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Research Article

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When bladders were exposed to vasopressin on the serosa and subsequently fixed with 1% glutaraldehyde on the mucosa, they were found to retain 74% of their prefixation permeability to urea. Net urea flux across these fixed bladders (in the absence of vasopressin) was markedly inhibited by thiourea, whereas osmotic water flux was not inhibited. These studies suggest that vasopressin induces the formation of “urea-channels” in the membrane that can be preserved by glutaraldehyde and blocked by thiourea.

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ABSTRACT The mammalian antidiuretic hormone, 8-arginine-vasopressin, was found to increase net mucosal-to-serosal urea flux across the isolated toad urinary bladder 13-fold. This urea flux was accompanied by a 24-fold increase in solute-linked water movement across the membrane. Net urea flux and urea-linked volume flux were inhibited by 50% or more when thiourea was added to the mucosal medium at concentrations equal to those of urea. In contrast, thiourea did not inhibit osmotic water flux across the bladder in the presence of vasopressin. These observations are consistant with the view that thiourea and urea compete for a common site on a membrane carrier molecule.

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INTRODUCTION

The antidiuretic hormone, 8-arginine-vasopressin, increases the permeability of the renal medullary-collecting duct of mammals to urea (1) and water (2) by a process which is regarded as similar to the permeability changes observed more readily in the isolated urinary bladder of the toad, *Bufo marinus*. Maffly, Hays, Lamdin, and Leaf (3) found that vasopressin increased labeled urea flux across the toad bladder wall 10-fold without affecting thiourea flux detectably. They found the two unidirectional fluxes of ^{15}N-labeled and ^{14}C-labeled urea across the membrane to be equal, and there was no evidence for self-depression of the labeled urea flux when nonlabeled urea was added to the medium up to concentrations as high as 50 mM. Leaf and Hays (4) raised the medium concentration of nonlabeled urea up to 100 mM without observing a significant depression of labeled urea transport across the bladder. Moreover, these investigators were unable to demonstrate competition for possible carrier sites between acetamide and dimethylformamide as well as between acetamide and nicotinamide, even up to molar ratios of 11,000 in the latter instance. In addition they observed a solvent drag effect of water on urea, which indicated that urea and water moved along the same pathways, at least for a portion of their transit through the bladder membrane. All of these observations seemed to suggest that urea moves simply by passive diffusion through aqueous channels in the membrane.

In spite of the lack of direct evidence for a urea-carrier molecule in the toad bladder, Leaf (5) has pointed out that the striking selectivity of the membrane for urea and thiourea must involve some form of interaction, possibly by hydrogen bonding, between the moving species and the bladder wall. On the other hand Schmidt-Nielsen has suggested that the possibility of a urea-carrier for the toad bladder has not been adequately ruled out since the urea-carrier of shark renal tubules shows no evidence of saturation at concentrations as high as 750 mM urea (6). Franki, Levine, and Hays (7) have recently shown that phloretin markedly inhibits the movement of urea without altering osmotic water flow across the bladder in the presence of vasopressin. This observation is consistant with the view that urea transport across the toad bladder is a carrier-mediated process. The present study was undertaken to learn whether the urea-permeability response of the bladder to vasopressin could be preserved with glutaraldehyde fixation of the bladder wall, and to determine whether the movement of urea

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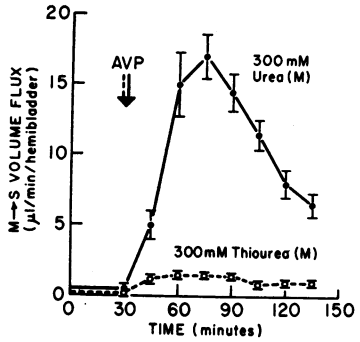

FIGURE 1 Vasopressin action on amide-linked volume flux. Control hemibladders (●) were filled on the mucosa (M) with a 300 mM urea solution; contralateral, experimental hemibladders (○) were filled with a 300 mM thiourea solution. These hemibladders were immersed in an equiosmotic solution of NaCl-supplemented Ringers to which vasopressin (AVP) was added at t = 30 min. Net mucosal-to-serosal (M → S) volume flux is given as the mean and the standard error of the mean of experiments on eight hemibladder pairs.

across fixed and nonfixed bladders is retarded by the urea analogue, thiourea.

METHODS

Experiments were carried out on adult female toads, *Bufo marinus,* which had been purchased from the National Reagents Inc., Bridgeport, Conn., and maintained without feeding on moist peat moss at room temperature for 3–4 wk. Toads were doubly pithed and their urinary bladders were then resected and tied to the ends of glass cannulas with the mucosa on the inside and the serosa on the outside of the bladder sac (8, 9). The bladder was filled with 7 ml of fluid containing urea, thiourea, and (or) mannitol in various proportions as indicated in the text. In the experiments depicted in Figs. 1 and 2 bladders were suspended in groups of four in a 100 ml serosal bath which had the following composition,[1] in grams per liter: NaCl, 3.74; KCl, 0.32; $NaH_2PO_4 \cdot H_2O$, 0.10; $MgSO_4 \cdot 7H_2O$, 0.16; $CaCl_2$ (anhyd.), 0.16; glucose, 0.80; $NaHCO_3$, 1.76. This solution was buffered at pH 7.4 by 4.8 g/liter *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, Sigma Chemical Co., St. Louis, Mo.) and 6.8 ml/liter of 1 N NaOH. The osmolality of this solution, which was 204 mosmol/kg H_2O, was raised either to 300 or to 400 mosmol/kg H_2O by addition of NaCl. The precise osmolality of both the serosal and the mucosal fluids was determined with a Fiske Osmometer (Fiske Associates, Inc., Uxbridge, Mass.) just before their use, and the osmolalities were matched by adding a little distilled water to the mucosal or the serosal solution when required. These solutions are hypertonic when compared with the tonicity of plasma of hydrated toads, but not necessarily when compared with the tonicity of plasma from dehydrated animals (10). Hypertonic Ringers solutions were employed in the present study to balance osmotically the high concentrations of urea and of thiourea used on the mucosal side of the bladder wall. Bladders were weighed periodically at 15- or 30-min intervals with a Mettler H43 balance (Mettler Instrument Corp., Princeton, N. J.); the net weight loss of the bladder assembly was taken as a measure of the net mucosal-to-serosal volume flux across the bladder wall. Bladders were wiped and weighed with a consistency of ±5 mg.

To measure the net mucosal-to-serosal movement of urea across the bladder wall (Tables I–V), bladders were filled with 7-ml urea solutions and suspended each into separate 10-ml serosal fluid baths that were changed at 15- or 30-min intervals. The urea nitrogen concentration of the serosal bathing fluid was measured on 10- or 50-μl aliquots by a modified Urease-Berthelot reaction method (Sigma Technical Bulletin no. 640) with a Bausch & Lomb Spectronic 20 at 570 nm (Bausch & Lomb Inc., Scientific Instrument Div., Rochester, N. Y.). Thiourea at concentrations as high as 200 mM did not produce a color reaction with this method nor did it interfere with the measurement of urea nitrogen.

Glutaraldehyde has been used previously to lock the water permeability barrier of the toad bladder in various stages of response to hormone (10–12). The same procedure was used here to preserve the effect of vasopressin on the permeability of the bladder to urea. Bladders were fixed by replacing the mucosal fluid by 7 ml of 1% glutaraldehyde in 0.05 M cacodylate buffer. After 5 min at room temperature the fixative was removed and the bladder mucosa rinsed twice with mucosal urea solution. Net urea and (or) water flux across this fixed bladder was then measured in the absence of vasopressin in the serosal bath. The 8-arginine-vasopressin employed before fixation and in the other studies mentioned above was prepared by Meienhofer, Trzeciak, Havran, and Walter (13); the sample used had a rat antidiuretic activity equivalent to 503 U/mg.

RESULTS

Effect of vasopressin on urea- and thiourea-linked volume fluxes across the toad bladder. The action of vasopressin on urea- and thiourea-linked volume fluxes across the isolated toad bladder is illustrated in Fig. 1. One set of hemibladders was filled with a 300 mM urea solution, whereas the other set from the same animals was filled with a 300 mM thiourea solution. Both sets of hemibladders were suspended in 300 mosmol/kg H_2O NaCl-supplemented Ringers fluid, so that there was no osmotic pressure difference between mucosal and serosal fluids to promote osmotic water flux. Hemibladders filled with the urea solution lost 0.6 mg/min (or μl/min) in the absence of vasopressin. Upon adding vasopressin to the serosal bath at a final concentration of 1×10^{-7} M net volume flux across the bladder wall increased to a maximal value of 17 μl/min, reaching a half-maximal response 20 min after the initial challenge with hormone. The net mucosal-to-serosal urea-linked volume flux then declined to half-maximal response levels within 90 min of bladder exposure to vasopressin. Hemibladders which had been filled with the thiourea solution lost only 0.2 μl/min in the absence of hormone (this value could not be accurately assessed because of the slow flow rate) and showed a small, i.e. 1.5 μl/min, but significant ($P < 0.01$) re-

[1] This medium contains the salts of Wolf and Quimby amphibian culture medium (Grand Island Biological Co., Grand Island, N. Y., 1973).

TABLE I

Effect of Vasopressin on Urea Transport and on Urea-Linked Volume Flux across the Toad Bladder

	Serosal fluid	Mucosal fluid	Period	Net M → S volume flux	Net M → S urea transport	Molarity of volume flux
			min	*μl/min/ hemibladder*	*mg/min/ hemibladder*	*mM/liter urea*
No AVP			0–15	0.47±0.32	0.017±0.004*	603
10^{-7} M AVP	300 mosmol/kg H_2O NaCl-Ringers	300 mosmol/kg H_2O urea solution	15–30	3.43±0.29	0.122±0.013	593
			30–45	10.33±1.00	0.215±0.014‡	347
			45–60	11.23±2.03	0.207±0.015	307
			60–75	9.33±1.46	0.175±0.021	313

Values are given as the mean and the standard error of the mean of experiments on six hemibladders.
* This value corresponds to a K_{trans} urea of 9.23×10^{-7} cm/s.
‡ This value corresponds to a K_{trans} urea of 116.66×10^{-7} cm/s.

sponse to vasopressin. The time-course of the thiourea-linked volume flux across the bladder with vasopressin appeared to be roughly similar to the urea-linked volume flux response to hormone.

In Table I the urea-linked volume flux across the bladder wall has been compared with the net urea flux measured simultaneously in the same bladder. In this experiment urea-linked volume flux increased 24-fold in the presence of vasopressin, whereas net urea flux increased only 13-fold. The molarity of the urea solution which moved across the bladder wall could be estimated from the net volume and net urea fluxes. In the absence of hormone and during the first 15 min interval of bladder exposure to hormone the molarity of the urea solution penetrating the bladder wall was approximately twice the molarity of the urea solution in the mucosal bath. After bladders had been exposed to vasopressin in excess of 30 min, the molarity of the volume flux rapidly approached the molarity of the mucosal fluid. In other words, the urea-linked volume flux across the bladder wall was initially a hypertonic volume flux, and only after a lag period became an isotonic volume flux. The osmotic composition of the 7 ml mucosal fluid bath was not detectably altered during a 60 min interval of net

TABLE II

Antagonism between Urea- and Thiourea-Linked Volume Fluxes

Period	Serosal fluid	Mucosal fluid	M → S volume flux	Percent inhibition
min			*μl/min/ hemibladder*	%
0–30	400 mosmol/kg H_2O NaCl-Ringers with 1×10^{-7} M AVP	Control: 200 mM urea 200 mM mannitol	6.3±0.9	29
		Exp.: 200 mM urea 183 mM mannitol 17 mM thiourea	4.5±0.2	
30–60		Control: 200 mM urea 200 mM mannitol	6.4±0.8	55
		Exp: 200 mM urea 150 mM mannitol 50 mM thiourea	2.9±0.9	
60–90		Control: 200 mM urea 200 mM mannitol	5.3±0.6	76
		Exp: 200 mM urea 100 mM mannitol 100 mM thiourea	1.3±0.6	

Values are given as the mean and the standard error of the mean of experiments on eight contralateral hemibladders.

TABLE III

Inhibition of Urea Transport across the Bladder by Thiourea

Serosal fluid	Mucosal fluid	M → S urea flux	Percent Inhibition
		mg/min/hemibladder	%
300 mosmol/kg H_2O NaCl-Ringers 1×10^{-7} M AVP	Control: 200 mM urea 100 mM mannitol	0.154±0.036 (4)	
	Experimental: 200 mM urea 75 mM mannitol 25 mM thiourea	0.132±0.029 (4)	14.3±5.2 ($P < 0.05$)
300 mosmol/kg H_2O NaCl-Ringers 1×10^{-7} M AVP	Control: 200 mM urea 100 mM mannitol	0.257±0.030 (4)	
	Experimental: 200 mM urea 50 mM mannitol 50 mM thiourea	0.194±0.023 (4)	24.5±6.6 ($P < 0.01$)
400 mosmol/kg H_2O NaCl-Ringers 1×10^{-7} M AVP	Control: 200 mM urea 200 mM mannitol	0.109±0.009 (8)	
	Experimental: 200 mM urea 150 mM mannitol 50 mM thiourea	0.078±0.012 (8)	28.4±8.7 ($P < 0.01$)
300 mosmol/kg H_2O NaCl-Ringers 1×10^{-7} M AVP	Control: 200 mM urea 100 mM mannitol	0.194±0.032 (4)	
	Experimental: 200 mM urea 100 mM thiourea	0.112±0.023 (4)	42.3±4.7 ($P < 0.001$)
400 mosmol/kg H_2O NaCl-Ringers 1×10^{-7} M AVP	Control: 200 mM urea 200 mM mannitol	0.115±0.020 (4)	
	Experimental: 200 mM urea 200 mM thiourea	0.059±0.012 (4)	48.7±5.2 ($P < 0.001$)

Values are given as the mean and the standard error of the mean on the number of experiments in parenthesis.

mucosal-to-serosal volume flux, i.e. its osmolality was 298±1 (SEM) mosmol/kg H_2O.

Inhibition of urea transport across the bladder by thiourea. To determine whether urea and thiourea compete for a common binding site in the membrane, the experiments shown in Tables II and III were carried out. In the experiments depicted in Table II, the effect of mucosal thiourea on the net mucosal-to-serosal urea-linked volume flux was examined. Control hemibladders were filled with a 200 mM urea plus 200 mM mannitol solution which was balanced osmotically by a 400 mosmol/kg H_2O NaCl-supplemented Ringers fluid containing a final concentration of 1×10^{-7} M vasopressin on the serosal side of the bladder wall. Experimental hemibladders were treated identically, except that progressively increasing amounts of mannitol in the mucosal solution were replaced by isosmotic concentrations of thiourea. Hemibladders were weighed for 30-min intervals, the mucosal solutions in both control and experimental hemibladders being changed at the end of the weighing period. The net mucosal-to-serosal urea-linked volume flux was found to decrease progressively as the concentration of thiourea in the mucosal fluid was raised from 17 to 50 to 100 mM. In the presence of 100 mM thiourea net urea-linked volume flux was depressed by

76%, and half-maximal inhibition was achieved with concentrations of less than 50 mM thiourea.

Since the net urea-linked volume flux measured gravimetrically is not necessarily directly related to the net urea flux across the membrane, the effects of thiourea on urea flux *per se* were determined by measuring changes in the urea concentration of the serosal bathing fluid with the urease reaction method (Table III). These studies were carried out under somewhat different experimental conditions as those mentioned above, i.e. (*a*) each concentration level of thiourea was tested on different sets of hemibladders during the first 30 min period of bladder exposure to vasopressin, and (*b*) the osmolality of the mucosal and serosal solutions were balanced not only at the 400 mosmol/kg H_2O level, but also at the 300 mosmol/kg H_2O level. As can be seen from the effects of 50 mM thiourea on net urea flux in a 300 and a 400 mosmol/kg H_2O environment, the percent inhibition was quite similar, i.e., 24.5 and 28.5% respectively. In the presence of 100 mM thiourea net urea flux was depressed by 42%. Thus, although it is clear that thiourea inhibits both the urea flux and the urea-linked volume flux across the bladder in the presence of vasopressin, in these experiments the urea flux was not inhibited to the same extent as the urea-linked volume flux. It must be pointed out, however, that these studies (Tables II and III) were carried out on different batches of toads, and that the urea-permeability response of the bladder to vasopressin varies markedly from one toad to the next.

Effect of thiourea on osmotic water flux across the bladder. To learn whether the inhibitory action of thiourea on urea flux across the bladder wall was specific or whether osmotic water flux could also be inhibited by thiourea, the experiment depicted in Fig. 2 was carried out. Control and experimental hemibladders were placed in 400 mosmol/kg H_2O NaCl-supplemented Ringers fluid containing a final concentration of 1×10^{-7} M vasopressin, and filled with 200-mM mannitol or 200-mM thiourea solutions, respectively. The osmotic pressure gradient across both bladders was, therefore, approximately 200 mosmol/kg H_2O. Net water flux was found to increase about 40-fold in the presence of vasopressin, both across control and experimental hemibladders. Moreover, the time-course of the response of both bladders to vasopressin were similar over a 60 min interval.

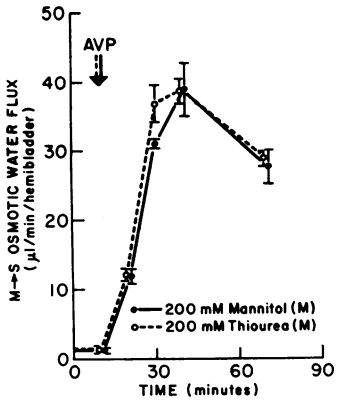

FIGURE 2 Effect of thiourea on osmotic water flux. Control hemibladders (●) were filled with a 200 mM mannitol solution on the mucosa (M); contralateral, experimental hemibladders (○) were filled with a 200 mM thiourea solution. Both sets were suspended into a 400 mosmol/kg H_2O NaCl-supplemented Ringers to which vasopressin was added at $t = 10$ min. Net mucosal-to-serosal ($M \rightarrow S$) water flux is given as the mean and the standard error of the mean of experiments on eight hemibladder pairs.

Effect of glutaraldehyde-fixation on urea movement across the bladder. To determine whether the urea permeability change of the bladder wall induced by vasopressin could be preserved by fixing the bladder with glutaraldehyde, the experiment depicted in Table IV was

TABLE IV

Urea Transport across Glutaraldehyde-Fixed Bladders

Serosal fluid	Mucosal fluid	Prefixation		Postfixation	
		M → S urea transport	M → S volume flux	M → S urea transport	M → S volume flux
		mg/min/ hemibladder	*μl/min/ hemibladder*	*mg/min/ hemibladder*	*μl/min/ hemibladder*
300 mosmol/kg H_2O NaCl-Ringers no AVP	300 mM urea	0.066±0.026	0.32±0.11	0.062±0.015	1.17±0.56
300 mosmol/kg H_2O NaCl-Ringers 10^{-7} M AVP (before fix only)	300 mM urea	0.388±0.068	18.03±3.66	0.289±0.049	10.35±1.92

Values are given as the mean and the standard error of the mean of experiments on six contralateral hemibladders.

TABLE V

Inhibition of Urea Transport across Glutaraldehyde-Fixed Bladders by Thiourea

Serosal fluid	Mucosal fluid	M → S urea transport	M → S volume flux	Osmotic water flow
		mg/min/ hemibladder	*μl/min/ hemibladder*	*μl/min/ hemibladder*
300 mosmol/kg H_2O NaCl-Ringers without hormone	Control: 200 mM urea 100 mM mannitol	0.247±0.010 $P < 0.001$ (8)	7.08±0.60 $P < 0.005$ (8)	
	Experimental: 200 mM urea 100 mM thiourea	0.160±0.007	4.91±0.29	
	Control: 100 mosmol/kg H_2O mannitol			37.26±2.56
	Experimental: 100 mosmol/kg H_2O thiourea			42.56±2.91

Before this experiment all hemibladders were filled with 300 mosmol/kg H_2O NaCl-supplemented Ringers and suspended into identical Ringers fluid containing 1×10^{-7} M vasopressin. Bladders were fixed on the mucosa with 1% glutaraldehyde as described in the text. These vasopressin-stimulated, glutaraldehyde-fixed bladders were exposed to the conditions listed in the Table. Values are given as the mean and the standard error of the mean on the number of experiments given in parenthesis.

carried out. Two sets of hemibladders were filled with 300 mM urea solution. One set of bladders was suspended in 300 mosmol/kg H_2O NaCl-supplemented Ringers containing a final concentration of 1×10^{-7} M vasopressin, the other set was suspended in hormone-free Ringers. Net urea and urea-linked volume fluxes were measured for two 15-min periods, and the mucosal fluid was then exchanged for a 1% solution of glutaraldehyde in 0.05 M cacodylate buffer. The bladders filled with fixative were kept in hormone-free Ringers fluid for 5 min. The fixative was then removed, the bladders rinsed twice with 300 mM urea solution, and filled with 300 mM urea solution. These fixed bladders were then suspended in hormone-free 300 mosmol/kg H_2O NaCl-supplemented Ringers fluid, and the net urea and urea-linked volume fluxes across the fixed bladders were measured for two 15-min intervals. In Table IV the results from the flux measurements during the 15 min interval just before and just after glutaraldehyde fixation have been summarized. The second and third 15-min intervals were chosen for comparison in this study, because the experiments shown in Table I had suggested that the urea flux response of the bladder to vasopressin tends to stabilize between 15 and 45 min after the initial challenge. The results indicate that net urea flux across the vasopressin-stimulated, glutaraldehyde-fixed bladder amounts to 74% of the net urea flux recorded just before fixation. Thus, the effect of vasopressin on urea permeability of the bladder wall is largely preserved by glutaraldehyde fixation. Moreover, the rather slow rate of urea movement across the bladder in the absence of vasopressin is not measurably affected by the fixative. While the observations on urea-linked volume flux roughly paralleled those on urea flux, it is noteworthy that glutaraldehyde fixation diminished urea-linked volume flux more than it did urea flux alone.

Inhibition of urea transport across glutaraldehyde-fixed bladders by thiourea. The purpose of this experiment was to see whether a bladder, which had been made permeable to urea with vasopressin and subsequently fixed with glutaraldehyde would still exhibit the competitive phenomenon between urea and thiourea transport observed in nonfixed bladders. In order to obtain two sets of urea-permeable, fixed bladders, bladders were filled with 300 mosmol/kg H_2O NaCl-supplemented Ringers fluid and suspended into an identical solution to which had been added vasopressin at a final concentration of 1×10^{-7} M. Bladders were then fixed on the mucosa with glutaraldehyde as described above. A control set of fixed bladders was filled with a solution containing 200 mM urea plus 100 mM mannitol, whereas the contralateral set of fixed bladders was filled with a solution of 200 mM urea plus 100 mM thiourea. Both sets of bladders were immersed in 300 mosmol/kg H_2O NaCl-supplemented Ringers fluid without vasopressin. Net urea and urea linked volume fluxes were measured for one 30 min interval. As is shown in Table V the urea flux was inhibited by 35% and the urea-linked volume flux by 31% in bladders containing thiourea instead of mannitol on the mucosa. Thiourea did not interfere with the osmotic

water flux across these fixed bladders. This was tested by omitting the urea from the mucosal fluid to establish an osmotic pressure difference between the mucosal and the serosal fluid baths of approximately 200 mosmol/kg H_2O. Control fixed bladders with 100 mM mannitol on the mucosa lost 37 μl/min and experimental fixed bladders with 100 mM thiourea on the mucosa lost 43 μl/min.

DISCUSSION

In the present study vasopressin was found to increase the permeability of the isolated toad urinary bladder to urea from a basal value of $K_{trans} = 9$ to 117×10^{-7} cm/s (Table I). This effect of the hormone on net urea movement as measured with a urease reaction method is similar to the results obtained by Maffly et al. (3) for isotopic urea movement across the bladder wall, i.e., the permeability coefficient of the bladder for urea increased from 26 to 239.7×10^{-7} cm/s with Pitressin. The net mucosal-to-serosal movement of urea along a concentration gradient was accompanied by net water movement. The experiments in Table I have shown that less water is coupled to the net solute flux in the absence of vasopressin than in the presence of vasopressin, i.e., the urea-linked volume flux is hyperosmotic with regard to the mucosal fluid in the absence of hormone and approximately isosmotic after the bladder has been exposed to hormone for 15 min or longer.

The experiment in Fig. 1 shows that the bladder is quite impermeable to thiourea, a finding which supports earlier observations by Maffly et al. (3) on isotopically labeled thiourea movement across the toad bladder. However, this compound was found to inhibit the urea-linked volume flux (Table II) as well as the net urea flux (Table III) across the bladder wall in the presence of vasopressin. In contrast, osmotic water flux across the membrane was not inhibited by thiourea in the presence of hormone (Fig. 2). These observations suggest that thiourea does not interfere with urea transport by inhibiting the action of vasopressin on urea permeability of the bladder, but that thiourea interferes with the movement of urea per se. Moreover, the finding that only urea movement and not water flux was affected by thiourea is consistent with the view that the rate-limiting permeability barriers for water and for urea differ.

Glutaraldehyde is an effective cross-linking agent of proteins and polyhydroxyalcohols that was introduced by Sabatini, Bensch, and Barrnett (14) into enzyme histochemistry as a fixative that preserves cellular fine structure without destroying the activities of a number of tissue enzymes. We had observed previously (10–12) that the water permeability barrier of the toad bladder could be virtually "locked" in various stages of response to vasopressin by exposing the mucosal surface of the bladder wall for 5 min to a 1% solution of glutaraldehyde. These fixed bladders were found to retain about 78% of their prefixation permeability to water when removed to hormone-free solutions. In the present study it has been shown (Table IV) that a bladder can be exposed to vasopressin, fixed, and removed to a hormone-free environment and still retain 74% of its prefixation permeability to urea; whereas, a bladder, which has not been exposed to vasopressin and fixed, remains relatively impermeable to urea. Vasopressin presumably induces a structural change in the rate-limiting urea permeability barrier of the bladder which is then stabilized by the cross-linking of proteins and (or) polyalcohols with glutaraldehyde. The movement of urea across vasopressin-stimulated, glutaraldehyde-fixed bladders was markedly inhibited by thiourea (Table V). Since vasopressin was not present in the medium during the urea flux studies across fixed bladders, thiourea could not have inhibited the urea flux by interfering with an early stage of hormone action on the membrane. Moreover, osmotic water flux across fixed bladders was not inhibited by thiourea.

Wyssbrod, Scott, Brodsky, and Schwartz (15) use the term "membrane carrier" to refer to any molecule or reactive group, fixed or mobile, that mediates the movement of a substance across the membrane. The results of the present study suggest that urea and thiourea compete for a common binding site in the membrane. The possibility that this binding site resides on a mobile urea-carrier is not excluded by the finding that glutaraldehyde-fixation does not abolish urea transport across the membrane. However, until more evidence for a mobile carrier molecule can be obtained, it seems that the available data can be explained by movement of urea through "urea channels" in the rate-limiting barrier that become partially obstructed when thiourea binds with reactive sites in the channel wall.

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