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Research Article

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Effect of Phloretin on Water and Solute Movement in the Toad Bladder

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ABSTRACT It is generally believed that urea crosses the cell membrane through aqueous channels, and that its movement across the membrane is accelerated in the direction of net water flow (solvent drag effect). The present report presents evidence for a vasopressin-sensitive pathway for the movement of urea, other amides, and certain non-amides, which is independent of water flow. Phloretin, when present at 10^{-4} M concentration in the medium bathing the luminal surface of the toad bladder, strongly inhibits the movement of urea, acetamide, and propionamide across the toad bladder, both in the absence and presence of vasopressin. The vasopressin-stimulated movement of formaldehyde and thiourea is also reduced. Osmotic water flow, on the other hand, is not affected; nor is the movement of ethanol and ethylene glycol, or the net transport of sodium. On the basis of these studies we would conclude that the movement of many, if not all, solutes across the cell membrane is independent of water flow, and that a vasopressin-sensitive carrier may be involved in the transport of certain solutes across the cell membrane.

INTRODUCTION

The mechanism of urea reabsorption by the renal tubule has been a problem of major interest since the studies of Shannon (1, 2), who concluded that urea reabsorption in the dog kidney is a passive process, dependent on glomerular filtration and the rate of tubular reabsorption of water. Support for this view was provided by the observation that acetamide and thiourea movement across toad skin (3) and urea movement across toad bladder (4)

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appeared to be accelerated in the direction of net water flow. This "solvent drag" effect not only suggested that urea and other small molecules move passively across the cell membrane, but that true aqueous channels exist in the membrane, large enough to permit water-solute interaction.

There is however, abundant evidence that urea movement is more than a passive process, influenced primarily by water flow. Active urea secretion by the frog kidney has been clearly demonstrated by Forster (5). Under conditions of protein depletion, a number of species appear able to reabsorb urea from tubular urine against a concentration gradient (6-10). Goldberg, Wojtczak, and Ramirez (11) have described uphill urea movement in the dog renal medulla. Intrarenal control of urea reabsorption by the elasmobranch kidney occurs independent of the filtered load of urea, and against a concentration gradient (12, 13). While questions remain about the exact nature of the process involved (14), it appears that under certain conditions and in some species, carrier-mediated transport may account for a significant fraction of urea reabsorption.

In the present paper, we have reexamined the movement of amides and other small molecules across the toad bladder, and the relationship of the movement of these solutes to water flow. These studies were prompted by two recent observations: (a) the solvent drag effect on amide diffusion across the bladder is abolished when the bathing medium is effectively stirred (15); (b) Macey and Farmer have reported that phloretin inhibits urea movement across the erythrocyte without decreasing osmotic water flow (16). We have found that 10^{-4} M phloretin, when present in the luminal bathing medium of the toad bladder, virtually abolishes urea movement, with no inhibition of water flow or active sodium transport. This selectivity of drug effect is seen both in the presence and absence of vasopressin. The inhibitory action of phloretin extends to amides in general, and to formaldehyde and thiourea. Ethanol and ethylene glycol, on the other hand,

are not affected. We would conclude that the movement of certain solutes, notably the amides, is independent of water flow and active sodium transport, and may be mediated by a vasopressin-sensitive carrier in the luminal membrane.

METHODS

Female toads (National Reagents, Bridgeport, Conn.) were doubly pithed, and glass bungs tied into both bladders *in situ*. The bladders were removed, and filled with either full strength glucose-free phosphate-buffered Ringer's solution (120 mM Na⁺, 4.0 mM K⁺, 0.5 mM Ca⁺⁺, 116 mM Cl⁻, 1.0 mM H₂PO₄⁻, 4.0 mM HPO₄⁻, pH 7.4, 230 mosm/kg H₂O); or, when osmotic water flow was to be determined, with Ringer's diluted 1:10 with distilled water. The bladders were washed inside and out three times with fresh Ringer's to remove any endogenous vasopressin. They were finally refilled with 5 ml of Ringer's or diluted Ringer's containing the isotope to be tested, and, in the test bladder, a sufficient volume of phloretin (K & K Laboratories, Inc., Plainview, N. Y.) dissolved in ethanol to achieve the desired phloretin concentration. The control bladder contained the isotope and ethanol alone. The final ethanol concentration within the bags was 0.5% or less; the surface area of the bags was 14.1 cm². The bladders were then suspended in 30–35 ml of Ringer's solution stirred by a magnetic stirrer, and aerated by compressed air. Stirring was not provided inside the bag, reducing to some extent K_{trans}urea in the control bladders. The permeability coefficient (K_{trans}) of the isotopic species was determined by sampling the inner and outer bathing medium at timed intervals; samples were pipetted into Bray's solution and counted in a Tri-Carb liquid scintillation counter (Packard Instrument Co., La Grange, Ill.). Water movement was determined by carefully blotting the bags and weighing them (17) on a Mettler B-6 balance (Mettler Instrument Corp., Hightstown, N. J.). Short-circuit current was determined by the method of Ussing and Zerahn (18), using a divided Lucite chamber in which one-half of the bladder served as a control for the test half (19). [¹⁴C]urea, -thiourea, -ethanol, -formaldehyde, and -propionamide were supplied by the New England Nuclear Corp., Boston, Mass.; [¹⁴C]acetamide and -ethylene glycol by the International Chemical and Nuclear Corp., Irvine, Calif. In

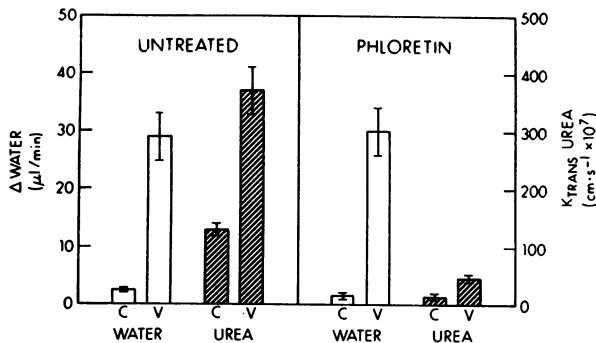


FIGURE 1 Effect of 10⁻⁴ M phloretin on the osmotic flow of water (open bars) and K_{trans}urea (hatched bars), in untreated bladders (left-hand panel) and phloretin treated bladders (right-hand panel). Nine paired experiments. C, control period (vasopressin absent); V, following vasopressin. Vertical bars, ±1 SEM.

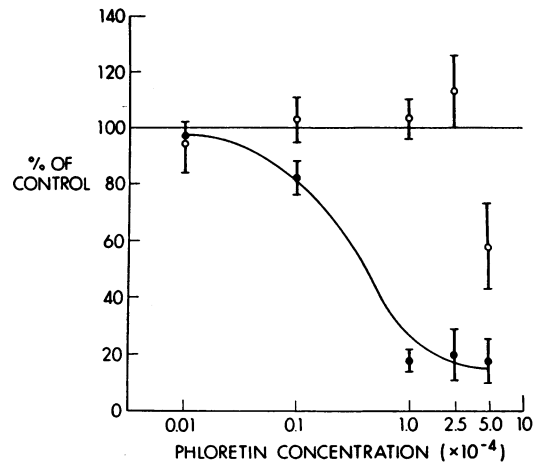


FIGURE 2 Effect of phloretin concentration on osmotic water flow (open circles) and K_{trans}urea (closed circles). Each point is the mean ± SEM of at least five experiments. The protocol in Fig. 1 was employed; values following vasopressin are shown.

all experiments, results obtained in the test bladder were compared with control bladders by the method of paired analysis (20).

RESULTS

Effect of phloretin on water and urea movement. The effect of 10⁻⁴ M phloretin on osmotic water flow and K_{trans}urea is shown in Fig. 1. Paired bladder halves, filled with 1/10 Ringer's solution, were used. The test bladder (right-hand panel) had 10⁻¹ M phloretin in 0.5% ethanol in the luminal bathing medium; the untreated bladder had 0.5% ethanol alone. When vasopressin was added to the untreated bladder, the usual sharp increase in net water flow and K_{trans}urea was seen. In the presence of phloretin, water flow was unimpaired; there was, however, a significant reduction in K_{trans}urea both before and following vasopressin. An increase in K_{trans}urea following vasopressin was still apparent in the phloretin-treated bladder.

Effect of varying concentrations of phloretin. The effect of varying luminal concentrations of phloretin on water and urea movement following vasopressin is shown in Fig. 2. 10⁻⁶ M phloretin was without effect on urea movement, while 10⁻⁵ M phloretin produced a small but significant decrease in K_{trans}urea compared with the untreated control. Urea permeability was maximally inhibited at 10⁻⁴ M phloretin; higher phloretin concentrations had no further effect. Water movement, on the other hand, was unimpaired up to and including 2.5 × 10⁻⁴ M phloretin; at 5 × 10⁻⁴ M, water movement dropped significantly to approximately 50% of control.

Symmetry of effect of phloretin on urea movement. To determine whether phloretin in the luminal bathing me-

dium inhibited the movement of urea across the bladder in both directions (lumen to serosa and serosa to lumen), a bladder was mounted in a Lucite diffusion chamber with a central dividing partition. [^{14}C]urea was placed in the luminal bathing medium of one chamber half, and the serosal medium of the adjoining half. After one 15 min control period, 2.5×10^{-4} M phloretin was placed in the luminal bathing medium of both chamber halves. There was a prompt and symmetrical fall in $K_{\text{trans}}^{\text{urea}}$ in both directions, and only a small increase in K_{trans} following vasopressin (Fig. 3).

Effect of phloretin on diffusion of other molecules. The effect of 10^{-4} M phloretin on the movement of other amides and non-amides was determined, using the same protocol employed for the urea studies. All molecules studied were those whose permeability is increased by vasopressin. The results are shown in Tables I and II. Phloretin (Table I) significantly decreased the permeability of acetamide and propionamide, both in the absence of vasopressin (period I) and following vasopressin (periods II and III). As in the case of urea, there was an increase in the K_{trans} of the phloretin treated bladders following vasopressin, but the values were considerably below those of the untreated controls. The inhibitory effect of phloretin was not restricted to the amides, however; there was a significant inhibition in $K_{\text{trans}}^{\text{formaldehyde}}$, apparent in both postvasopressin periods, and a small but significant reduction in period III of $K_{\text{trans}}^{\text{thiourea}}$.¹

Table II lists the molecules whose permeability was unaffected by phloretin. These include water, ethanol, and ethylene glycol. Phloretin also failed to depress net sodium transport (see below).

¹ The appearance of a small, but definite vasopressin effect on $K_{\text{trans}}^{\text{thiourea}}$ was unexpected, and can be attributed to the improved method of determining permeabilities, in which the "edge effect" is minimized (see Discussion).

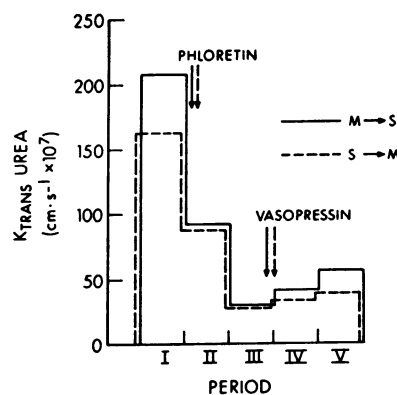


FIGURE 3 Effect of phloretin on the bidirectional $K_{\text{trans}}^{\text{urea}}$. M \rightarrow S, mucosa to serosa; S \rightarrow M, serosa to mucosa.

Effect on active sodium transport. Fig. 4 shows the mean values for short-circuit current in four experiments in which 10^{-4} M phloretin in ethanol was added to the luminal bathing medium of one-half of a divided Lucite chamber, and ethanol alone was added to the luminal medium of the other half. The addition of these agents followed a control period of 30 min. There was no reduction in short-circuit current following phloretin; indeed, there was a small, but consistent increase over the untreated bladder. $K_{\text{trans}}^{\text{urea}}$, determined simultaneously in the chamber, showed the usual inhibition by phloretin.

40 minutes after the addition of phloretin, vasopressin was added to the serosal medium of both half-chambers. The increase in short-circuit current in the untreated bladder exceeded that in the phloretin-treated bladder in two of the four experiments; in the other two, the increases were identical. In view of the inconsistency of this finding, and the possibility that enough phloretin had accumulated within the epithelial cells in the course of the experiment to depress active sodium transport

TABLE I
Permeability (K_{trans}) Decreased by Phloretin

Compound	Untreated			10^{-4} M Phloretin		
	I	II	III	I	II	III
	$\text{cm} \cdot \text{s}^{-1} \times 10^7$			$\text{cm} \cdot \text{s}^{-1} \times 10^7$		
Amides						
Urea (9)	104 \pm 16	392 \pm 36	579 \pm 39	18 \pm 4*	68 \pm 24*	114 \pm 22*
Acetamide (6)	34 \pm 6	151 \pm 21	252 \pm 27	16 \pm 1*	40 \pm 6*	69 \pm 7*
Propionamide (11)	58 \pm 10	102 \pm 14	157 \pm 18	43 \pm 6*	67 \pm 7*	90 \pm 8*
Non-amides						
Formaldehyde (4)	72 \pm 11	108 \pm 12	113 \pm 18	63 \pm 6	87 \pm 11*	88 \pm 12*
Thiourea (12)	2.2 \pm 0.5	3.5 \pm 0.5	5.2 \pm 0.5	2.0 \pm 0.4	2.5 \pm 0.3	3.5 \pm 0.5*

Vasopressin added to both bladders after period I.

* Difference (untreated vs. phloretin) statistically significant.

TABLE II
Permeability (K_{trans}) Not Decreased by Phloretin

Compound	Untreated			Phloretin (10^{-4} M)		
	I	II	III	I	II	III
	$\text{cm} \cdot \text{s}^{-1} \times 10^7$			$\text{cm} \cdot \text{s}^{-1} \times 10^7$		
Water (24) ($\mu\text{l} \cdot \text{min}^{-1}$)	2.3±0.2	27.5±2.2	38.0±2.4	1.8±0.1	32.0±2.5	39.0±1.9
Ethanol (3)	550±62	693±100	843±94	652±95	803±137	922±102
Ethylene Glycol (6)	10.1±1.5	21.7±2.6	25.5±1.8	10.7±0.9	21.3±2.2	23.6±2.1

(see below), three experiments were carried out with a shorter (10 min) interval between the addition of phloretin and the addition of vasopressin. There was no significant difference between the increase in short-circuit current in the treated and untreated bladders (Fig. 5).

Effect of phloretin on the serosal side. The effect of 10^{-4} M phloretin in the serosal rather than the luminal bathing medium was determined in six paired experiments. The findings for osmotic flow and for $K_{\text{transurea}}$ were the same as for the luminal administration of phloretin: vasopressin-stimulated water flow was unimpaired (93±15% of control values), while $K_{\text{transurea}}$ was significantly depressed, both in the absence of vasopressin (68±10% of control) and following vasopressin (64±9% of control). The inhibitory effect of serosal phloretin on $K_{\text{transurea}}$ following vasopressin was not as great as that of luminal phloretin; in four paired experiments, $\Delta K_{\text{trans}}(\text{serosal-luminal phloretin})$ was 58 ± 16 (SE) $\times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$ ($P < 0.05$).

The effect of serosal phloretin on active sodium transport differed significantly from its luminal action: there was a consistent reduction in short-circuit current both in the absence and presence of vasopressin (Fig. 6).

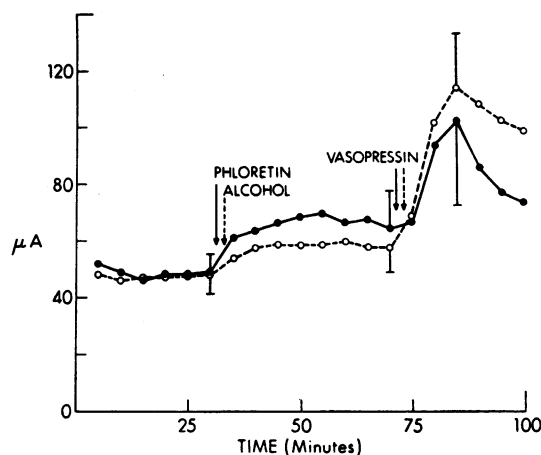


FIGURE 4 Effect of phloretin in the luminal bathing medium on short-circuit current. Vasopressin added 40 min after phloretin.

Reversibility of the phloretin effect. The extent to which the inhibitory action of phloretin on $K_{\text{transurea}}$ could be reversed was determined by exposing one of a pair of bladders to 10^{-4} M phloretin (luminal side); phloretin was then removed by three serial washouts of the contents of the bag with phloretin-free Ringer's containing 0.5% ethanol. The control bag (no exposure to phloretin) was subjected to the same washout procedure. Fig. 7 shows the results of four paired experiments in which phloretin was present in the test bag for one period prior to, and two periods following vasopressin. Phloretin was then washed out of the test bag with vasopressin remaining in the serosal solution. The usual depression of $K_{\text{transurea}}$ was seen in the presence of phloretin; following washout, $K_{\text{transurea}}$ rose to the level of the control bladder. The phloretin effect, therefore, was entirely reversed by the washout.

Effect of unlabeled urea. To determine whether phloretin was reducing $K_{\text{transurea}}$ by simply binding the isotope in the bathing medium and preventing it from penetrating the bladder, the effect of introducing unlabeled urea into the medium was determined. 5×10^{-3} M unlabeled urea was added to Ringer's solution containing 10^{-4} M phloretin in 0.5% ethanol, giving a 50-fold greater concentration of urea than phloretin. [^{14}C]urea was then added to the Ringer's and the solution was in-

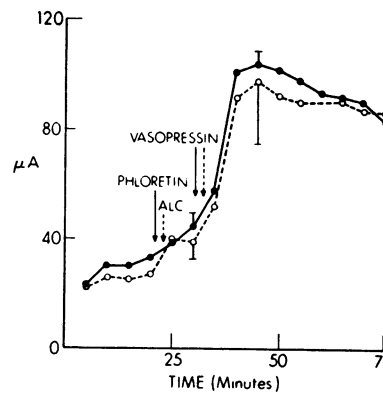


FIGURE 5 Effect of phloretin in the luminal bathing medium on short-circuit current. Vasopressin added 10 min after phloretin.

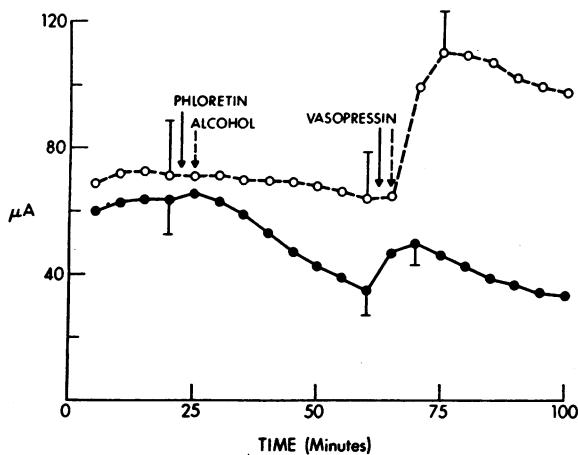


FIGURE 6 Effect of phloretin in the serosal bathing medium on short-circuit current. Points are means of four experiments.

troduced into the lumen of one of a pair of bladders. The control bladder half contained Ringer's, 0.5% ethanol, 5×10^{-3} M unlabeled urea, and $[^{14}\text{C}]$ urea. The results are shown in the first part of Table III. Despite the large excess of unlabeled urea, both with respect to phloretin and isotopic urea, there remained a significant inhibition of $K_{\text{trans}}[^{14}\text{C}]$ urea by phloretin.

To determine whether unlabeled urea had any effect at all on $K_{\text{trans}}\text{urea}$ in the presence of phloretin, a second set of paired experiments was carried out, with 10^{-4} M phloretin in both bags, and 5×10^{-3} M unlabeled urea in the test bag. The results are shown in the second part of

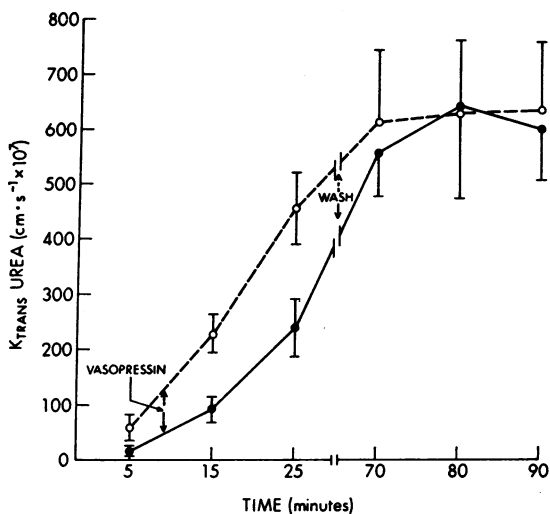


FIGURE 7 Reversibility of the phloretin effect. Bladders were initially treated with phloretin (closed circles) or 0.5% ethanol (open circles). At "wash" the contents of both bladders were washed out with Ringer's solution and 0.5% ethanol.

TABLE III
Interaction of 10^{-4} M Phloretin and 5×10^{-3} M Urea

	$K_{\text{trans}}[^{14}\text{C}]$ urea		
	I	II	III
	$\text{cm} \cdot \text{s}^{-1} \times 10^7$		
Effect of phloretin in presence of urea (4)			
Urea	196 ± 28	355 ± 69	413 ± 60
Phloretin + urea	51 ± 10	173 ± 67	203 ± 72
	$P < 0.01$	$P < 0.01$	$P < 0.01$
Effect of urea in presence of phloretin (6)			
Phloretin	26 ± 10	105 ± 24	185 ± 35
Phloretin + urea	29 ± 11	98 ± 20	173 ± 26
	NS	NS	NS

Vasopressin was added after period I.

Table III. There was no significant increase in $K_{\text{trans}}\text{urea}$ in the presence of unlabeled urea.

In another variation of this protocol, shown in Fig. 8, one bladder half received $[^{14}\text{C}]$ urea and 10^{-4} M phloretin, while the paired half received $[^{14}\text{C}]$ urea alone. After three vasopressin periods, 5×10^{-3} M unlabeled urea was added to the luminal medium of both bladders. There was no increase in $K_{\text{trans}}[^{14}\text{C}]$ urea in the phloretin-treated bladder. Additional isotope was then added to the luminal solutions of both bladders. $K_{\text{trans}}\text{urea}$ was unchanged in test and control bladders after the addition of isotope, indicating that binding of the original $[^{14}\text{C}]$ urea by phloretin was not a factor in the phloretin effect. Ultraviolet spectrophotometry also failed to demonstrate

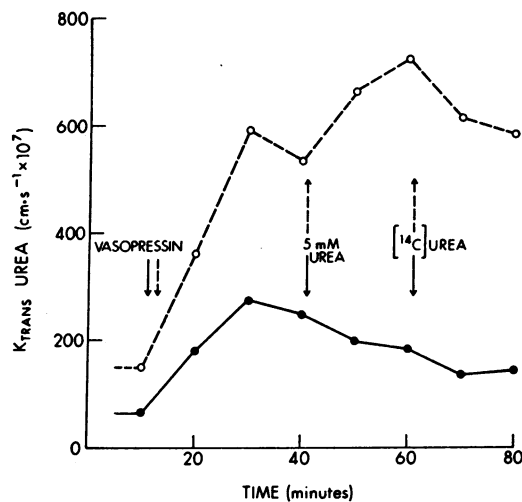


FIGURE 8 Effect of treatment with 5×10^{-3} M unlabeled urea, followed by additional $[^{14}\text{C}]$ urea, on $K_{\text{trans}}[^{14}\text{C}]$ urea in bladders containing 10^{-4} M phloretin (closed circles) or 0.5% ethanol (open circles). Points are the means of two paired experiments.

any alteration in the characteristic phloretin spectrum after the addition of urea.

DISCUSSION

Earlier studies on the toad bladder showed that vasopressin increased both the osmotic flow of water and the movement of a restricted group of solutes across the luminal membrane of the epithelial cell (4, 21, 22). The amides, notably urea, showed a particularly sharp increase in permeability. There was no evidence that an active transport system for urea was involved; studies of the simultaneous bidirectional movement of [¹⁴C]- and [¹⁵N]urea showed equal permeability in the two directions (21). Nor could evidence be obtained for a saturable urea carrier, since $K_{\text{trans}}[^{14}\text{C}]\text{urea}$ was unaffected by concentrations of unlabeled urea up to 50 mM (21). While the unlabeled urea concentration that could be used in the toad bladder was limited,² active reabsorption of urea in the dogfish kidney had failed to show saturation up to 750 mM/liter plasma concentration (23). It was concluded that in the toad bladder, urea moved passively in the same aqueous channel through which the osmotic flow of water took place.

The demonstration by Macey and Farmer (16) that phloretin inhibited the movement of urea into the erythrocyte without diminishing osmotic flow showed that in the erythrocyte membrane the mechanisms, and possibly the pathways, for water and urea movement were separate. Our findings in the toad bladder confirm and extend these observations.

Several features of the action of phloretin on the toad bladder deserve comment. Phloretin inhibits urea movement at a concentration in the luminal bathing medium as low as 10^{-5} M; maximal inhibition (approximately 80%) is at 10^{-4} M. Active sodium transport is maintained, both in the absence and presence of vasopressin, when phloretin is present in the luminal medium, but is depressed by phloretin in the serosal medium. The depression of sodium transport probably represents a direct inhibitory action of the drug on the sodium pump, which is believed to be located at the basolateral cell membrane. Phloretin at either surface of the bladder inhibits urea movement, and shows no effect on water movement; the inhibitory effect on $K_{\text{trans}}\text{urea}$, however, is less striking when the drug is on the serosal side. These findings suggest that phloretin acts at a site in the luminal membrane, rather than at an intracellular or basolateral site, and are consistent with earlier evidence that the luminal membrane is the rate-limiting barrier for urea movement across the cell (21). Phloretin in-

hibits the movement of urea in both directions across the bladder, as would be expected in this passive transport system. An effect of vasopressin on $K_{\text{trans}}\text{urea}$ was still apparent in the phloretin-treated bladder; indeed, there was an increase of approximately six-fold ($K_{\text{trans}}\text{period III}/K_{\text{trans}}\text{period I}$) in both the untreated and phloretin-treated bladders (Table I). This finding suggests that urea movement both in the presence and absence of vasopressin may proceed through the same pathway, and that phloretin inhibits this pathway to the same extent before and after vasopressin. It is also apparent that urea transport can be greatly depressed with no accompanying reduction of active sodium transport, indicating that the close correlation between urea and sodium transport reported in the dogfish (24) is not present in the toad bladder.

The movement of a number of non-electrolytes in addition to urea is inhibited by phloretin. There is a significant decrease in the K_{trans} of acetamide and propionamide, and it is possible that the transport pathway modified by phloretin is one involved in the passive movement of all amides. In addition to the amides, phloretin inhibits the movement of formaldehyde, and, in the second vasopressin period, the movement of thiourea. With the small number of molecules tested, it is not yet possible to explain on the basis of structure either the inhibition of amide, formaldehyde, and thiourea transport by phloretin, or the failure of phloretin to inhibit the transport of ethylene glycol and ethanol (Table II). The extent to which these non-electrolytes are capable of forming hydrogen bonds may be of importance in determining whether they move through the phloretin-sensitive pathway. It is also of interest that ethylene glycol and ethanol are molecules that appear to penetrate human red cells by simple diffusion, rather than facilitated diffusion (25), and that, as in the toad bladder, phloretin has no effect on the movement of ethylene glycol in the red cell (16). Thiourea, on the other hand, which is inhibited by phloretin, may be related closely enough to the amides in structure to have an affinity for the amide pathway; there is evidence, for example, that thiourea as well as true amides are actively secreted by the frog renal tubule (26). However, no evidence exists for an active mucosal to serosal transport system for thiourea in the renal tubule of the shark (13) or of the protein-depleted sheep (13). While no obvious relationship exists between thiourea structure and transport in the toad bladder, its inclusion in the group of compounds that may be carrier-mediated could explain our earlier finding that thiourea has a reflection coefficient significantly less than 1.0 (27). Indeed, the observation by Diamond and Wright (28) that many compounds, including the amides, have reflection coefficients below those predicted from their low lipid solubility could be explained

² Recent studies (Franki, Levine, and Hays, unpublished observations) have shown that at 150 mM unlabeled acetamide, [¹⁴C]acetamide transport shows significant inhibition. This provides direct evidence for a carrier-mediated system.

by the existence of carrier-mediated movement of these compounds across the epithelium under study.

An unexpected finding in these studies was the small but significant effect of vasopressin on K_{trans} thiourea, a molecule whose permeability had been thought to be unchanged by the hormone (4, 21). The increase in K_{trans} is apparent in Table I, and has been confirmed in an independent group of paired studies (Franki, Levine, and Hays, unpublished observations), in which one bladder half received vasopressin and the control half did not. ΔK_{trans} (vasopressin-control) was 2.2 ± 0.4 (SE) $\times 10^{-7}$ $\text{cm} \cdot \text{s}^{-1}$; $P < 0.02$. Our failure to detect a vasopressin effect in the earlier experiments was probably due to edge damage associated with the Lucite chambers used in these studies (29, 30); K_{trans} thiourea was three to six times greater than with the present bag technique, and the small vasopressin effect was probably obscured by the leak at the bladder edge. Our results are in accord with those of Lippe, Micelli, and Galluci (31), who found a significant increase in K_{trans} thiourea across the bladder of *Rana esculenta* following vasopressin.

Little can be said at this time about the composition or kinetics of the carrier or mechanism involved in non-electrolyte transport. It may simply be a component of the membrane for which certain non-electrolytes have a high affinity. It is not clear whether such a component is a lipid or a protein, although it is of interest that the extent to which phloretin inhibits the more water-soluble molecule urea is far greater than the more lipid-soluble propionamide (Table I). This would be consistent with an action of phloretin at a protein rather than at a lipid site. Handler, Sugita, Preston, and Orloff (32) have reported that cycloheximide and puromycin, inhibitors of protein synthesis, reduce the permeability of the bladder to urea to a relatively greater extent than the permeability to water, again suggesting that a protein component may be involved in vasopressin-stimulated urea movement. In view of the complex structure of membranes, however, and the number of steps outside the membrane involved in the action of vasopressin, neither finding is conclusive. With regard to the location of the non-electrolyte transport pathway, it could be in series with, or parallel to the water pathway. The present studies do not distinguish between these two possibilities.

How phloretin inhibits the movement of non-electrolytes is obscure. The inhibition is easily reversible, making it unlikely that covalent bonding is involved in its action. It also seems unlikely that phloretin acts as a competitive inhibitor of non-electrolyte transport, in view of the extraordinary capacity of the transport system. Phloretin may alter the properties of the transport system at a point other than the amide receptor site; with the limited information now at hand, this would be a reasonable possibility. There is no evidence

for either an interference with cell metabolism or a non-specific effect on membrane permeability, since sodium transport and water flow are preserved, and the permeabilities of ethanol and ethylene glycol are unchanged. As in the red cell (33), we have found that the inhibitory effect of phloretin is abolished when the pH of the luminal bathing medium is raised to 9.0. This is the result of a shift from the physiologically active ketonic to the inactive enolic form of the compound (33).

Phloretin, and the naturally occurring glucoside phlorizin, have both been shown to inhibit transport of a variety of compounds across cell membranes. They both inhibit monosaccharide transport across the cat proximal tubule (34) and the hamster small intestine (35); phlorizin is markedly more potent in both preparations. In the human red cell, on the other hand, phloretin is a more potent inhibitor of glucose entry than phlorizin (33), and also inhibits sodium uptake by the dog red cell (36). Alvarado (35), in discussing the mode of action of the two compounds in the small intestine, has suggested that phlorizin competes with glucose and its analogs for a common membrane binding site, while phloretin inhibits sugar transport allosterically at a different, although closely associated binding site. He has proposed a model in which the glucose carrier is assumed to have two different binding sites: one for phenols, to which both phloretin and phlorizin bind, and one for sugars, to which glucose and phlorizin bind. The existence of two binding sites for phlorizin in the model could account for the finding that phlorizin has a higher affinity for the carrier than glucose, and is a competitive inhibitor of intestinal sugar transport (35). While phloretin may occupy an allosteric site in the intestinal and renal tubular glucose carriers, there is evidence that it is a competitive inhibitor of erythrocyte glucose transport (37), and therefore its relationship to the carrier in the erythrocyte membrane may differ in important respects from that in gut and renal tubule. It is not yet clear whether the inhibition by phloretin of both urea and glucose transport in the red cell represents an action on the same, or different carriers. However the demonstration in both the toad bladder and the erythrocyte of a phloretin-sensitive pathway for solutes other than sugars adds a problem of considerable interest to the area of carrier-mediated transport.

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