

3-0-methyl-D-glucose, as previously reported, could not be confirmed in this study; C₂-OH is not required. This investigation was supported in part by USPHS Grant AM-12619.

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THE EFFECT OF PHLORETIN ON RENAL UREA AND SODIUM TRANSPORT IN *Squalus acanthias*

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The dogfish kidney is capable of reabsorbing urea against a large concentration gradient and can produce a urine with a urea concentration substantially lower than that of blood independent of the urinary sodium concentration. The purpose of the present study was to examine the effect of phloretin, a potent inhibitor of amide movement across several biological membranes, on the ability of the dogfish kidney to reabsorb urea.

Free-swimming, inulin-loaded fish were studied in tanks having a brisk flow of running sea water. Catheters were placed in the urinary sinus and dorsal aorta for collection of samples and injection of test substances. After a two-hour equilibration period and one 2-hour baseline period, the fish were injected with one to 1.2 ml of a test solution consisting of phloretin (20-23 mg/kg) dissolved in ethanol (0.14-0.16 ml/kg) and dogfish Ringer's (0.08-0.09 ml/kg), over a period of 10-15 minutes. Following the injection urine and blood were collected for two additional two-hour periods. A second group of fish was treated similarly but received only ethanol and Ringer's, without phloretin in order to ascertain the effect of ethanol alone on urea reabsorption.

On each plasma or urine sample, volume, urea, sodium, and inulin concentrations were determined. Analysis of data was done by comparing the mean of second and third periods with the first by the method of pair analysis.

Data for four control fish are shown in the left half of Table 1 for one period before and the average of two periods after the addition of ethanol. Ethanol did not lead to a significant alteration in urinary volume (V), GFR, fractional urea excretion or urinary excretion of urea or sodium. A small although significant decrease was seen in fractional excretion of sodium.

Results for the five fish receiving phloretin are shown in the right half of Table 1 for one period before and the mean of the two periods after the administration of phloretin. Urinary urea excretion increased markedly after phloretin administration. Fractional urea excretion also increased greatly while absolute urea reabsorption fell. Therefore phloretin significantly decreased urea reabsorption by the dogfish kidney. Urinary sodium excretion did not change significantly after phloretin but a decrease in absolute reabsorption and an increase in fractional excretion were observed. Urinary osmolality changed insignificantly from 705 ± 19 before phloretin to 723 ± 42 after phloretin. There was a small increase in urinary volume and a small decrease in GFR neither of which was significant. However fractional water excretion did increase significantly from 24 ± 2 to 37 ± 2 percent after phloretin ($p < 0.01$).

Thus phloretin causes a marked decrease in urea reabsorption in the dogfish kidney associated with a decrease in sodium reabsorption as well. Its action here is similar to that seen in the toad

TABLE 1
EFFECT OF PHLORETIN ON UREA AND SODIUM EXCRETION IN *Squalus acanthias*

Period	CONTROL (n = 4)		PHLORETIN (n = 5)	
	I	II [†]	I	II [†]
GFR (ml/kg/hr)	3.50 + 0.44	3.03 + 0.09	3.09 + 0.17	2.29 + 0.22
V (ml/kg/hr)	0.89 + 0.06	0.78 + 0.06	0.74 + 0.09	0.81 + 0.03
<u>Urea</u>				
UV (mM/kg/hr)	76.2 + 6.9	85.5 + 15.7	44.5 + 9.3	116.3 + 15.6**
Absolute reabsorption (mM/kg/hr)	1148 + 141	1019 + 47	1022 + 78	670 + 96*
Fractional excretion (%)	6.3 + 0.7	8.0 + 1.3	4.0 + 0.6	16.0 + 2.8**
<u>Sodium</u>				
UV (mEq/kg/hr)	232 + 18	194 + 24	198 + 28	186 + 6
Absolute reabsorption (mEq/kg/hr)	691 + 101	608 + 35	619 + 30	422 + 55*
Fractional excretion (%)	26.0 + 2.7	24.1 + 3.0*	24.2 + 2.2	31.6 + 2.4**

*p<0.05

**p<0.01

[†]Period I was used as baseline in both groups.

Ethanol (in control fish) or phloretin was added at the start of period II.

bladder: luminal phloretin decreases amide permeability alone, while serosal phloretin causes a fall in both amide permeability and short-circuit current. In the dogfish intravenous phloretin would gain access to both sides of the tubular cell, the serosal via the blood and the luminal via the glomerular filtrate, and could affect the cell by decreasing both urea permeability and sodium transport.

A second possibility should also be considered however in view of the fact that phloretin does not disturb the close relationship between absolute urea and sodium reabsorption demonstrated by Schmidt-Nielsen, et al (Comp. Biochem. Physiol. 42A, 13-25, 1972). In the baseline period the ratio of urea to sodium reabsorption was 1.64 ± 0.06 . After phloretin the ratio remained unchanged at 1.59 ± 0.10 . The preservation of this relationship after phloretin suggests that the drug's two effects in the dogfish may not be the result of two independent actions. The interdependence of urea and sodium reabsorption after phloretin might mean that their fluxes are so closely coupled as to be affected equally by a single action of phloretin on either urea or sodium. Supported by Grants AM-03858 and HL-05928 from the U.S. Public Health Service.