interlamellar system. Indeed, when India ink is injected into the circulation of the dogfish, the collateral circulation at the tip of the filament and under the water channels is seen to turn gray while the lamellae remain red. This indicates a separation of red cells from plasma as the collateral circulation is filled. The collateral circulation supplies areas rich in chloride cells before returning to the venous system. During hypoxic vasoconstriction then, perhaps the respiratory function of the gill is attenuated without impairing the osmoregulatory function. An alternate mechanism may be suggested for control of flow in the collateral and respiratory circulations. Since the control of the gill flow resistance rise in hypoxia has been shown to be neural and not local (Kent & Peirce, Comp. Biochem. Physiol. 60C, 37-44, 1978), the nerves running in the interlamelar space may control the amount of constriction of the AVA. Dilated AVA's would tend to fill the interlamellar vessels and might even restrict flow through the prelamellar arteriole by a sluice effect at the point where the two circulations interdigitate. Constriction of the AVA's would reverse those circumstances and enhance lamellar flow.

The small vessels from the efferent filamental artery deliver oxygenated blood to the interlamellar space and vessels and are nutritive in nature. Because the direction of blood flow from the small efferent nutritive vessels is from efferent to afferent across the width of the filament, there may be opportunity for counter current exchange with blood running the opposite direction in the inner marginal channel of the adjacent lamella.

The presence of AVA's in dogfish gill vasculature opens many new possibilities of an important role of blood flow control in the regulation of respiration and osmotic equilibrium. This work was supported by Research Project #4901-01 and 02, Veterans Administration Medical Center, Bronx, New York and by NSF grant PCM 76-16840.

## RENAL HANDLING OF PEPTIDE ANTIFREEZE IN NORTHERN FISHES

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Seasonal studies of freezing avoidance of marine coastal fishes from New England have revealed the presence of blood peptides with antifreeze properties (Duman and DeVries, Comp. Biochem. Physiol. 52A: 193-199,1975, Fletcher, Can. J. Zool. 55: 789-795, 1977, Petzel and DeVries, J. Exp. Zool (in press)). These antifreeze compounds lower the freezing point of the body fluids without significantly affecting the osmolarity. This non-colligative lowering of the freezing point results from adsorption of the antifreeze to the ice which then inhibits crystal growth (Raymond and DeVries, Proc. Natl. Acad. Sci. 74: 2589-2993, 1977). The peptide antifreezes have been isolated from winter flounder, Pseudopleuronectes americanus (Duman and DeVries, Comp. Biochem. Physiol. 54B: 375-379, 1976) and the shorthorn sculpin, Myoxocephalus scorpius. Their average molecular weight is 5,000 daltons and they make up two to three percent of the blood and other body fluids. However they are not found in the urine although these fishes have been shown to freely filter the glomerular markers inulin and polyethylene glycol which are similar in size. We have attempted to compare the clearance of the glomerular marker polyethylene glycol and tritium labeled peptide antifreeze in an attempt to explain why the antifreeze molecules are not found in the urine.

### Materials and Methods

The fishes were otter trawled from waters south of Mt. Desert Island, Maine at a depth of 60 meters. One week after being kept in a continuously circulating open sea water system, the fish were bled for serum chemistry analysis. Serum ions, melting and freezing points were determined as described by Duman and DeVries (Comp. Biochem. Physiol. 52A: 193-199, 1975).

Renal clearance studies were conducted in unanesthetized, free-swimming fish maintained in 80 liter aquarium at ambient sea water temperatures (13 to 15°C). A PE 10 caudal vessel cannule and urinary bladder catheter were sutured in place under MS 222 anethesia. Urine was collected continuously by securing the free end of the catheter in a collection vial placed 5 cm below the level of the fish. Blood was periodically sampled from the caudal vessel cannulae or in the case of the flounder directly from the caudal vessel via a 30 guage needle. Glomerular filtration markers including $^{14}$C-inulin (5,000 daltons) and $^{14}$C-polyethylene glycol (PEG, 4,000 daltons) were purchased from New England Nuclear.

The winter flounder antifreeze peptide was synthesized in vitro with mRNA isolated from winter flounder liver in a cell free wheat germ translation system with $^{3}$H-alanine incorporation (Lin, J. Biol. Chem. 254: 1422-1426, 1979). The winter flounder antifreeze peptide translation product (WFAFPTP) consists of the peptide having a molecular weight of 5,000 daltons plus a pro-peptide sequence of 20 to 30 amino acids giving the translation product a molecular weight of 7,000 to 8,000 daltons. The WFAFPTP has an isoelectric point of between pH 4 and 5.

## Results and Discussion

The results of serum chemistry analysis of fishes from Mount Desert Island waters are contrasted with those of Halifax, Nova Scotia in Table 1. The surface water temperature at MDIBL at the time of collection was 13°C while

Table 1. The serum freezing points, melting points, amount of thermal hystersis (freezing point minus melting point), $Na^+$, and $Cl^-$ concentrations, and the percentage of the freezing point depression due to NaCl in several species of fishes from the Gulf of Maine. Values are expressed as the mean ± one standard deviation of the mean.

| Species | Date | n | $Na^+$ mEq/l | $Cl^-$ mEq/l | m.p. °C | f.p. °C | f.p.-m.p. °C | %f.p.d. due to NaCl |
|---|---|---|---|---|---|---|---|---|
| Myoxocephalus scorpius | 3/72[a] | 12 | 252±14 | 200±8 | -0.81±.01 | -1.69±.11 | 0.87±.10 | 49.7 |
| | 7/78[b] | 6 | 185± 3 | 167±4 | -0.62±.02 | -0.94±.08 | 0.30±.07 | 71.5 |
| | 7/79[b] | 6 | - | - | -0.69±.06 | -1.03±.22 | 0.35±.17 | - |
| Hemitripterus americanus | 3/72 | 4 | 196± 8 | 173±3 | -0.65±.06 | -1.11±.15 | 0.48±.08 | 61.8 |
| | 3/78 | 8 | 172± 2 | 166±4 | -0.60±.04 | -0.90±.03 | 0.30±.03 | 70.3 |
| | 7/79 | 4 | - | - | -0.65±.03 | -0.90±.09 | 0.26±.07 | - |
| Macrozoarces americanus | 3/72 | 1 | 232 | 188 | -0.80 | -1.21 | 0.41 | 64.6 |
| | 7/78 | 1 | 196 | 173 | -0.67 | -0.94 | 0.27 | 70.2 |
| Pseudopleuronectes americanus | 3/72 | 12 | 250±10 | 174±4 | -0.75±.03 | -1.37±.31 | 0.62±.35 | 58.1 |
| | 7/78 | 5 | 252±14 | 200±8 | -0.61±.04 | -0.76±.06 | 0.15±.05 | 85.1 |

[a] Nova Scotia, Duman and DeVries (Comp. Biochem. Physiol. 52A: 193-199, 1975)

[b] MDIBL

that of Nova Scotia was -1.2°C. Despite this warm water temperature, the fishes at MDIBL still retained some peptide antifreeze as indicated by the substantial difference between the melting and freezing points of the serum. This thermal hystersis has been shown to be an indication of antifreeze activity (Duman and DeVries, Comp. Biochem. Physiol. 54B: 375-380, 1976). The level of antifreeze and the corresponding percentage of freezing point depression due to NaCl as compared with Nova Scotia fishes revealed an increase of the antifreeze compound during the winter months, thus substantiating earlier reports.

Renal clearances of glomerular filtration markers indicated that all the fishes studied have glomerular kidneys based on a comparison of the U/P ratio of an aglomerular fish (Table 2). Clearance of the WFAFPTP in both the case of the flounder and the eelpout, which also processes a peptide antifreeze, was well below that of PEG suggesting that the translation product is either filtered into the formative urine and reabsorbed as it moves down the proximal segment of the nephron or that it is not filtered at all because of the polyanionic surface charge of the WFAFPTP.

Table 2. The urine flow rates, urine to plasma ratios and clearance values of inulin, polyethylene glycol (PEG) and winter flounder antifreeze peptide translation product (WFAFPTP) in several species of fishes from the Gulf of Maine. Values expressed as the mean of n ± one standard error of the mean.

| species | marker | injected | n[a] | fish weight (kg) | urine flow (ml/hr/kg) | urine/plasma ratio | clearance (ml/hr/kg) | clearance ratio[b] |
|---|---|---|---|---|---|---|---|---|
| Trematomus bernacchii[c] | inulin | i.v. | 1(-) | 0.20 | - | 0.06 | | |
| Myoxecephalus scorpius | inulin | i.v. | 2(10) | 0.56±.27 | 0.39±.04 | 0.93±0.22 | 0.36±.04 | |
| Hemitripterus americanus | inulin | i.v. | 2(16) | 1.12±.05 | 0.14±.01 | 6.31±1.29 | 0.88±.12 | |
| Macrozoarces americanus | PEG | i.v. | 4(29) | 0.45±.06 | 0.11±.01 | 3.34±0.61 | 0.37±.05 | |
| | WFAFPTP | i.v. | 2(5) | 0.34±.02 | 0.21±.20 | 0.18±0.04 | 0.04±.04 | 0.11 |
| Pseudopleuronectes americanus | PEG | i.v. | 4(19) | 0.44±.06 | 0.35±.08 | 1.94±0.48 | 0.68±.19 | |
| | WFAFPTP | i.v. | 1(5) | 0.23 | 0.16 | 0.20 | 0.03 | 0.04 |
| | WFAFPTP + PAN[d] | i.v. | 1(7) | 0.28 | 0.44 | 0.15 | 0.07 | 0.10 |
| | WFAFPTP + PS[e] | i.v. | 1(9) | 0.28 | 0.32 | 0.25 | 0.08 | 0.12 |

[a] number of fish (number of clearance periods)

[b] clearance of WFAFPTP/clearance of PEG

[c] aglomerular (Dobbs et al., Science: 185, 793-794, 1974)

[d] puromycin aminonucleoside, 1 mg every 3 days for 12 days

[e] protamine sulfate, single injection of 2 mg

Although the results are preliminary, both puromycin aminonucleoside and protamine sulfate appear to enhance the clearance of the WFAFPTP. This increase in clearance of the antifreeze suggests that the mechanism of renal conservation results from a charge repulsion which occurs between the acidic peptide antifreeze and the polyanionic glomerular basement membrane. This research was supported by NSF Grant PCM 77-25166.

## A THYROID NEOPLASM IN THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The reported incidence of spontaneous tumors and tumor-like lesions in elasmobranch fishes is very low. In a survey made in 1968, Wellings cited fourteen instances (Wellings, Nat. Cancer. Inst. Monog. 31, 59-128); that number has since been doubled (in the yearly Accession Lists, Registry of Tumors in Lower Animals, Smithsonian Institute), but still remains far below the incidence for bony fishes. Most of the recorded tumors of elasmobranchs are melanomas and external tumors, but five lesions of the thyroid gland have been listed. Cameron and Vincent (J. Med. Res. 27, 251-256, 1915) described a thyroid carcinoma in the dogfish, Squalus suckleyi, and a possible adenoma was found in a nurse shark, Ginglystoma cirratum (#RTLA 1836. Registry of Tumors). Recently, thyroid lesions have been noted in three Chondrichthyes from the Ueno Zoo Aquarium, Japan, including thyroid hyperplasia, colloid goitre and adematous goitre or follicular adenoma (RTLA 1851, 1852, 1853).

During August 1979, we dissected a large sexually mature female dogfish in the second year of gestation, weighing 7.2 kg which was carrying 15 young in utero. The thyroid gland was greatly enlarged and weighted 1.1 g; this is about 5-10 times the usual weight for the thyroid of fish of this size. The gland was symmetrically enlarged, translucent and flabby. There were no nodules, but the connective tissue sheath was thickened. The microscopic appearance of the gland was variable. Some areas, which tended to be at the edges of the gland, were composed of normal thyroid follicles, spherical in shape, bordered by low cuboidal epithelium, and filled with eosinophilic colloid. There was no conspicuous increase in vascularity or interfollicular stroma in these areas. In most of the gland, however, the thyroid