

respiratory lamellae, which are the sites of gaseous exchanges and presumably also *passive* ion fluxes, will be entirely absent.

We have carefully dissected the skin on the inside of the operculum of large (2 kg) specimens of the King O'Norway (*Hemirhamphys americanus*) and examined (1) the histology--six skins--and (2) several electrical properties--four skins. We have established that chloride cells are present (figure 1) in this opercular sheet and that respiratory lamellae are absent. Our studies with the Ussing chamber revealed that the membrane is especially hearty and survives well in both short term (four hour) and long term (up to 24 hours) experiments. Additionally the opercular skin is characterized by high electrical resistance (about 1000 ohm-cm<sup>2</sup>) suggesting that it is not "leaky." The potential difference is on the order of several millivolts, mucosal side positive to the serosal side.

On the basis of these preliminary observations we feel that the opercular skin preparation warrants serious attention as a possible tool for studying detailed mechanisms of teleost osmoregulation.

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#### BLOOD FLOW DISTRIBUTION IN *Squalus acanthias*: A STUDY WITH ACID LOADING

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In a preliminary study of blood flow distribution in *S. acanthias* (Bull. MDIBL. 11,53, 1971) the pattern of cardiac output distribution through the organs of the free swimming dogfish was described. In the present study fish were made acidotic and the resulting distribution of the cardiac output at the lower pH was measured.

Six male dogfish (1.8± 0.35 Kg), allowed to swim freely in a 0.3 M<sup>3</sup> tank, were used. An injection catheter (PE 20) was threaded through a # 15 Touhuy needle in the caudal artery, pushed up the dorsal aorta to the point of confluence of the efferent arteries from the fifth gill arch and there was used for injection or withdrawal of blood samples for pH determinations. Blood samples were taken at time 0 (cannula in position), and after one hour. Immediately after the one-hour sample, a control injection of 1 cc of 50μ - diameter microspheres (3M Brand Tracer Microspheres) isotopically tagged with either <sup>141</sup>Ce or <sup>85</sup>Sr was given and was washed in with 1 cc of dogfish Ringers. The activity per dose was approximately 1 μCi. Fifteen minutes later the dogfish was made acidemic by injection of 5 - 7 cc's of dogfish Ringers to which lactic acid had been added to lower the pH of the Ringers to 3.2. Within the next minute following the acid load, a second dose of 50μ diameter microspheres tagged with either <sup>141</sup>Ce or <sup>85</sup>Ce after the acid load; three fish received the tags in opposite order. Blood samples were taken after injection of the second set of microspheres and final samples were taken 15 to 60 minutes later. The animal was sacrificed, autopsied, and organ samples analyzed for radioactivity as described in reference above. Several samples were

taken from each organ. The cpm/gram of tissue was calculated for both isotopes for each sample and total organ flow determined. The percent of cardiac output found in each organ was calculated as follows: 
$$\frac{\text{average activity/gm} \times \text{organ weight}}{\text{total dose activity}}$$

There was no significant difference in the distribution pattern between the isotopic tags given in the control period. The acidemia which lowered pH from  $7.36 \pm 0.109(\text{SD})$  to  $7.151 \pm 0.328(\text{SD})$  produced a variety of blood flow shifts in various organs of individual fish. The only consistent pattern within the group was an increase in blood flow to the kidneys after the acid load in five out of six fish. Excluding the one fish in which flow fell from 3.4 to 1.9 percent of cardiac output, the average of the remaining five fish rose from 5.17 percent  $\pm 2.76$  SEM to 6.53 percent  $\pm 2.55$  SEM after the acid injection. By the time of the terminal sample the pH of the fish had returned to  $7.39 \pm 0.23$  (SD).

The lack of consistent changes in blood flow distribution to all parts of the body excluding the kidney indicates the degree of acidemia produced in this study is not a factor in controlling the amount of blood flow to most of the vascular beds of the dogfish. This may be due in part to the fact that acidosis was already present in the control periods (pH =  $7.36 \pm 0.109$ ); moreover the time course of a change in blood flow due to changing pH may not coincide with our period of observation. It has been found that a fall in arterial pH comparable to the one used in this study is not reflected in the urinary pH (Sharks, Skates, and Rays, 1967, p.254) and that the gills play an important role in non-respiratory acid excretion in dogfish (#28, MDIBL, 1968). However the trend for the kidneys to receive a larger portion of the cardiac output with a fall in pH is of interest and should be examined in a larger group of fish exposed to a variety of conditions in which pH is lowered.

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#### SUGAR TRANSPORT ACROSS THE PERITUBULAR FACE OF RENAL CELLS OF THE FLOUNDER *Pseudopleuronectes americanus*

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Previous studies on the transport of sugars in teased tubules of flounder kidney (Bull MDIBL 10:34, 1970) have been extended. An improved analytical technique (to be published) allowed determination in the same tissue extract of both free and total sugars; the difference between these values represents phosphorylated sugar. As opposed to the previously employed  $\text{ZnSO}_4 + \text{Ba}(\text{OH})_2$  deproteinizing procedure, the new technique prevents breakdown of sugar phosphate to free sugars.

The uptake of  $\alpha$ -methyl-D-glucoside- $^{14}\text{C}$  ( $\alpha$ -MGLU), 2-deoxy-D-glucose- $^{14}\text{C}$  (2-DGLU), D-galactose- $^{14}\text{C}$  (GAL) and 2-deoxy-D-galactose- $^3\text{H}$  (2-DGAL) were tested.  $\alpha$ -MGLU entered the cells as free sugar only, whereas the other sugars were present in the tissue both in free and phosphorylated form. The cellular uptake of all sugars was inhibited practically completely by 0.5 mM phlorhizin, 0.3 mM phloretin and also by 1 mM N-ethylmaleimide, but not by 0.5 mM ouabain or