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Methylurea and Acetamide: Active Reabsorption by Elasmobranch Renal Tubules

Abstract. *The renal tubules of the shark actively reabsorb urea. They also can reabsorb acetamide and methylurea, but there is no evidence for active reabsorption of thiourea. The specificity of the transport system thus appears to be different from the urea secretory system in the frog in which thiourea is secreted but acetamide and methylurea are not secreted.*

The renal tubules of elasmobranchs actively reabsorb urea from the tubular fluid with the result that the concentration of urea in the urine is lower than that of plasma (*1–3*). This mechanism helps in maintaining a high concentration of urea in the blood (approximately 350 m*M*/liter) which in turn makes it possible for the elasmobranch to be in osmotic equilibrium with the surrounding seawater in spite of lower concentrations of electrolytes in the plasma.

We have studied the renal excretion patterns of compounds related to urea in chemical structure. This was done in order to be able to compare the carrier specificity of the urea transport system in the shark tubules with that of other species, such as frog and mammal. The compounds used were methylurea ($CH_3NHCONH_2$), acetamide (CH_3CONH_2) and thiourea (NH_2CSNH_2).

Ten sharks, representing five species of elasmobranchs, were studied (*4*). A polyethylene catheter was inserted into the urinary papilla in the females or urogenital papilla in the males and tied securely in place. A narrow side tube opened into the part of the catheter nearest the papilla. By blowing air through the side tube the catheter could be emptied at the end of each urine collection period. The shark was placed in running seawater in a narrow wooden box. The C^{14}-labeled test compounds, together with the same nonlabeled compounds, were dissolved in seawater and injected with a hypodermic needle into the caudal vein or artery 2 to 3 hours prior to the first urine collection period. When inulin was given, it too was injected into the caudal vein 3 hours prior to the first urine collection. Blood samples from the caudal vein or artery were taken before and after each urine collection period, which lasted from 1 to 12 hours. (The length of the collection period had no effect on the results.) The urine and blood samples were analyzed for urea by the Conway method; for inulin, by the diphenylamine method; and the C^{14}-labeled test compounds, by liquid scintillation spectrometry.

Data for all fish studied are presented in Table 1. In four sharks (three different species) injected with methylurea, the ratio of the methylurea concentration in the urine (U_m) to that in the plasma (U_m/P_m) was less than unity in all 11 clearance periods (average, 0.62). In three sharks injected with acetamide, the ratio for acetamide, U_a/P_a, was likewise lower than unity in all nine periods (average 0.76). The ratio for urea, U_u/P_u, was also less than unity and less than the simultaneously measured ratios U_a/P_a and U_m/P_m in all collection periods.

Table 1. Concentration of urea or test compound in plasma (P). Concentration of compound in urine divided by its concentration in plasma (U/P). The species *Heterodontus francisci* and *Rhinotriacis henlei* were caught in the coastal waters of Baja California; the species *Mustelus canis* and *Squatina dumerili*, in the coastal waters of North Carolina; and *Squalus acanthias*, in the coastal waters of Maine.

Species	Period	Test compound		Urea	
		P (mM/l)	U/P	P (mM/l)	U/P
		Methylurea			
Squatina dumerili	1	10.5	0.67	376	0.44
	2	10.2	.64	374	.47
	3	9.9	.70	378	.48
	4	9.8	.73	376	.54
Mustelus canis	1	21.7	.82	288	.68
Mustelus canis	1	21.0	.72	273	.57
Squalus acanthias	1	10*	.49		
	2	10	.52		
	3	10	.56		
	4	10	.60		
Squalus acanthias	1	1.0*	.39		
Average			0.62	343	0.53
		Acetamide			
Heterodontus francisci	1			367	0.20
	2	0.01	0.84	368	.20
	3	.01	.76	370	.23
Heterodontus francisci	1	.01	.75	419	.14
	2	.01	.68	397	.18
	3	.01	.77	402	.16
Rhinotriacis henlei	1	.01	.76		
	2	5.8*	.89	345	.60
	3	5.8*	.68	341	.34
	4	13.5*	.71	341	.39
Average			0.76	372	0.27
		Thiourea			
Rhinotriacis henlei	1	0.01	2.69	323	0.56
	2	.01	2.85	315	.56
	3	.01	3.25	313	.57
Mustelus canis	1	20.0	2.15	296	.89
Average			2.74	312	0.64

* Represents approximate plasma concentration, calculated from the amount injected and the estimated total body water.

Since a urine-to-plasma ratio of less than 1.0 for an uncharged molecule indicates tubular reabsorption against a concentration gradient, these results constitute evidence for the active reabsorption of methylurea and acetamide. The data for urea are similar to those reported by others (*1, 3*) and also show active reabsorption.

The ratio for thiourea, U_t/P_t, measured in two fish was greater than 1.0 (average, 2.74). These results for thiourea confirm those of Clarke and Smith (*5*) and provide no evidence for active reabsorption of thiourea. The ratio for inulin, U_i/P_i, as measured in four fish was always greater than 1.0 (average, 6.6; range, 2.12 to 15.0), while urine flow varied from 0.03 to 1.69 ml/hr.

If the results obtained for the shark are compared with those for the frog (*6*) it is apparent that the substance thiourea, which is actively secreted in frog tubules (from blood to tubular lumen), is not actively reabsorbed (from tubular lumen to blood) in the shark tubules; while the two substances acetamide and methylurea, which are not secreted by the frog tubules, are actively reabsorbed by the shark tubules.

In the mammalian kidney, Rabinowitz and Kellogg (*7*) found that acetamide and methylurea can enhance the ability to produce a concentrated urine. The effect of these compounds were similar to the well-recognized effect of urea, whereas no effect was found with thiourea. Truniger and Schmidt-Nielsen (*8*) found that methylurea, acetamide, and urea accumulate in the renal medulla in concentrations higher than that in the urine in rats on a diet low in protein and high in salt, while thiourea does not accumulate in the medulla.

The various results suggest that the two mechanisms for transporting urea across the renal epithelium, namely, the secretory mechanism in the frog tubules and the reabsorptive mechanism in the shark tubules, do not share a common carrier since they handle urea-related compounds differently. The similarity between the results in mammals and shark are in agreement with the concept of an active reabsorption in the collecting ducts as suggested by previous results (*8, 9*).

A facilitated diffusion system for urea in elasmobranch erythrocytes was described recently by Murdaugh *et al.* (*10*), but the results of those authors cannot readily be interpreted on the same basis as our results.

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Inulin and Albumin Absorption from the Proximal Tubule in Necturus Kidney

Abstract. *In the kidney of the amphibian,* Necturus, *appreciable quantities of inulin and human serum albumin are transported from the tubular lumen of the proximal tubules into the blood. These findings suggest that inulin may not be a satisfactory indicator for measuring net water movement in the kidney of this species.*

The time course of fluid absorption from the proximal tubules of the *Necturus* kidney was measured by sequential photography of isolated columns of fluid in the tubule. Water absorption in these experiments, which were all performed on adult "summer" necturi obtained in April, May, and June, 1964, was found to be considerably in excess of that previously determined in the same species by the stopped flow microperfusion technique (*1*) in which C^{14}-labeled inulin is used to measure net water movement. To investigate this discrepancy, we examined the possibility that inulin may be absorbed from the tubular lumen. Although it has been widely accepted by renal physiologists that inulin is not reabsorbed by the renal tubule, evidence in support of this viewpoint is entirely indirect (*2*). Our results show that appreciable quantities of this molecule are indeed absorbed in the *Necturus* proximal tubule, but, conversely, inulin initially introduced into the blood does not cross into the tubule. These findings indicate that inulin may not be a satisfactory indicator for measuring net water movement in the proximal tubule of *Necturus*.

Segments of the proximal and the distal portions of the proximal tubule are usually visible on the ventral surface of the *Necturus* kidney. These can be filled with oil, and the column then can be split by injection of isosmotic perfusion fluid containing 100 m*M* NaCl. This procedure allows the isolation of columns of perfusion fluid 0.5 to 0.6 mm long in either of these segments of the proximal tubule. To record the changes in volume of the perfusate, we took photographs throughout a 20-minute period at 2-minute intervals, using a condenser-actuated flash discharge, according to the procedure described by Gertz (*3*). The length and the diameter of the column of perfusion fluid were measured on projections of the photographic negative, so that the perfusate volume could be calculated. After applying a suitable meniscus correction, the logarithm of the volume of perfusate relative to its initial volume [ln (v/v_0)] was found to be a linear function of time over the course of the experiments. The relative volumes at 20 minutes, expressed as percentage water absorption [$(1-v_{20}/v_0)$ 100], were, for the proximal segment, 73, 70, 37, 48, 35; and for the distal segment, 44, 51, 43. These data show that there was no significant difference in water absorption between the proxi-