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Microviscosity of Mucosal Cellular Membranes in Toad Urinary Bladder: Relation to Antidiuretic Hormone Action on Water Permeability

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Summary. The microviscosity of cellular membranes (or membrane fluidity) was measured in suspensions of single mucosal cells isolated from the urinary bladder of the toad, *Bufo marinus*, by the technique of polarized fluorescence emission spectroscopy utilizing the hydrophobic fluorescent probe, perylene. At 23 °C, 5 mM dibutyryl cyclic 3′,5′-AMP decreased the apparent microviscosity of the cell membranes from 3.31 to 3.07 P, a minimum decrease of 7.3 % ($P < 0.001$) with a physiological time course. Direct visualization of the cell suspension indicated that 98 % of the cells were viable, as indicated by Trypan Blue dye exclusion. The fluorescent perylene could be seen only in plasma membranes, suggesting that the measured viscosity was that of plasma membrane with little contribution from the membranes of cellular organelles. Addition of antidiuretic hormone to intact hemibladders stained with perylene produced changes in fluorescence consistent with a similar 7 % decrease in apparent microviscosity with a physiological time course. However, finite interpretation of the findings in intact tissue cannot be made because the location and the fluorescent lifetime of the probe could only be conducted on the isolated cells. Comparison with previously determined relationships between water permeability and microviscosity in artificial bilayers suggests that the 7 % (a lower limit) decrease in microviscosity would produce only a 6.5 % increase in water permeability.

The urinary bladder of the toad *Bufo marinus* responds to antidiuretic hormone (ADH) by an increase in its rate of active transport of sodium and by an increase in its permeability to water and small nonelectrolytes, such as urea. Because these actions are similar to those of ADH on the distal tubule and collecting duct of the mammalian nephron and because the anatomy is less complex, the toad bladder is a useful model for studying these actions of ADH. From observations on cellular volume, Dibona, Civan and Leaf (1969) concluded that the action of ADH on osmotic water flow resides at

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the apical plasma membrane of the granular cells, the predominant type of cell. Considerable evidence indicates that adenosine 3′,5′ monophosphate (*c*-AMP) is the intracellular mediator of this ADH response (Orloff & Handler, 1962). However, the sequence of biochemical, molecular, and biophysical events subsequent to the increase in *c*-AMP are still not known.

We report on a biophysical property of the toad bladder, the microviscosity of cellular membranes, and examine the effects of ADH and *c*-AMP derivatives upon this parameter (Masters, Fanestil & Yguerabide, 1976; 1977). Two earlier observations are consistent with an effect of ADH upon membrane microviscosity (or "fluidity"). Grantham (1970) found that ADH increased the deformability of the luminal surface of cells in the rabbit kidney collecting tubule. Later, Pietras and Wright (1974) observed that ADH produced about a 1.3-fold increase in the permeability of the toad bladder to lipophilic nonelectrolytes and a decrease in the discrimination between straight and branched chain isomers. Although both of these results are consistent with an action of ADH on membrane microviscosity, the quantitative significance of such a decrease in microviscosity has not been examined in the toad bladder.

Therefore, we utilized the technique of polarized fluorescence emission spectroscopy to provide an independent approximation of the microviscosity of toad bladder cellular membrane and to quantitate the effect of ADH and *c*-AMP. We studied the intact urinary bladder of the toad as well as suspensions of isolated mucosal cells. Our results suggest that *c*-AMP and ADH induced a modulation of membrane microviscosity, with a physiological time course, and that the 7% change in microviscosity is only a minimum value of the change in apparent microviscosity since the actual value depends on the value of the fraction of the probe in the apical membrane.

Materials and Methods

Male toads (*Bufo marinus*) from Nicaragua were obtained from Pet Farm, Miami, Florida, and kept in cages on peat moss moistened with tap water. Toads were doubly pithed, given an initial injection of 0.3 ml of heparin (beef lung, 1000 U/ml, Upjohn) and their hearts perfused with heparin containing Ringer's solution for 20 min in order to remove all visible blood from the bladder. The bladders were excised as sacs, everted and mounted onto the end of a 3-mm glass tube. Each everted hemibladder was filled with amphibian Ringer's solution free of calcium and containing 1 mM ethylenediaminetetraacetic acid (EDTA-Ringer's). The outer or mucosal surface was washed with this solution and blotted with tissue paper to remove mucous and desquamated cells. The sacs were immersed in an aerated solution of EDTA-Ringer's in a 30-ml beaker for 40 min at room temperature. The EDTA-Ringer's

solution was exchanged for fresh solution after an initial 10-min period. The epithelial cells were isolated by gently rotating the everted hemibladder in a petri dish containing normal amphibian Ringer's solution. The cells were twice separated from the suspension by low speed centrifugation and resuspended in normal amphibian Ringer's solution. The normal amphibian Ringer's solution had the following composition in mmoles/liter: NaCl 111, KCl 3.35, sodium bicarbonate 2.4, $CaCl_2$ 2.7, glucose 5, osmolarity 220 mosM and pH 8.

Two methods for labeling the mucosal cell membranes with perylene were investigated. In one method the mucosal surface of the bladder was covered with small glass beads on which perylene had been deposited. After 15 min the glass beads were removed and the bladder was washed several times with normal amphibian Ringer's solution. The labeled cells were then isolated from the bladder by the procedure described above. In the second method, isolated cells were stained with the fluorescent probe by incubating a 2-ml suspension of cells (at a concentration of one million cells per ml) in aerated normal amphibian Ringer's solution, which was stirred with an aliquot of millimolar perylene in acetone for 15 min in the dark. The final concentration of perylene was 2.5 μM and the maximum concentration of acetone was 0.25 %. The constant aeration rapidly removed the acetone from the incubation mixture. The cell suspension was then spun in a low speed centrifuge to separate cells from the perylene dispersion. The cells were twice resuspended in normal amphibian Ringer's solution. The morphology of the cells labeled by either method was studied by phase contrast microscopy and the uniformity of fluorescence intensity of the cells was observed visually by fluorescence microscopy using epi-illumination. Cell viability prior to and after staining with perylene was determined by trypan blue dye exclusion tests. A 0.4 % solution of trypan blue in normal amphibian Ringer's was mixed with a cell suspension (1 : 3, v/v), and after 10–15 min a drop of the suspension was placed in the hemocytometer and the number of stained and unstained cells in a given area was counted.

Fluorescence polarization measurements were performed on a spectrofluorometer constructed by one of the authors (Yguerabide). The labeled cell suspension was contained in a 2-ml quartz cuvette which was maintained at a temperature of 23 ± 0.5 °C. The cell suspension was gently aerated by an air stream and stirred with a magnetic flea. A beam of vertically polarized light was used for excitation at 404 nm. This beam was obtained by passing the light from a 150 W Xenon lamp through a high intensity Bausch and Lomb monochromator (band pass 5 nm), a 404-nm interference filter, and a polarizer (Polacoat, Edmund Scientific Co., New Jersey) oriented in a vertical direction. The emitted light was detected through an analyzing polarizer and a Corning 3-72 cut-off filter by two matched 1P28 photomultiplier tubes. One photomultiplier tube detected the vertical component of the polarized emission I_{VV} while the other detected the horizontal component I_{VH}. The first and the second subscripts in these intensities refer to the orientation of the excitation and the analyzing polarizers respectively. The recording system gave values of I_{VH} and I_{VV} as well as the ratio: $R = I_{VH}/I_{VV}$. The values could be read from a recorder or digital voltmeter. Fluorescence anisotropy was calculated from Eq. (1):

$$A = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}} = \frac{1 - R}{1 + 2R}. \tag{1}$$

The intensities were corrected for scattered light using a suspension of unlabeled cells to establish the scattering background. The corrections were always small since the emitted light was 40–50 times greater than the scattered light.

Membrane fluidity, quantitatively measured as membrane microviscosity was calculated from the method developed by Shinitzki *et al.* (1971). According to theory, the fluorescence anisotropy of perylene is a function of the ratio $T\tau/\eta$, where T is the absolute temperature, τ is the fluorescent lifetime and η is the viscosity (Weber, 1953). The anisotropy

decreases with an increase in T or τ and increases with increasing η. The exact relation between anisotropy and microviscosity, however, depends upon the shape of the fluorescent molecule. Therefore, membrane fluidity or microviscosity was evaluated from the measured value of anisotropy by comparison with a calibration graph obtained by measuring the fluorescence anisotropy and lifetime of perylene in a series of oils of known viscosity at specific temperature (Cannon Instrument Co., State College, Pennsylvania). The calibration data was a plot of $1/A$ *vs.* $T\tau/\eta$ (Shinitzki *et al.*, 1971). Fluorescent lifetime of perylene in the membrane and in the calibration oil was measured by a single photon counting technique on an Ortec nanosecond fluorimeter (Yguerabide, 1972).

In a typical experiment in which the effects of an agent on the membrane fluidity were to be evaluated, the ratio I_{VH}/I_{VV} for a perylene-labeled cell suspension was first monitored for at least 15 min prior to the addition of the agent. During this time, the balance of the two detecting photomultiplier tubes was carefully checked by measuring the ratio I_{VV}/I_{VV} obtained by orienting the analyzing polarizer for each photomultiplier tube in a vertical direction. When necessary, the photomultiplier tubes were rebalanced by changing their applied voltages so that the ratio I_{VV}/I_{VV} was equal to one. If the ratio I_{VH}/I_{VV} for the labeled cell suspension was stable over a period of at least 15 min, in the sense that the ratio was not undergoing large random fluctuations, the agent was added and the ratio was monitored for at least half an hour. In experiments where a change in I_{VH}/I_{VV} occurred upon the addition of an agent, it was necessary to establish whether the change was due to a change in the microviscosity or to a change in the fluorescent lifetime of the perylene. In principle, this could be accomplished by a direct measurement of the fluorescent lifetime of the perylene labeled-cells in the presence and in the absence of the agent. However, for the small changes in I_{VH}/I_{VV} detected in our experiments, the direct measurement of the fluorescent lifetime does not have sufficient precision to identify the parameter responsible for the change in the fluorescence anisotropy. However, small changes in fluorescent lifetime can be detected by measuring the fluorescence intensity $I_{V,54}$ detected through an analyzing polarizer oriented at 54° with respect to the vertical direction. This intensity is independent of changes in rotational motion of the fluorescent molecule but does depend on its fluorescent lifetime. An increase in the fluorescent lifetime increases the intensity $I_{V,54}$. In order to determine whether there was a small change in the fluorescent lifetime of the perylene due to the addition of the agent, we measured the steady state intensity of the probe with vertically polarized excitation and with the analyzer oriented at 54° with respect to the vertical. All inorganic chemicals were analytical grade, perylene (99 % pure) was obtained from Aldrich Chemical Co.; Adenosine 3′,5′-cyclic phosphate, ethylene diamine tetraacetic acid, tetra sodium dihydrate were obtained from Calbiochem; N^6,O^2-dibutyryl-adenosine 3′-5′-cyclic phosphate sodium was obtained from P.L. Biochemicals; synthetic arginine vasopressin was from Sigma; Pitressin (arginine vasopressin) was from Park-Davis; 8-Bromoadenosine 3′,5′ cyclic monophosphoric acid was from Aldrich, adenosine-5′-monophosphate was from ICN Biochemicals; and didansyl-L-cystine was from Pierce Chemical Co.

Results

Initial experiments attempted measurement of fluorescence anisotropy from intact urinary bladders which had been stained from the mucosal surface by the glass bead method. Despite extensive and variable background fluctuations due to light scattering, in six bladders we observed that 100 mU/ml ADH caused a 6.2 ± 0.7 % decrease in the apparent anisotropy

(a lower limit or minimum value of the change in anisotropy occurring at the luminal membrane; *see Discussion*), a finding indicative of a decrease in microviscosity. However, interpretation of these data were compromised because: (i) they were selected from bladders in which measurement was not possible because of excessive backround fluctuation, (ii) we could not directly measure the lifetime of the probe in functional bladders and (iii) we were not able to determine whether the probe was confined to the plasma membrane or in all cellular membranes. Therefore, all additional studies were conducted on isolated cells.

Single mucosal cells isolated from the toad urinary bladder were studied with and without labeling by perylene to search for changes in morphology. As estimated from Trypan Blue dye exclusion, approximately 98% of the cells were viable. The percent viability and the appearance of the cells under phase contrast microscopy were unchanged by staining with perylene. The cells also had a uniform fluorescence when labeled with didansyl-L-cystine and appeared to be spherical. The few nonviable cells took up Trypan Blue dye, had a very high fluorescence when incubated with didansyl-L-cystine, and had a flat appearance. We had postulated that addition of perylene to the intact bladder from glass beads would permit labeling of the mucosal surface of the toad bladder, while addition of the perylene dispersion to the isolated cells would label the entire surface of the cells. However, when viewed in the fluorescence microscope, cells labeled by both methods were similar. Either the perylene penetrated the intercellular regions of the intact bladder to label the entire cell surface or the perylene on the mucosal surface migrated over the remaining cell surface during the one hour required to isolate the cells from the intact urinary bladder.

In the fluorescence microscope, the cell surface was uniformly fluorescent and the perylene seemed to be restricted to the cell plasma membrane in the sense that no fluorescent structure or organelles could be discerned within the cell. However, we cannot rule out the possibility that some small fraction of the label was transported from the plasma membrane to other internal membranes.

Figure 1 shows the time course of the effect of 5 mM dibutyryl cyclic 3′,5′-AMP on the apparent fluorescence anisotropy of perylene-labeled single mucosal cells isolated from toad urinary bladder. The graph shows that anisotropy was fairly constant prior to the addition of the agent. In some cases, however, the apparent anisotropy slowly changed during the course of the experiment. The addition of the compound produced a definite and rapid decrease in the apparent anisotropy, after which the apparent anisotropy either remained constant or changed slowly.

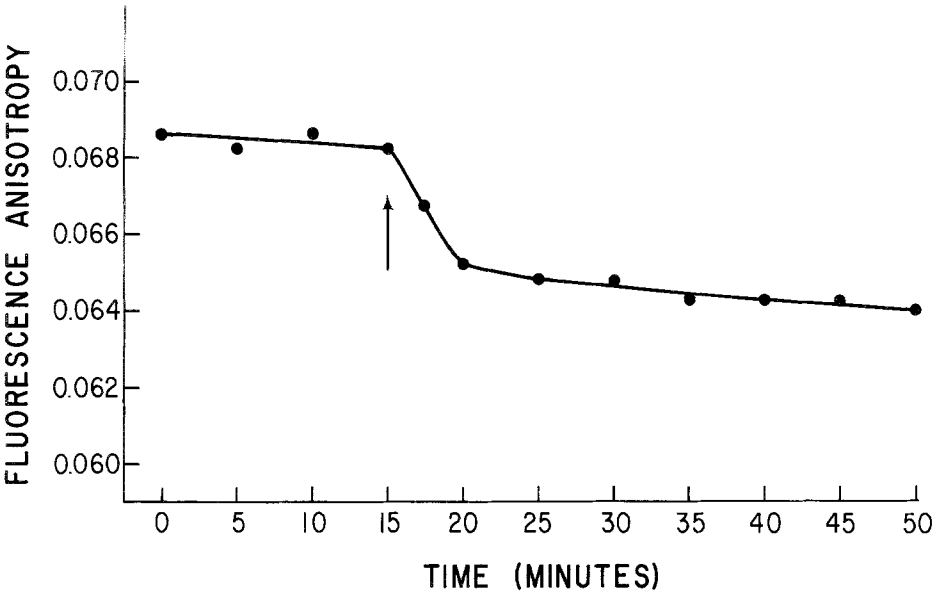

Fig. 1. The time course of the effect of 5 mM dibutyryl cyclic 3′,5′-AMP on the fluorescence anisotropy of perylene-labeled single mucosal cells isolated from the toad urinary bladder. The arrow indicates the application of the agent. The line connects the experimental points

To determine whether the decrease in the fluorescence anisotropy was due to a decrease in the microviscosity in the environment around the probe or an increase in the fluorescent lifetime of the probe, we measured the effect of dibutyryl cyclic 3′,5′-AMP on the time course of $I_{V,54}$ (*see* Methods). No change in $I_{V,54}$ was detected, which indicates that the fluorescent lifetime of the probe did not change and that the decrease in anisotropy resulted from a decrease in membrane microviscosity. The measured values of the fluorescence anisotropy before and after the addition of dibutyryl cyclic 3′,5′-AMP were therefore converted to microviscosities using the calibration graph for perylene and a fluorescent lifetime of 7.5 nsec (determined by direct measurement of perylene-labeled cells in a nanosecond fluorometer). The results are shown in Table 1. The calculated microviscosity decreased from 3.31 to 3.07 P upon the addition of 5 mM dibutyryl cyclic 3′,5′-AMP. Since the microviscosity varied from one cell preparation to another, the change in anisotropy and microviscosity for each cell preparation was analyzed by the paired *t*-test. This indicates that the effect of cyclic 3′,5′-AMP was statistically significant ($P < 0.001$).

In contrast to the results with intact tissue, the addition of ADH at a concentration of 200 mU/ml to perylene-labeled cells did not significantly

Table 1. The effect of dibutyryl cyclic 3′,5′-AMP on the anisotropy and microviscosity of perylene-stained single mucosal cells isolated from toad urinary bladder[a]

	Anisotropy	Membrane-microviscosity (P)
Control	0.0719 ± 0.0029 (17)	3.31 ± 0.16
Dibutyryl cyclic 3′,5′-AMP (5 mM)	0.0671 ± 0.0027 (17)	3.07 ± 0.15
Mean difference	0.0048 ± 0.0007 (17)	0.24 ± 0.04

[a] Numbers are means ± SE. Temperature was 23 °C; the fluorescent lifetime of the emission from the stained cells was 7.5 nsec. Student's t-test (paired), $n = 17$. Significant at $P < 0.001$. Number of experiments in parentheses.

Table 2. Effect of various compounds on the scope of I_{VH}/I_{VV} for a 10-min period before and after addition to perylene-stained single mucosal cells[a]

Compound	Concentration	Slope before addition $\times 10^4$ $(\text{min})^{-1}$	Slope after addition $\times 10^4$ $(\text{min})^{-1}$	Comments
ADH (8)	200 mU/ml	−8.37 ± 2.23	− 6.50 ± 1.81	NS
Dibutyryl cyclic 3′,5′-AMP[b] (8)	5 mM	−4.13 ± 0.81	+11.4 ± 2.97	Significant effect $P < 0.005$
AMP (10)	5 mM	−4.70 ± 1.15	− 3.70 ± 0.93	NS
8 Bromo-cyclic 3′,5′-AMP (5)	2.5 mM	−8.40 ± 1.43	+ 4.20 ± 4.07	Significant effect $P < 0.025$

[a] Standard conditions as given in test.

[b] Dibutyryl cyclic 3′,5′-AMP was added 30 min after the addition of ADH.

Values are means ± SE. Number of experiments in parentheses.

affect the fluorescence anisotropy. However, subsequent addition of dibutyryl cyclic 3′,5′-AMP 30 min after ADH resulted in the expected decrease of fluorescence anisotropy, indicating that the cells were responsive to dibutyryl cyclic 3′,5′-AMP but not to ADH.

Several other compounds were tested for effects on the anisotropy of perylene-labeled mucosal cells. Studies on toad urinary bladder usually employ adenosine 5′-monophosphate (AMP) as a test for nonspecific nucleotide effects. Therefore, we added AMP (5 mM) and found no significant effect on the anisotropy. Furthermore, 8-Bromo cyclic 3′,5′-AMP (2.5 mM) did produce a decrease in anisotropy similar to that produced by dibutyryl cyclic 3′,5′-AMP.

To correct for any change in anisotropy with time of incubation in the cuvette, the slope of I_{VH}/I_{VV} *vs.* time was calculated for the 10 min before and after addition of the various agents. A summary of these results,

presented in Table 2, indicates that the small effect of the *c*-AMP derivatives was highly statistically significant and was not due to a change in microviscosity with time. The lack of effect of AMP and ADH was confirmed.

Discussion

Our study used a fluorescent probe to test the hypothesis that *c*-AMP and ADH induced osmotic water flow is mediated by a decrease in the microviscosity (i.e., an increase in the fluidity) of the membranes of the responsive cells in the toad urinary bladder. The application of fluorescent probes to the study of membranes is well documented (Radda, 1975). We chose perylene as the fluorescent probe because: (i) it is a small, disk-like and water insoluble molecule which is highly fluorescent in a hydrophobic environment such as the lipid bilayer region of a cell membrane, yet barely fluorescent in aqueous solution; (ii) it readily partitions into the hydrocarbon phase of membrane phospholipids from a dispersion in aqueous solution; and (iii) it does not photoisomerize, (in contrast, 1,6-diphenyl 1,3,5,-hexatriene reversibly bleaches upon exposure to ultraviolet light which did not permit measurement of the kinetics of the anisotropy change). In our experiments the isolated cells were incubated with a micromolar dispersion of perylene for 15 min. We assume that the perylene partitioned into the plasma membrane of the cells and that all three types of mucosal cells (granular, mitochondria rich, and goblet) were labeled with perylene. Extensive microscopic studies were performed to localize the position of the perylene in the membrane; we only observed fluorescence from the periphery of the cells suggesting that little, if any, perylene gained access to intracellular membranes.

It would have been advantageous to conduct our entire study on intact epithelial tissue, since (i) the tissue is more physiologically functional than cellular or subcellular fractions, (ii) the intact tissue maintains a polarity which permits water permeability to be measured, (iii) the intact tissue may be specifically stained from one side, which might increase the specificity of the staining and the signal-to-noise ratio of the fluorescence measurements, (iv) ADH may be applied specifically to the serosal side of the tissue, and (v) the simultaneous measurement of membrane fluidity and water permeability in intact toad urinary bladder might be possible and would yield information about the time course of the relation between these two parameters. These factors would reduce the ambiguity in the interpretation

of experimental results and lead to more definitive conclusions. However, our preliminary studies on the ADH-induced changes in the anisotropy of the perylene emission from stained intact tissue indicated that light scattering and motion due to smooth muscle contractions severely complicated the measurements. Moreover, methods are not available to localize the probe in the tissue or determine the lifetime. Nevertheless, we were able to measure a 6% decrease in the anisotropy in some intact urinary bladders after the addition of ADH. This decrease in anisotropy is similar to the 6% decrease in anisotropy which was later produced in isolated mucosal cells by the addition of dibutyryl cyclic 3′,5′-AMP. If the fluorescent lifetime of the probe is the same in the intact tissue as in the isolated cells, there is about a 7% decrease in microviscosity in the intact bladder upon the addition of ADH and in isolated cells upon addition of *c*-AMP derivative. The time course of the decrease in anisotropy after the addition of the agents was similar for both intact tissue and the isolated mucosal cells.

Because measurements on isolated mucosal cells are not complicated by excessive light scattering and by smooth muscle contractions we conducted more extensive studies on suspensions of isolated mucosal cells. Several experimental methods release mucosal cells from the intact tissue. A method involving the application of collagenase to the mucosal surface of the urinary bladder yields sheets of cells, which preclude fluorescence methods due to light scattering properties. Therefore, we used incubation of the everted bladder in calcium-free amphibian Ringer's solution containing 1 mM EDTA. This produced a suspension of individual cells, in high yield, with the required optical properties. This suspension of single isolated cells appeared similar to those previously reported (Eggena, Christakis, & Deppisch, 1975) in that the cells were spherical and vacuolated when viewed in the phase contrast microscope. Single cells isolated by calcium chelation do not respond to vasopressin with an increase in oxygen consumption (Gatzy & Berndt, 1968; Masters, *unpublished results*). Thus, it is not surprising that ADH also failed to elicit a change in fluorescence anisotropy. However, as noted above, the 7.3% decrease in microviscosity produced by cyclic AMP derivatives may correspond with the 6% decrease in anisotropy produced by addition of ADH to the intact tissues. Although not conclusive, this correspondence of findings suggests that the observations on both intact tissue and isolated cells might be biologically relevant.

Therefore, we now discuss the possible quantitative relationship between the small decreases in microviscosity and alterations in membrane

permeability caused by ADH and *c*-AMP. We have measured a change in the apparent anisotropy and therefore a change in the apparent microviscosity since we don't know what fraction of the probe entered the apical membrane. We have theoretical expressions (Yguerabide, *in preparation*) which relate the change in anisotropy (and therefore the change in microviscosity) of the apical membrane to the experimentally measured change in apparent anisotropy and the fraction of the probe in the apical membrane:

$$\Delta a_{AP} = \Delta A / f_{AP} \tag{2}$$

where the change in anisotropy at the apical membrane Δa_{AP}, is related to the total measured change in anisotropy ΔA, and the fraction of the probe in the apical membrane, f_{AP}. Thus, the 7.3 % decrease in microviscosity is only a lower limit or *minimum value* and could be larger depending on the value of f_{AP}, which requires information on the location of the probe. Next we determine a relation between the change in microviscosity and water permeability, and ask whether this minimum value of 7.3 % change in apparent microviscosity could account for the change in water permeability. We estimate the predicted change in permeability of a membrane for water from the measured change in microviscosity by using permeabilities for water measured by Finkelstein (1976*a*) and microviscosities measured by Shinitzky and Inbar (1976) in bilayer membranes. This comparison of permeabilities and microviscosity in bilayers of defined composition shown in Fig. 2 suggests that permeability (P) and microviscosity (η) are related by Eq. (3), where a is a constant. From Eq. (3) we can derive Eq. (4) which relates the fractional change in permeability $\Delta P/P(0)$ and the fractional change in microviscosity $\Delta \eta/\eta(0)$ where the negative sign indicates that ΔP decreases as $\Delta \eta$ increases and the bracketed (A) and (0) refer to the presence and the absence of the agent.

$$P = a/\eta \tag{3}$$

$$\frac{P(A) - P(0)}{P(0)} = \Delta P/P(0) = \frac{-\Delta \eta/\eta(0)}{1 + \Delta \eta/\eta(0)}. \tag{4}$$

The 7 % fractional decrease in the apparent microviscosity (a minimum value) observed with both the intact bladder (upon the application of ADH) and single isolated mucosal cells (upon the application of dibutyryl *c*-AMP) corresponds to only a 6.5 % fractional change in the water permeability (from Eq. (4)). Thus, if the relationship between water permeability and microviscosity in toad bladder membrane is similar to that in bilayers, the

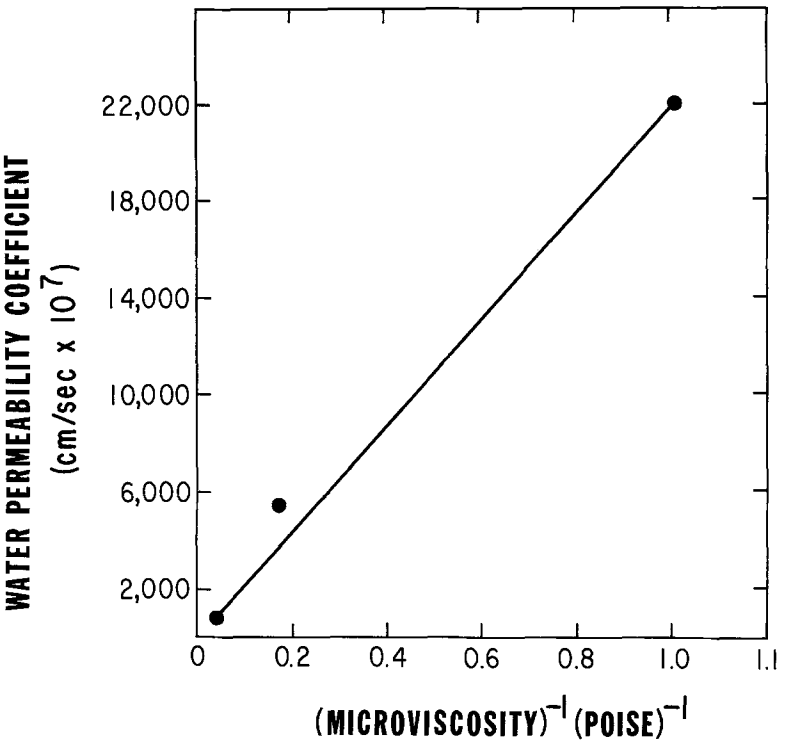

Fig. 2. The relation between the diffusive water permeability coefficient and the reciprocal of the lipid microviscosity. The water permeability coefficients (THO) across planar lipid bilayers measured by Finkelstein (1976*a*) were combined with measurements of the microviscosity of lipid vesicles which were determined by Shinitzky and Inbar (1976). Both sets of measurements were made at 25 °C. Our plot of their data suggests the proportionality between the diffusive water permeability coefficient and the reciprocal of the microviscosity

observed 7% decrease in apparent microviscosity (a minimum value) could not account for the tenfold or greater change in water permeability which ADH produces.

On the other hand, the changes in the apparent microviscosity are consistent with the data presented by Finkelstein (1976*b*) which led him to suggest that aqueous pores are responsible for the effects of ADH on water flow and not changes in fluidity. The apparent changes in microviscosity are also in line with the 1.3-fold increase in the permeability of lipophilic solutes observed by Pietras and Wright (1974).

These correlations, however, are open to several reservations resulting from our present lack of knowledge concerning what fraction of the probe is present in the membrane responsible for the permeability change. First, the anisotropy of the perylene emission is sensitive only to the fluidity of the membrane in the immediate environment of the probe molecule. Thus, it is

conceivable that domains of the plasma membrane which have a low partition coefficient and therefore exclude perylene might undergo larger changes in microviscosity without being sensed in our experiment. Second, several conditions tend to cause underestimation of the overall fractional change in microviscosity: (i) probably no more than 85% of the cells were responsive granular cells, (ii) the probe labeled both the potentially responsive apical plasma membranes and the unresponsive basolateral plasma membranes, and (iii) some fraction of intracellular membranes may have been labeled.

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