Legends for Figures on page 86.

Figure 1. Section of hagfish brain showing capillary and adjacent neuron (N). Note wide lumen (L) of capillary, thick endothelial cytoplasm, and prominently stained basement membrane (arrow). One micron plastic embedded section, toluidine blue. 1000X

Figure 2. Cytoplasm of two adjacent endothelial cells (E). Note vesicles (v) and tubules (t), some of which appear to coalesce with the lumenal plasma membrane (bent arrow) and others which appear to communicate with the space between adjacent endothelial cell plasma membranes (straight arrows). Note also the area of apparent fusion between the two endothelial cells (star). 88,000X.

Figure 3. Endothelial cytoplasm with many vesicles. Note prominent basement membrane (arrow). 20,000X

Figure 4. Complexly interdigitated junction between two adjacent endothelial cells (star). 56,000X

Figure 5. Tubules in endothelial cytoplasm (arrows) communicating with basement membrane (BM). Note trilaminate structure of basement membrane. 88,000X.

Figure 6. Spoke like processes of basement membrane (arrow) extending into brain tissue. Note small dense granules (g), presumably glycogen. 34,000X

Figure 7. Clefts between adjacent glial cells contacting ablumenal surface of basement membrane. 88,000X

Figure 8. Endothelial cells process extending into lumen which contains glycogen granules similar to those found in ablumenal tissue. 88,000X

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FURTHER STUDIES ON THE EFFECTS OF DIURETIC DRUGS ON RENAL FUNCTION IN *Squalus acanthias*.

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In 1971 data were presented which established that furosemide and ethacrynic acid in doses of 40 mg and 50 mg per dogfish respectively produced a striking diuresis with marked augmentation of sodium, urea, and osmolar outputs. Further experiments have been conducted utilizing the same general procedures to determine the effects of smaller doses of the diuretics with particular emphasis on furosemide. Dose-response curves have been derived.

Control observations, using the same experimental protocol but without drug, have been expanded. In the control studies dogfish Ringers solution, 4 ml intravascularly, were injected instead of diuretic drug and the usual sequence of clearance periods was followed.

The control data are presented in Table 1. Urea excretion does increase with time to a moderate degree but is associated with no significant change in plasma urea concentration. The calculated urea clearance is moderately increased. In the face of a slight fall in inulin clearance and in filtered loads of urea and sodium, the ratios $U_{urea}V/F_{urea}$ and $U_{Na}V/F_{Na}$ are increased. Otherwise all measured parameters are steady.

Table 2 provides the data for the various doses of furosemide. A striking diuresis, natruresis, and increase in urea excretion occurred with all doses from 0.1 mg through 40 mg but diminished effect was noted on a total dose below 1 mg for water and sodium and below 2 mg for urea. Plotting

TABLE 1

CONTROL DATA ON *Squalus acanthias* (7)

(Values except for ratios expressed as unit/Kg/hour and given as mean ± standard error.)

	BASELINE VALUES	DIFFERENCES AFTER 4 ML DF RINGERS
Volume, ml	0.67 ± 0.052	0.11 ± 0.060
C_{in}, ml	2.80 ± 0.202	-0.08 ± 0.267
$U_{Na}V$, u Eq.	176 ± 14.9	14 ± 19.9
F_{Na}, u Eq.	734 ± 54.3	-14 ± 83.6
$U_{Na}V/F_{Na}$	0.239 ± 0.0104	0.024 ± 0.0097*
$U_{urea}V$ u M	58 ± 6.1	12 ± 4.8*
P_{urea} u M/L	329 ± 8.1	-5 ± 3.2
C_{urea}, ml	0.18 ± 0.024	0.05 ± 0.015**
F_{urea}, u M	924 ± 76.3	-41 ± 112.1
$U_{urea}V/F_{urea}$	0.062 ± 0.0077	0.019 ± 0.0029**
C_{osm}, ml	0.47 ± 0.038	0.06 ± 0.046
C_{H_2O}, ml	0.20 ± 0.018	0.05 ± 0.021*
U_{osm}/P_{osm}	0.70 ± 0.013	-0.04 ± 0.018

* $p < 0.05$
** $p < 0.01$
All other differences are statistically insignificant.

of dose-response curves indicated that full response in promoting water and sodium excretions was obtained at 0.2 mg per kg and for urea at 0.45 mg per kg. Below those dosages there was the expected "straight-line" diminution of effect. Inulin clearance and the filtered loads of sodium and urea were not consistently affected. Osmolar clearance paralleled the increases in sodium and urea excretion and showed similar dose relationship. Alterations in free water clearance were small and seemingly insignificant.

Two experiments using 0.5 mg and 1 mg respectively of ethacrynic acid demonstrated definite augmentation of water, sodium, and urea excretions but of lesser degree than with the previously reported 50 mg dose.

Squalus acanthias is highly sensitive to the diuretic effects of furosemide and ethacrynic acid which presumably exert their effects by interference with active tubular reabsorption of sodium and urea.

TABLE 2

RENAL RESPONSE IN *Squalus acanthias* TO VARIOUS DOSES OF FUROSEMIDE.
DIFFERENCES INDUCED BY THE DRUG AS COMPARED TO CONTROL PERIODS FOR EACH INDIVIDUAL FISH.
Units/kg/hour given as means ± S.E.

FUROSEMIDE, DOSE, MG	2	1	0.4	0.2	0.1
NO. OF FISH	3	2	2	2	2
NO. OF DIURETIC PERIODS	12	8	8	8	5
Volume, ml	0.83 ± 0.127	0.84 ± 0.062	0.54 ± 0.108	0.36 ± 0.088	0.45 ± 0.115
p	<0.001	<0.001	<0.01	<0.01	<0.02
C_{in}, ml	-0.14 ± 0.168	+0.25 ± 0.280	+0.13 ± 0.268	-0.54 ± 0.248	+0.01 ± 0.211
p	Insig.	Insig.	Insig.	Insig.	Insig.
$U_{Na}V$, μ Eq	234 ± 36.0	237 ± 02.6	176 ± 28.1	102 ± 25.0	107 ± 31.2
	<0.001	<0.001	<0.001	<0.01	<0.05
F_{Na}, μ Eq	-32 ± 44.4	+71 ± 73.3	+40 ± 68.2	-133 ± 63.7	+10 ± 54.2
p	Insig.	Insig.	Insig.	Insig.	Insig.
$U_{Na}V/F_{Na}$	0.471 ± 0.0464	0.496 ± 0.0727	0.378 ± 0.0452	0.302 ± 0.1471	0.239 ± 0.2653
p	<0.001	<0.001	<0.001	<0.001	Insig.
$U_{urea}V$, μ M	335 ± 43.0	257 ± 22.3	129 ± 22.4	146 ± 25.3	139 ± 34.7
p	<0.001	<0.001	<0.001	<0.001	<0.02
C_{urea}, ml	1.06 ± 0.141	0.83 ± 0.062	0.42 ± 0.063	0.46 ± 0.084	0.42 ± 0.177
p	<0.001	<0.001	<0.001	<0.001	Insig.
$U_{urea}V/F_{urea}$	0.553 ± 0.0473	0.463 ± 0.0627	0.251 ± 0.0493	0.283 ± 0.0391	0.250 ± 0.1179
	<0.001	<0.001	<0.01	<0.001	Insig.
C_{osm}, ml	0.88 ± 0.123	0.83 ± 0.063	0.56 ± 0.076	0.35 ± 0.081	0.35 ± 0.168
p	<0.001	<0.001	<0.001	<0.01	Insig.
C_{H_2O}, ml	-0.05 ± 0.019	+0.01 ± 0.016	-0.02 ± 0.040	+0.01 ± 0.014	+0.10 ± 0.036
p	<0.02	Insig.	Insig.	Insig.	Insig.
U_{osm}/P_{osm}	0.20 ± 0.021	0.17 ± 0.040	0.10 ± 0.057	0.10 ± 0.025	0.06 ± 0.063
p	<0.001	<0.01	Insig.	<0.01	Insig.

FIG. I - RENAL RESPONSE IN SQUALUS ACANTHIAS TO VARIOUS DOSES OF FURSEMIDE. DIFFERENCES INDUCED BY THE DRUG AS COMPARED TO CONTROL PERIODS FOR EACH FISH.