of the collateral circulation is its proximity at the base of the filament to cells described by Doyle (Bull. MDIBL · 15:27-28, 1975) as analogous to teleost chloride cells. Perhaps the collateral circulation is an inter-gill lymphatic venous system designed to aid in osmoregulation.

Although many interesting new aspects of the dogfish gill microvasculature were found, there was no evidence of an anatomic A-V shunt of the respiratory lamellae. Perhaps the anatomic basis for physiological shunting lies in the lamellae themselves. Preferential flow through the outer margins may influence gas exchange characteristics, or simply thickening of the blood layer within the lamella might result in a difference in gas exchange and a change in physiological shunt. 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.

## PLATINATE INHIBITION OF WINTER FLOUNDER (*Pseudopleuronectes americanus*) RENAL TUBULAR FUNCTION

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Thirty years ago, Forster suggested that the isolated flounder tubule preparation could provide a rapid screening test for agents that modify tubular secretion (Science 108:65-67, 1948). Since then, this preparation has been used to correlate tubular dysfunction with histopathological lesions after exposure to nephrotoxins and to study subcellular sites of drug and pollutant toxicity (J. Exp. Zool. 199:365-382, 1977; Miller, unpublished data). We present here data which indicate that this preparation could be useful in assessing platinate nephrotoxicity. cis-Dichlorodiamineplatinum (II) (cisPt) and certain analogues have been shown to possess antitumor activity in animal test systems. In the search for new analogues with a higher therapeutic index, nearly 1000 platinates have emerged. Since, testing all of these drugs for nephrotoxicity in whole animal systems would be prohibitively expensive, a rapid in vitro screening test could aid in selecting the most promising for further study.

TABLE 1

Inhibition of renal tubular PAH transport and Na,K-ATPase activity by platinates *

| NSC No. | Brief Name | $I_{50}$ Flounder PAH Uptake | $I_{50}$ Flounder ATPase | $I_{50}$ Rat ATPase |
|---|---|---|---|---|
| 4958 | $PtCl_3$ | 0.01 | 0.001 | 0.06 |
| 119,875 | cis Pt | 0.8 | 5.0 | 2 |
| 131,558 | trans Pt | 0.5 | - | - |
| 224,964 | cyclohexanediamine propanediato Pt | 0.15 | 1.0 | - |
| 247,541 | Discreet | 1.0 | - | - |
| 250,427 | cyclohexanediamine sulfato | 0.1 | 0.2 | 0.5 |
| 256,927 | di Cl di OH propanamine | 2.5 | 0.5 | - |
| 263,158 | cyclohexanediamine methanesulfanato | 0.05 | 0.005 | 0.04 |
| 267,583 | diammine guanosine di Cl | 4.0 | - | - |
| 268,252 | cyclohexanediamine di OH | 0.25 | - | - |
| 271,674 | cyclohexanediamine benzene tricarboxylato | 0.2 | 0.1 | - |

* $I_{50}$ values (the concentration of inhibitor causing 50% inhibition) calculated from plot of % inhibition vs. log platinate concentration. Transport data reflects inhibition of active component only; thus, 1.0 was subtracted from both control and experimental T/M values before calculation of % inhibition. Na,K-ATPase data generated by addition of drug to enzyme assay medium.

Flounder kidneys were teased into 20 mg batches of tubules and each bath was preincubated in 5 ml of Forster's marine teleost buffer (FB); containing 140 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 7.5 mM $NaHCO_3$, 0.5 mM $NaH_2PO_4$, 0.5 mM $CaCl_2$, at pH 8.25, with or without (control) 0.001-10 mM drug. After 0.24 hr, 0.5 ml of FB containing both $^{14}C$ and unlabeled p-aminohippuric acid (PAH) was added to each vial; the final PAH concentration was 10 µM. After a 45 min incubation with PAH, tissues in each vial were divided into two 10 mg samples and each sample was weighed, solubilized and counted. Both incubation and preincubation were carried out at 17°C under 100% $O_2$. With control tissue, 45-min incubations yielded steady state values for PAH uptake and thus reflected the ability of the tubules to concentrate organic anions. Control uptakes were nearly constant after 0-6 hr preincubations, with uncorrected tissue to medium ratios (T/M) averaging

about 9. We found significant (T/M > 1), but substantially reduced, PAH accumulation after 24 hr preincubations. Reduction of the preincubation temperature to 10° or 4° dramatically improved tissue viability. These data indicate that, in contrast to mammalian tissue, the teleost renal preparation may be useful for testing drugs with long time courses of action.

Preincubation of tubules with 1 mM cisPt caused some initial stimulation of PAH transport, and maximal inhibition after 6 hr; with 5 mM cisPt, the stimulatory phase was not observed. Table 1 shows 6 hr $I_{50}$ values (drug concentration causing 50% inhibition of transport) for 11 platinates. Chloroplatinic acid ($PtCl_3$), a potent nephrotoxin with no antitumor activity, was the strongest inhibitor and three compounds (NSC 247, 541, 256, 927 and 267, 583) were less toxic than the parent drug (cisPt). Comparison of the present results with available whole animal toxicity data shows the same rank order for drug-induced elevation of plasma BUN, renal histopathology and inhibition of flounder tubule PAH transport (Guarino et al., in preparation).

In the search for mechanisms of platinate nephrotoxicity, one must consider heavy metal sensitive sites within the proximal tubular epithelial cell, i.e.: 1) apical or basal lateral membrane organic acid carriers, 2) ATPases, which maintain essential gradients for cations, and/or 3) cytoplasmic or mitochondrial enzymes which are involved in metabolic processes. Our initial experiments have focused on the enzyme, Na,K-ATPase, since inhibitors of this enzyme, e.g., ouabain and heavy metals, also reduce PAH transport (Miller, unpublished data). As shown in Table 1, all platinates tested are flounder kidney Na,K-ATPase inhibitors. Comparison of these data with those for the rat kidney enzyme, shows that although the mammalian enzyme is less sensitive, the relative inhibitory potency of the drugs tested is similar (Table 1). The rat enzyme is also less sensitive to ouabain, since $I_{50}$ values for rat and flounder are 1 mM and 1 μM, respectively. We have thus far tested only one of several possible sites of drug action using whole tissue homogenates. One, therefore, might not expect to see a perfect correlation between inhibition of enzyme activity and organic acid transport. Nevertheless, our data do show that the most potent inhibitors of transport are indeed the strongest inhibitors of flounder kidney, Na,K-ATPase. Supported by USPHS Grant ES 01678.

## ION TRANSPORT PROPERTIES OF THE ISOLATED OPERCULAR EPITHELIUM OF *Fundulus grandis*

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The opercular epithelium of the killifish (*Fundulus heteroclitus*) has been used as an in vitro preparation for the study of ion transport and osmoregulation in teleosts fishes (Karnaky et al. Science 195:203, 1977). In search for other species of teleosts with similar characteristics we have determined the electrical properties, chloride fluxes and the actions of some drugs on isolated operculi of *Fundulus grandis*. This teleost is native to the warm seawaters of the Gulf of Mexico and easily available in the State of Florida. It is a larger form of *Fundulus*, and offers a larger surface area of opercular epithelium to be placed as a membrane in Ussing type chambers and study its transport characteristics. The methods utilized were previously described (Degnan et al. J. Physiol. 271:155, 1977).

The results, shown in Table 1 indicate that the opercular epithelium of *Fundulus grandis* exhibits an electrical potential difference comparable to the one of operculi from *Fundulus heteroclitus*, but the short circuit current is substantially lower than in the latter. The average electrical resistance was higher in *Fundulus grandis*. The fluxes of chloride (Table 2) determined with $^{36}Cl$ indicated that an active transport of this anion from the serosal to the mucosal side exists in operculi of *Fundulus grandis* which is responsible for most of the electrically measured short circuited current.