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A SYSTEM TO STUDY THE TELEOST CHLORIDE CELL WITH THE USSING CHAMBER

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In the study of ion transport mechanisms in the gill of teleosts, isolated gill preparations offer distinct advantages over studies using whole animals.

Unfortunately however success with isolated gill perfusion preparations has been meager and data from this approach represents only a small fraction of the pertinent literature on gill function. Apparently the two major problems encountered with isolated gills are rapid deterioration and leakage. In addition, within the last eight years investigators have found that microcirculation in the teleost gill involves several different blood pathways whose relative contributions to the total gill blood flow are under hormonal control. Thus the investigator has the additional problem of simulating the *in vivo* blood hormone environment in gill perfusion experiments.

In light of these difficulties we are exploring an alternative approach to the study of the specialized electrolyte-transporting cell (the chloride cell) responsible for the excretion of sodium chloride across the teleost gill. Over two decades ago Burns and Copeland (Biol. Bull. 99,381, 1950) using the light microscope, showed that chloride cells are present in the skin of the inside face of the operculum of the minnow *Fundulus heteroclitus*. Significantly opercular skin chloride cells and gill chloride cells share a similar fine structure (Karnaky, unpublished observations on *Fundulus grandis*). We reasoned that if chloride cells were also present in the opercular skin in much larger teleosts, there then should be enough epithelial surface area so that the opercular skin, and therefore chloride cells, could be studied in the classical Ussing chamber. Most importantly the use of the Ussing chamber would eliminate entirely problems of shunting blood through various vascular pathways and would allow study of the chloride cell in a much simpler setting than the gill since

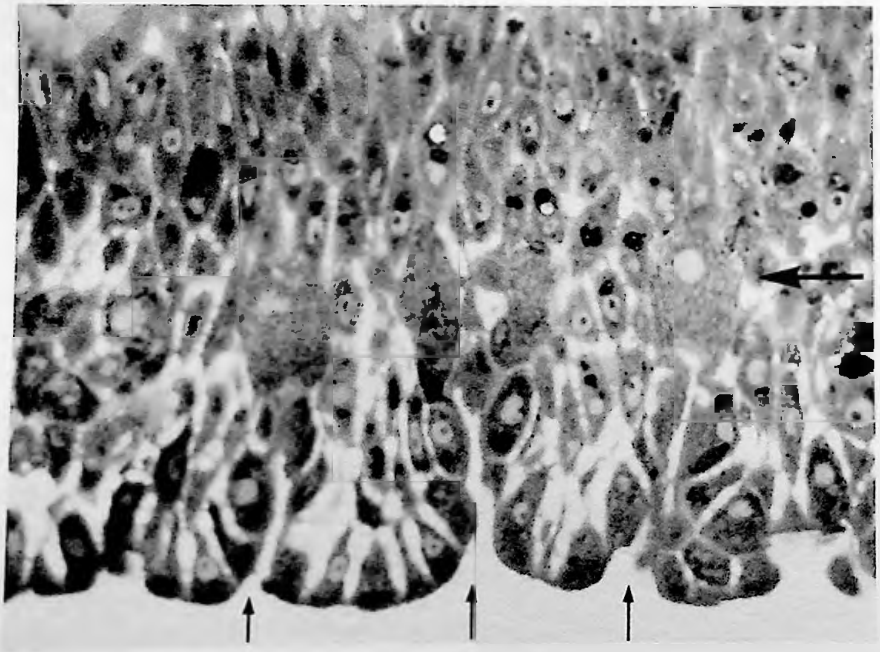

Figure 1. A one micron thick section of opercular skin from *Hemitripterus americanus*. Four prominent chloride cells (level of large arrow) are identified by their large size and granular (mitochondria-rich) cytoplasm. One or more chloride cells appear to be located at the bottom of large crypts (openings marked by small arrows) formed by other epithelial cell types. The cause of the large intercellular spaces between the distal epithelial cells has not been established. About 600x.

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 *(Hemitripterus 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^2) 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$\pm$ 0.35 Kg), allowed to swim freely in a 0.3 M^3 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 ^{141}Ce or ^{85}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 ^{141}Ce or ^{85}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