels so that the core of water they contain possesses the physical properties of ordinary bulk water. Urea penetrates the tissue *via* these aqueous channels while thiourea is limited by some other permeability barrier.

INTRODUCTION

The preceding two papers (1, 2) were concerned with the movements of water and small solutes through the isolated toad bladder and the effects of neurohypophyseal hormones thereon. Conventional analysis of the data leads to an apparent discrepancy between the porosity of the bladder wall necessary to account for the high rates of net water transfer achieved in the presence of vasopressin and the low degree of permeability retained to most small

solutes. Although the presence of series diffusion and porous permeability barriers may satisfactorily account for the observations (3), such analyses all tacitly assume that water in a living tissue in proximity to complex surfaces and in very small channels retains the properties of bulk water. The present study was undertaken as an initial attempt to examine this assumption since a considerable amount of recent evidence indicates that in close proximity to both polar and non-polar surfaces the properties of water may be markedly different from those of bulk water (4–8).

Both theory (9, 10) and experiment (10) support the view that viscosity nd self-diffusion in bulk water are largely determined by intermolecular hydrogen bonding. Deductions regarding the degree of hydrogen bonding and therefore the state of water can be made from the activation energies for diffusion and viscous flow in water. The temperature dependence of both processes has been shown by Wang and associates (10) to be essentially identical, yielding activation energies of 4.6 and 4.59 kcal per mole at 25°C, respectively. Therefore we have determined the temperature dependence for both diffusion of THO and net transfers of water through the toad bladder and also for the diffusion of urea and thiourea. The results indicate that in the absence of vasopressin activation energies considerably greater than the established figure for self-diffusion of water are observed. Following addition of vasopressin, by contrast, activation energies for both diffusion and net transfer approximate the values obtained in liquid water. These results have a bearing on the state of water and on the action of vasopressin in the bladder.

METHODS

The temperature dependence of diffusion of THO and of small solutes through the isolated toad bladder was determined by two different methods.

(*a*) *Chamber Technique* Lucite chambers of the type previously employed (11) were used; the toad bladder divided the two chamber halves, which were filled with 25 ml of phosphate-buffered, bicarbonate-free Ringer's solution, pH 7.3. No osmotic gradient was present in the chamber experiments. The chamber reservoirs were secured in a nalgene tank containing water maintained at a constant temperature (±0.2°) by a Wilkins-Anderson lo-temp bath (Wilkins-Anderson Company, Chicago). Short lengths of rubber tubing led from the reservoirs through water-tight fittings to the chambers. The Ringer's solution was circulated from the reservoirs to the chambers by a bubbling device (11). Circulation was rapid enough so that the temperature of the Ringer's solution in the reservoir did not differ measurably from the temperature of the solution bathing the membrane, as determined by a Tri-R electronic thermometer (Tri-R Instruments, Jamaica, New York) placed against the membrane. Short-circuit current was monitored continuously throughout the experiments. Measurements of diffusion were carried out over a temperature range of 5–35°C. A few measurements were made at 3°C and a few as high as 42°C. In

three experiments observations were made on the same half-bladder before and after the addition of vasopressin; in the remaining experiments, separate halves were used for the pre- and posthormone measurements. In carrying out the permeability measurements, the sequence of temperatures chosen was randomized, to avoid any systematic error introduced by a progressive rise or fall in temperature. After a given temperature was attained, an equilibration period of 20 to 40 minutes was allowed to elapse before sampling was begun. The sampling period was 15 minutes in the case of tritiated water and 20 minutes for C^{14}-urea. To determine whether or not the rate of diffusion of water or urea was changing as a result of changes in the membrane with time, it was the practice to repeat a measurement at room temperature at least once during the course of an experiment. Values for these duplicate determinations differed from the original by a mean value of 16.8 $\pm$5 per cent (SE).

(*b*) *Bag Technique* The alternative method used to determine the temperature dependence of diffusion and flow through the bladder was the *bag technique*. One half-bladder was tied over a short length of polyethylene tubing (inside diameter 5.0 mm) with serosal surface outward and filled after several rinses with 3.0 ml. of the appropriate fluid containing THO, C^{14}-urea, or C^{14}-thiourea. The bag and its contents were then dipped into a rinse of Ringer's solution, quickly weighed (precision torsion balance, Roller-Smith, Bethlehem, Pennsylvania), dipped once again into the rinse, and then suspended with the bag immersed in a beaker containing 40 ml Ringer's solution. The beaker in turn was immersed in a constant temperature bath at 5 or 10°C as the lower temperature used and then in another bath at 31°C. The duration of immersion at each temperature was exactly 10 minutes. With a micropipet 100 μl were removed from the bag contents at the midperiod for counting. At the end of the 10 minute period the bag was removed from the medium, drained by contact with the side of the beaker, quickly weighed, emptied, refilled again with 3 ml of solution kept at the same temperature at which the bag was to be incubated, rinsed on the serosal side, and reimmersed for the next experimental period. Mixing of bag contents and of the incubating medium was accomplished by suspending the tubing and bag by a loop of thread from an arm which slowly moved the bag back and forth through the medium in the beaker. Samples of 100 μl were taken from the 40 ml of outside incubating medium at the conclusion of each period and a separate immersing bath was used for each experimental period. For the net flow experiments the bag was filled with Ringer's solution diluted to four fifths (176 mOsM per kg. water) of the normal osmolality (220 mOsM per kg water) to provide a gradient as the driving force for net water movement (1). Two to three periods of observation were made successively at each temperature and experiments began and ended with observations at 31°C.

When vasopressin was used it was added to yield a concentration of 12.5 milliunits per ml to the 40 ml bathing medium in the beaker in all incubations and in the rinse bath as well. The bag was incubated 30 to 60 minutes in such medium before commencement of flux measurements.

Counting of tritium and C^{14} was accomplished with a Packard Tricarb liquid scintillation spectrometer as described (1). The radioactively labeled compounds used in these experiments (THO, C^{14}-urea, and C^{14}-thiourea) were supplied by New England

Nuclear Corporation, Boston. The vasopressin used was the commercial preparation (Parke, Davis and Company, Chicago).

RESULTS

Hormonal Effects on Activation Energies for Diffusion of Water

The influence of temperature on the diffusion permeability of toad bladder to labeled water, THO, is shown in Fig. 1 and Table I. Data shown in Fig. 1 were obtained by diffusion measurements made in a lucite chamber as described. Ten experiments were made without hormone and eight with

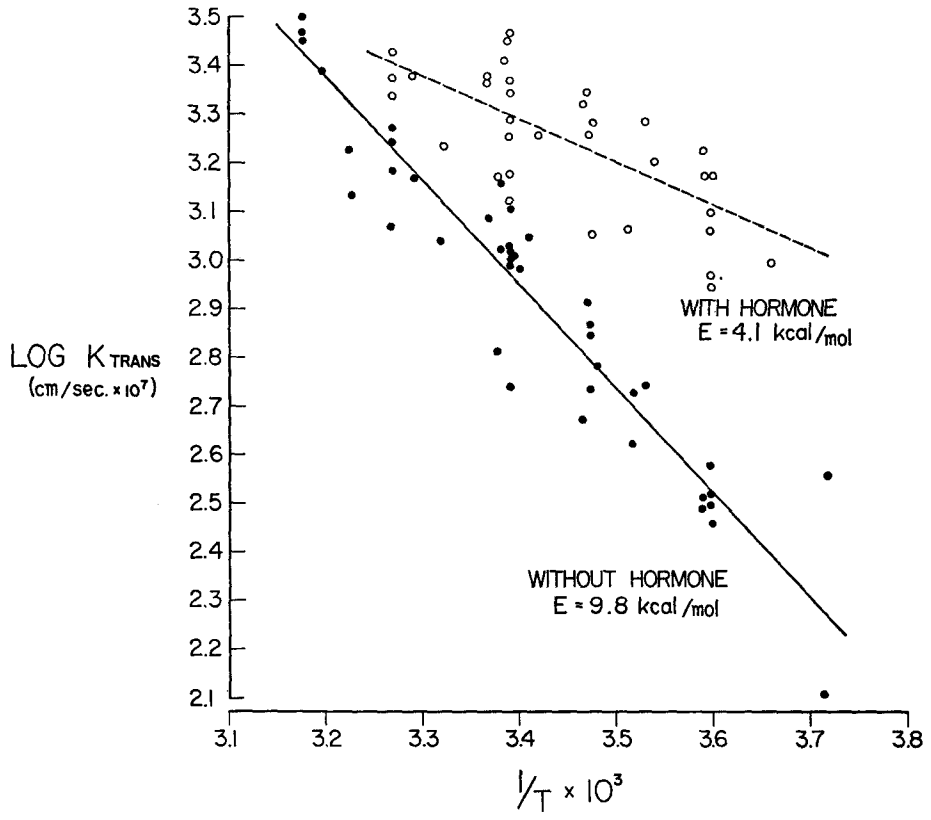

FIGURE 1. Arrhenius plot of the temperature dependence for diffusion of THO across the toad bladder, in the presence and absence of vasopressin. Measurements were made using the chamber technique. The open and closed circles represent individual determinations of permeability coefficients for tritiated water, plotted against the reciprocal of the absolute temperature. The solid and dashed lines were fitted by the method of least squares; the regression equations are: $y = -2.15\ x + 10.26$ (no hormone) and $y = -0.90\ x + 6.35$ (following hormone). The difference in calculated activation energies is highly significant ($p < 0.001$).

hormone present. Permeability coefficients were obtained at several different temperatures and the order of the observations with respect to temperature was randomized and in most experiments measurements were made twice at the same temperature. Although there is considerable scatter in the pooled data the slope of the temperature effect is significantly altered by the presence of vasopressin. The calculated experimental activation energy, ΔE, was 9.8

TABLE I
EFFECT OF TEMPERATURE ON DIFFUSION OF THO THROUGH TOAD BLADDER

	Activation energy Vasopressin		
Experiment	Absent	Present	Δ
		kcal per mole	
1	9.8	3.1	6.7
2	7.6	5.7	1.9
3	8.7	4.0	4.7
4	7.1	3.5	3.6
5	8.2	3.4	4.8
6	8.6	5.7	2.9
7	9.0	4.4	4.6
8	7.0	3.7	3.3
9	8.6	4.1	4.5
10	7.2	4.1	3.1
11	10.8	3.0	7.8
Means	8.4±0.4 (SE)	4.1±0.3 (SE)	4.3±0.5 (SE) $p < 0.001$

Measurements were made on the same half-bladder using the bag technique without and with vasopressin present in the bathing medium at two different temperatures, 5 and 31°C. Each figure is calculated from the mean of five or more 10 minute periods at the higher temperature and three or more 10 minute periods at the lower temperature.

± 0.4 (SE) kcal per mole of water in the absence of the hormone and 4.1 ± 0.7 (SE) in the presence of vasopressin. This difference is highly significant ($p < 0.001$).

Since the logarithm of the permeability coefficient appeared to be a linear function of the reciprocal of the absolute temperature over the temperature range of 5 to 30° or 40°C, a further set of measurements was made at two temperatures, 5 and 31°C using the bag technique. In these measurements the effects of presence and absence of vasopressin were observed in the same preparation. In each observation changes of permeability with time were controlled by ending with measurements at the temperature initially observed. In two experiments the hormone treatment was first and observations without hormone were made after rinsing the bag to remove hormone. The

order of control and hormone treatment had no effect on the results. The calculated values for ΔE, of 8.4 ± 0.4 (SE) and 4.1 ± 0.3 (SE) without and with hormone, respectively, in this series are comparable to the results shown in Fig. 1. Again the difference is highly significant statistically.

Activation Energies for Diffusion of Urea

Since vasopressin increases permeability of the toad bladder to urea (12), as well as to water, and urea appears to move across the bladder through aqueous

TABLE II

EFFECT OF TEMPERATURE ON DIFFUSION OF C^{14}-UREA THROUGH TOAD BLADDER

	Activation energy Vasopressin	
Experiment	Absent	Present
	kcal per mole	
1	3.4	3.5
2	3.8	4.5
3	7.4	4.3
4	5.1	2.9
5	3.4	3.8
6	4.0	4.5
7	3.1	5.4
8	2.9	3.6
9	4.6	3.0
10	2.9	3.9
Means	4.1±0.4 (SE)	3.9±0.2 (SE)

Measurements were made on the same half bladder using the bag technique without and with vasopressin in the bathing medium at two different temperatures, 5 and 31 °C. Each figure is the mean of five or more 10 minute periods at the higher temperature and three or more 10 minute periods at the lower temperature. The permeability of the bladder to C^{14}-urea was determined in the presence of 50 mM urea in the fluid within the bag and penicillin and streptomycin (0.1 mg of each per ml of medium) inside and outside the bag.

channels (2), the temperature dependence of diffusion of urea was similarly determined.

The results of the ten bag experiments shown in Table II indicate an activation energy comparable to that found for diffusion of water in the presence of vasopressin. However, with the exception of Experiment three there appeared to be no significant difference for the value obtained in the absence or presence of hormone and the respective means 4.1 ± 0.4 and 3.9 ± 0.2 kcal per mole are statistically the same. Thus, although vasopressin causes a tenfold increase in the rate of diffusion of urea through the bladder

(12), it has no effect upon the activation energies for this process. The activation energy is in the same range as the value for its free diffusion in water as reported by Longsworth (13).

Activation Energies for Diffusion of Thiourea

As vasopressin affects bladder permeability to urea but not to thiourea (2, 12) similar observations were made with the latter compound. ΔE was found to

TABLE III

EFFECT OF TEMPERATURE ON DIFFUSION OF C^{14}-THIOUREA THROUGH TOAD BLADDER

Experiment	Activation energy Vasopressin	
	Absent	Present
	kcal per mole	
1	6.9	7.0
2	8.8	7.3
3	8.1	8.6
4	7.4	6.1
5	3.6	6.0
6	4.9	4.9
7	5.0	5.0
8	7.9	7.0
9	5.3	7.5
10	5.5	6.5
Means	6.3±0.54 (SE)	6.6±0.36 (SE)

Measurements were made on the same half-bladder using the bag technique without and with vasopressin present in the bathing medium at two different temperatures, 5 and 31 °C. Each figure is calculated from the mean of five or more 10 minute periods at the higher temperature and three or more 10 minute periods at the lower temperature.

have a higher value than for urea and this value was likewise unaffected by vasopressin as shown in Table III. Mean figures are 6.3 ± 0.5 kcal per mole without vasopressin and 6.6 ± 0.4 with homone. The first four experiments were made with only tracer amounts of throurea, while the remainder were made in the presence of 91 mM thiourea to minimize any possible effect of chemical interaction between thiourea and bladder on the observed diffusion rates.

Activation Energies Simultaneously Determined for Diffusion and Net Movement of Water

As mentioned earlier the activation energies for diffusion and viscous flow in liquid water are identical (10). Since the purpose of this study was to examine

the validity of the assumption that the bulk properties of water were retained in the presence of the complex surfaces and small dimensions present in a biological system, an attempt was made to determine the activation energies simultaneously for both diffusion and net transfer of water through the toad bladder in the presence of vasopressin. The results are summarized in Table

TABLE IV

EFFECT OF TEMPERATURE ON DIFFUSION OF THO AND ON NET MOVEMENT OF WATER ACROSS ISOLATED TOAD BLADDER IN PRESENCE OF VASOPRESSIN

Experiment	Activation energy		Δ
	Diffusion	Net movement	
	kcal per mole		
1	3.8	3.4	+0.4
2	2.9	2.5	+0.4
3	2.5	3.8	−1.3
4	3.8	3.8	0
5	5.4	4.3	+1.1
6	3.5	4.4	−0.9
7	3.3	5.7	−2.4
4	3.1	4.3	−1.2
9	2.0	5.1	−3.1
10	2.8	4.7	−1.9
11	4.0	6.5	−2.5
12	3.1	3.7	−0.6
13	3.8	4.7	−0.9
14	2.8	5.1	−2.3
15	3.4	6.3	−2.9
Means	3.35±0.2 (SE)	4.55±0.3 (SE)	−1.20±0.34 (SE) $p < 0.01$

Measurements were made simultaneously in the same preparation using the bag technique with vasopressin in the bathing medium at two different temperatures 5 and 31°C or 10 and 31°C. Diffusion permeability was measured by the rate of loss of THO from the bag while net losses of water induced by an osmotic gradient were determined by changes in weight of the bag. Each figure is calculated from the mean of five or more 10 minute periods at the higher temperature and three or more 10 minute periods at the lower temperature.

IV. The value of 4.6 kcal for the activation energy for net movement was found to be slightly but significantly greater than that for diffusion in these simultaneous measurements. However, the increased complexity of the tracer measurement in the presence of net movements of water and the fair agreement between the value of 4.6 kcal for net movement and that of 4.1 kcal for diffusion from Fig. 1 and Table I when the measurements of diffusion were undisturbed by net transfers of water, are considered experimental confirmation that the same frictional forces determine diffusion and viscous

flow in the presence of the hormone in the toad bladder even as they do in liquid water.

The Effects of Vasopressin on Diffusion Permeability and Net Transfer of Water at High Temperatures

Fig. 1 suggests that at a temperature of some 44.5°C the diffusion permeability of the toad bladder should be the same in the presence or absence of vaso-

TABLE V

COMPARISON OF DIFFUSION PERMEABILITY AND NET MOVEMENT OF WATER AT 45°C WITHOUT AND WITH VASOPRESSIN

Diffusion flux Vasopressin		Net movement of water Vasopressin	
Absent	Present	Absent	Present
	μl/min./half-bladder		
214	144	0	13
164	170	5	22
227	273	0	29
263	279	6	19
248	260	4	24
246	224	9	29
262	275	4	28
246	220	0	19
228	230	4	33
233	230	4	24

Each of the nine experiments was done on a single half-bladder using the bag technique. Diffusion permeability was measured isotopically with 3.0 ml of THO-labeled Ringer's solution within the bag and net movements were measured gravimetrically with 3.0 ml Ringer's solution diluted 1 to 4 with water within the bag. Serosal medium was 30 ml of Ringer's solution. When hormone was used, 0.5 unit of vasopressin was added to the serosal bathing medium. Each value is the average of two consecutive 5 minute periods. In the last two experiments the observations with vasopressin were made first and after washing out the hormone the control values without hormone were obtained.

pressin. This was directly tested using the bag technique by measuring the diffusion permeability to THO at 44.5°C. The left portion of Table V indicates that the diffusion permeability is, in fact, essentially the same with or without hormone.

To determine further whether vasopressin still exerts an effect on *net* transfer of water at this high temperature, diluted Ringer's solution was placed within the bag and net water movements were measured first in the absence and then in the presence of vasopressin except for the last two experiments in which the order of treated and control periods was reversed. Table V shows

that there was still a large stimulatory effect of vasopressin on net transfers of water.

In these experiments the high temperatures were found to depress the electrical activity of the bladder with time. In order to minimize the possibility that the viability of the tissue was influencing the results, measurements were made on "vigorous" bladders and the order of control and hormone treatments reversed in some experiments. Out of a total of fifteen experiments the nine shown in Table V are presented because in these essentially equal diffusion permeability with or without vasopressin was attained. In the remainder diffusion permeability was still higher in the presence of the hormone in spite of the high temperature so that the finding of an effect of vasopressin on net water movement in these experiments was not pertinent to this study.

DISCUSSION

Since the classic study of Bernal and Fowler (14) it has been appreciated that even at room temperatures liquid water retains a semicrystalline structure. Frank (5) has picturesquely referred to this organization of water molecules as "flickering clusters of ice-like material." Glasstone and associates (9) and Wang and associates (10) have indicated that in liquid water at room temperature and pressure the dissociation of water molecules from the semicrystalline lattice is the rate-determining step in self-diffusion. Since the molecules in water are held together essentially by hydrogen bonding, the activation energy for self-diffusion in water should be related to the number of hydrogen bonds per molecule or, in other words, yield information regarding the extent of organization of the water in the system studied. The value of the activation energy for diffusion of THO through the isolated toad bladder in the absence of vasopressin obtained from Table I and Fig. 1 is approximately twice that of 4.6 kcal per mole obtained for the self-diffusion of water (10). This finding means that the frictional resistance which the diffusing molecule of THO encounters in the bladder is considerably greater than that encountered during self-diffusion in bulk water.

A criticism that may be raised to such an interpretation is that changes in structure of the tissue may occur with alterations in temperature. Whereas the physical chemists can measure activation energies for diffusion through an inert membrane which is itself little affected by temperature variations, the biologist must proceed with no such assurance. Thus a potential source of error in our interpretation lies in the possibility that temperature changes may themselves induce alterations in dimensions of the aqueous channels traversed by the THO molecules in crossing the bladder. Such an effect of temperature could give rise to high apparent activation energies for diffusion

without necessarily yielding information regarding the degree of bonding of water in the bladder. Thus

$$k = D\left(\frac{A}{dx}\right)$$

in which k is the permeability coefficient which was measured in this study, D is the conventional diffusion constant, A is the area per unit area of membrane actually available for penetration, and dx is the length of the diffusion path. An increase in $\frac{A}{dx}$ with increase in temperature would yield an increase in the apparent activation energy for diffusion, as calculated from Fig. 1 and Table I. The possibility of such a temperature effect cannot be excluded. However, in the presence of hormone satisfactory agreement was obtained between the measured activation energy for diffusion through the bladder and the known value for self-diffusion. Since an additional effect of temperature on the bladder would tend to cause divergence rather than agreement between these values, it is unlikely that temperature significantly alters $\frac{A}{dx}$ in the presence of hormone. We will assume that such variation in $\frac{A}{dx}$ is not the explanation for the high values of the activation energies observed in the absence of vasopressin.

If continuous aqueous channels are present through the limiting cell membrane in the absence of vasopressin as they appear to be in its presence (2), then two possibilities exist: (*a*) The channels are very small and the frictional force between water molecules and pore wall is greater than between water and water. The hormone would accordingly act by enlarging the small pores so that the central core of water in the channel would have the bulk properties of water and provide a lower resistance pathway for diffusing THO molecules than does the organized layer of water at the pore surface. (*b*) The diffusing THO molecule penetrates through a layer of structured water in the channels which is more highly organized than unconfined water at the same temperature, and the action of vasopressin would be to "melt" such a structured water. These two possibilities are depicted schematically in Fig. 2. It perhaps should be stated that diffusion through small channels does not in itself lead to high activation energies. The important determinant is the frictional resistance determined by bonding forces which the diffusing molecule encounters. Thus small holes will reduce the over-all rate at which diffusing molecules traverse a barrier but unless the diffusing species encounters a different molecular friction, the activation energy for diffusion through large and small pores would be the same.

In the absence of vasopressin both possibilities depicted in Fig. 2 have in common a highly bonded or organized water as the rate-limiting barrier to penetration of the bladder by water. Thus, if very small pores exist in which water is strongly associated with the pore wall, as pictured in A of Fig. 2, this would lead to a layer of ordered water adherent to the surface of the

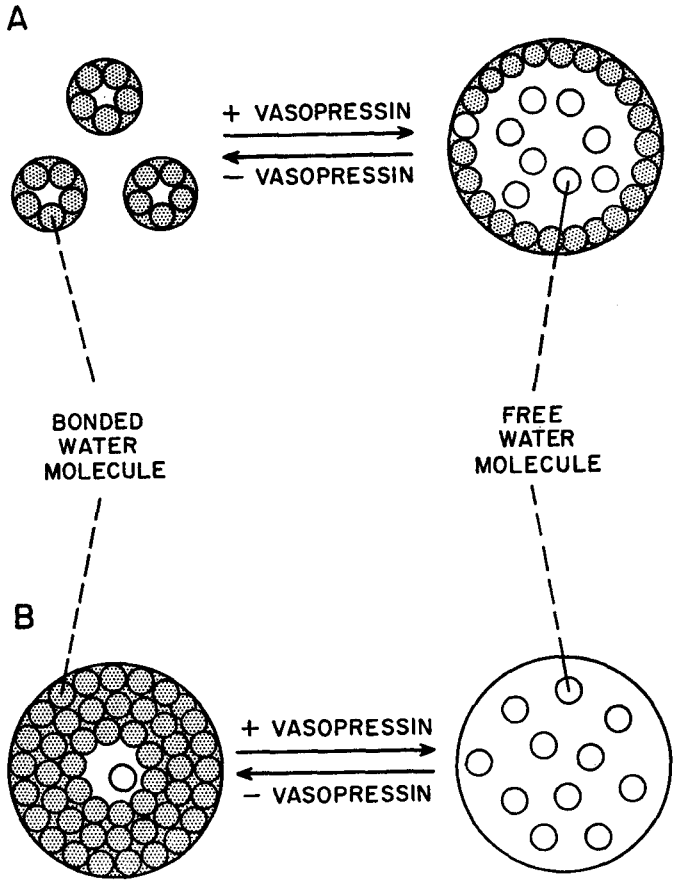

FIGURE 2. Schematic description of the two hypotheses of the hormonal action discussed in the text. In A the aqueous channels in the bladder are considered to be very small and the frictional force or bonding between water molecules and pore wall is greater than between water and water. Vasopressin is pictured as reversibly making several small channels into one large pore in which the central core of water possesses the physical characteristics of bulk water.

In B the actual confines of the aqueous channels through the bladder are considered fixed but in the absence of vasopressin the channels are clogged by a structured water which is more highly organized than unconfined water at the same temperature. The action of vasopressin is pictured as reversibly "melting" such a structured water perhaps through an effect on the walls of the pores but without actually altering the confines of the pores.

pore and extending its structure to block the small lumen. In B of Fig. 2 the larger lumen is also considered to be effectively obstructed by an "ice-like" water. In this case, however, water is bonded on the average more frequently to neighboring water molecules than to the pore surface.

From the data in Table V it appears possible to make a choice between the two alternative modes of action of vasopressin depicted in Fig. 2. Table V confirms the expectation from Fig. 1 that at approximately 45°C the diffusion permeability of the toad bladder to THO should be essentially the same in the presence or absence of vasopressin. According to B in Fig. 2 this would mean that the high temperature had broken down the structured water so that no further hormonal effect would be expected on diffusion of water. But at this temperature the net transfer of water should be increased and also unaffected by addition of vasopressin: Table V, however, indicates that net movement of water even at this temperature remains low and is distinctly augmented by addition of vasopressin. Therefore, from these results vasopressin must have some action on the structure of the membrane such as that depicted in A (Fig. 2) and as previously postulated by Koefoed-Johnsen and Ussing (14 *a*).

In his classic studies on Brownian movement, Einstein (15) demonstrated theoretically that the diffusion constant depends only on the size of the diffusing particle and the viscosity of the medium through which diffusion takes place. This theoretical identity of the frictional forces for diffusion and viscous flow received experimental confirmation by Wang and associates who found identical activation energies for diffusion and viscous flow in water (10), as mentioned. An attempt was made to measure the activation energies simultaneously for the diffusion permeability of the bladder to THO and for net movements of water induced by an imposed osmotic gradient. Such small net transfers of water occur in the absence of neurohypophyseal hormones that the temperature dependence of this process could not be determined. Satisfactory data could only be obtained with hormone present. The agreement between activation energies for diffusion and viscous flow is sufficiently close considering the experimental difficulties and error, as discussed, to constitute confirmation of equal frictional terms for these two processes in the toad bladder, just as is the case in ordinary, unconfined water.

Application of the theory of absolute reaction rates of Eyring (9) to the process of diffusion indicates that a large positive entropy change must have accompanied the hormonal effect in the membrane. Thus

$$D = e\lambda^2 \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta E/RT}$$

in which D is the diffusion constant, λ, the distance between the successive equilibrium positions or mean free path of the diffusing substance, e is the

base of the natural logarithm, k is the Boltzmann constant, T, absolute temperature, h, Planck's constant, ΔE is the apparent energy of activation as obtained in Fig. 1, and $\Delta S\ddagger$ is the entropy change associated with formation of the activated state. Since the value for ΔE dropped from 9.8 to 4.1 kcal with vasopressin an increase in D by a factor of 13,000 might have been expected if this change in ΔE represented an equal change in the free energy of activation for diffusion. In fact, the increase in diffusion coefficient observed upon addition of hormone was only a 1.7-fold change (1). Thus $\Delta S\ddagger$ for diffusing water molecules is 17.8 cal per mole per °C less with hormone present than in its absence as a consequence of the large increase in entropy of the system induced by the hormone. Such an increase in entropy would be interpreted as largely resulting from the structural changes in the membrane associated with the enlargement of pores and would include any accompanying disorganization of structured water which might occur.

Hempling (16) has recently reported the activation energy for net osmotic transfers of water across the Ehrlich ascites tumor cell to be 9.6 kcal per mole. High values of 13 to 17 kcal per mole for osmotically induced volume changes in *Arbacia* egg had previously been reported by Lucké and McCutcheon (17). In other biological systems energies of activation for permeability to water have been in a range compatible with that for self-diffusion or viscous flow in water reported by Wang (10). Thus Pappenheimer (18) found the temperature coefficient of filtration for fluid transfer across capillary membranes to approximate closely the temperature coefficient of viscosity. Nevis (19) found an apparent activation energy of 3 to 5 kcal per mole for efflux of THO from invertebrate peripheral nerve fibers. In human red cells, Jacobs, Glassman, and Parpart (20) measured the effect of temperature on hemolysis time and Hempling (16) has calculated an apparent activation energy of 3.9 kcal per mole for this process from their data.

The presence of cells and tissues in which high activation energies for diffusion of water exist makes it necessary to interpret with caution the physical significance of the calculation of $\frac{A}{dx}$ from the relationship

$$k = D\frac{A}{dx}$$

in which the true self-diffusion coefficient for water is assumed to be appropriate. The self-diffusion coefficient of water in ice has been stated by Kuhn and Thurkauf (21), to be 8×10^{-11} cm^2 per second at -1.5 to -2.0°C; less by a factor of nearly 10^{-6} than its value in water. Therefore the calculated value of $\frac{A}{dx}$ must be considered a formalism until the true state of the water is known through which the actual diffusion takes place.

In the preceding paper studies were made to determine whether urea and its amide analogs penetrate the toad bladder through aqueous pathways since these compounds, like water, are unique in having their rates of penetration accelerated by vasopressin. Solvent drag studies and the relationship between the hydrophilic nature of amides and the magnitude of the hormonal effect on their rates of penetration support the view that these compounds do traverse the membrane through aqueous channels in the presence of hormone. The activation energies for diffusion of urea also are in accord with this conclusion. In the absence of vasopressin a mean value of 4.1 ± 0.4 kcal per mole was obtained which remained essentially unchanged at 3.9 ± 0.2 kcal per mole when hormone was added. These values are sufficiently close to that of 4.7 kcal per mole for the activation energy for diffusion of urea in water (13) to indicate that urea and water traverse the same aqueous channels through the toad bladder. Unlike water, however, the activation energy in the absence of hormone is not increased although the rate of penetration is only one-tenth as great as in the presence of hormone (12). This suggests that even in the absence of vasopressin urea penetrates the bladder through pathways in which water retains its bulk characteristics but that there are many fewer such channels for it to move through.

The concept of structured water as an integral part of certain cell membranes would account for other observations that have been considered curiosities. Thus activated trout and salmon eggs were found (22–24) to be highly impermeable to water—even to penetration by DHO. On the other hand, the same eggs exposed to air lose weight rapidly by surface evaporation as though they were covered with water (25). The very slow rate of diffusion of water through ice (21) but the similarity in rates of evaporation of water and ice at the same temperature (26) suggests that the curious permeability properties of the trout egg could be accounted for by a layer of ice-like water near the surface of the egg. In this instance, as in the toad bladder, the limiting permeability barrier even to water itself is considered to be water but in an organized ice-like state.

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