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TELEOST URINARY BLADDER (WINTER FLOUNDER, *Pseudopleuronectes americanus*): ULTRASTRUCTURE AND Na-K-ATPase LOCALIZATION

KARL J. KARNAKY, JR., J. LARRY RENFRO, DAVID S. MILLER, HAROLD H. CHURCH, AND WILLIAM B. KINTER. MT. DESERT ISLAND BIOLOGICAL LABORATORY, SALSBURY COVE, MAINE AND UNIVERSITY OF CONNECTICUT, STORRS, CONNECTICUT

It is now generally accepted that the active transport of sodium and the net movement of water from the mucosal to the serosal face of the urinary bladder play an important water-conserving role in marine teleosts (Forster and Danforth, Bull. MDIBL, 12:35, 1972; Hogben et al, Bull. MDIBL, 12:52, 1972; Renfro, Bull. BDIBL, 12:81, 1972; Renfro, Amer. J. Physiol., in press, 1974). Recent studies have shown that spontaneous Na transport and Na-K-ATPase activity are significantly correlated (Miller and Renfro, Bull. MDIBL, 13:80, 1973) and that ouabain inhibits the flux of both ions and water (Renfro, 1974). To further evaluate the role of Na-K-ATPase we have undertaken a morphological and autoradiographic study to localize this enzyme by means of ^{3}H-ouabain binding.

For morphology, bladders from four flounder were excised and dissected into three narrow strips. One strip was osmium fixed immediately (normal morphology); the second and third (effects of fluid transport) were fixed after one hour incubation at 10°C in a modified Forster's saline (MFS) with or without 10^{-4} M ouabain (Renfro, 1974). For autoradiography, bladder strips from an additional three flounder were incubated for one hour in 5×10^{-6} M ^{3}H-ouabain, sufficient to give about 70 percent inhibition of active sodium transport and Na-K-ATPase activity (unpublished observations). Autoradiographs were prepared from freeze-dried, plastic embedded sections

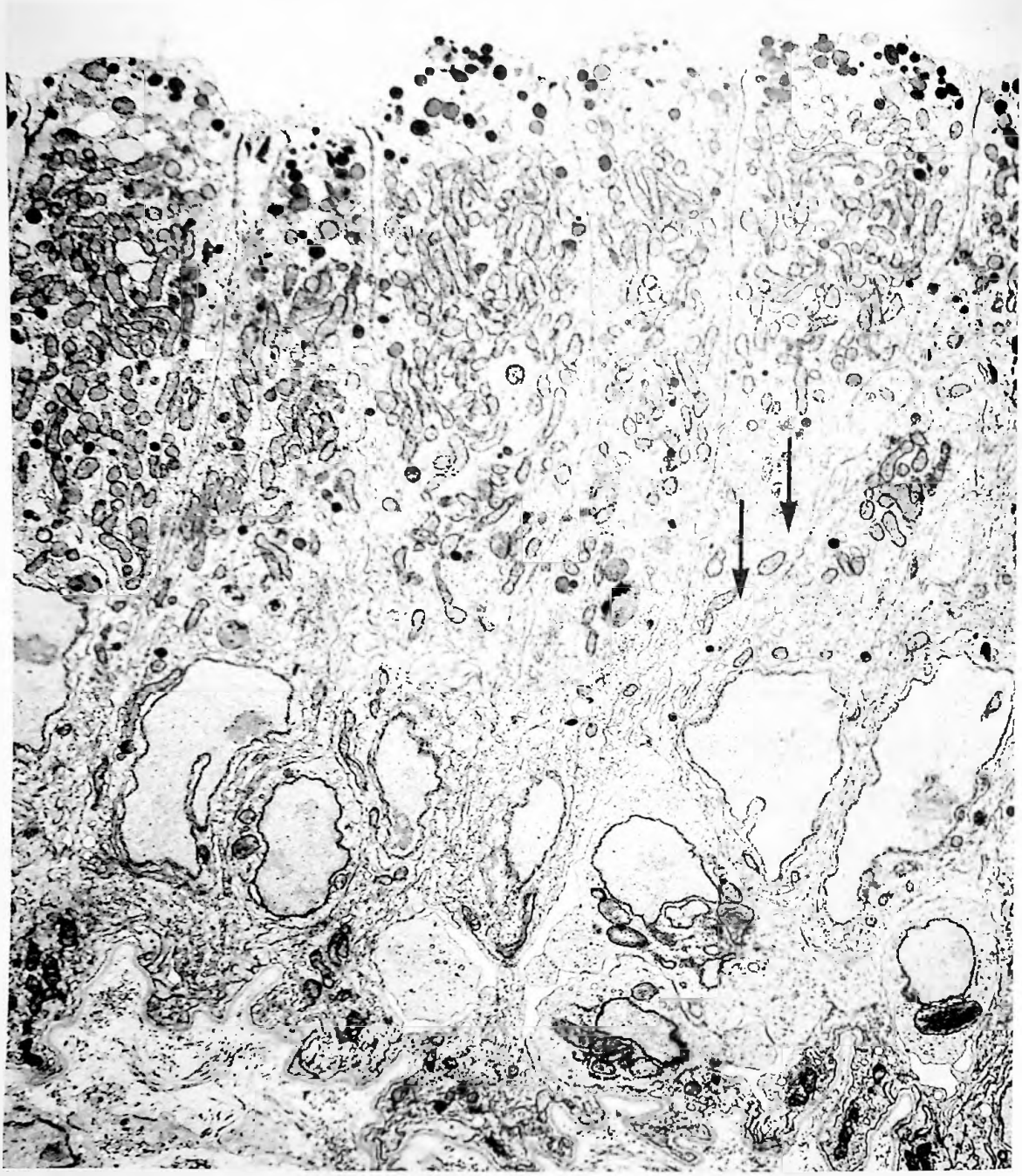

Figure 1: Low magnification electron micrograph of the epithelium of flounder urinary bladder oriented so that the lumen is at the top. The apical plasma membrane displays very few microvilli. The apical region of the cell is filled with mucous droplets. Directly beneath these are numerous mitochondria. The basal region contains numerous membrane profiles which may represent basal and lateral infoldings. Of utmost importance for interpreting the light microscope autoradiographs is the ultrastructural visualization of cells apparently stacked one upon another, resulting in plasma membrane profiles cutting across cells (arrows). Stained with uranyl and lead salts. 6700X.

according to the method described by Stirling (J. Cell Biol., 53:704, 1972).

The flounder urinary bladder is an epithelium-lined tube with valves at both ends. At the light microscope level the general structure follows closely that of rabbit gall bladder as described by Tormey and Diamond (J. Gen. Physiol., 50:2031, 1967). The wall (400-1000 μm thick) is composed of four layers: a mucosa, which is composed of an epithelium and a lamina propria; a smooth muscle layer; a subserosa; and a serosa. The epithelium of the bladder appears to consist of a unicellular layer of a single cell type of columnar cell 3-4 μm wide and 20-40 μm high. The large nucleus of each cell is basally located. The epithelium of tissue fixed immediately upon removal rarely exhibited large intercellular spaces. This finding may be significant because flounder bladder urine is usually quite low in sodium (3.3±0.9 SE mEq/l; data from three fish) and this morphological pattern may correspond to a nonabsorptive state. In contrast after one hour incubation with MFS (135 meq Na/l) intercellular spaces were wide open between most cells. These findings in flounder urinary bladder suggest that the intercellular spaces may be involved in the coupling of sodium transport and water movement.

Electron microscope examination (Figure 1) reveals that the apical cytoplasm is filled with numerous mucous droplets. Directly beneath is the major complement of the cell's mitochondria which are only sparsely represented in the immediate supranuclear region and in the basal cytoplasm. Of particular interest from the standpoint of the possible mechanism of Na transport is the pattern of surface amplification in this cell. The apical microvilli and extensive lateral interdigitations which characterize the mammalian gall bladder are poorly represented here. Numerous membrane profiles in the basal cytoplasm suggest extensive basal infolding. However,

Figure 2: Autoradiograph of urinary bladder incubated one hour with 5×10^{-6} M ^{3}H-ouabain on both mucosal and serosal sides (60 µCi/ml) and exposed for 8 days. Most autoradiographic grains, i.e., black dots, are over basal and lateral cell borders. Many of the grains that appear to be over cytoplasm are probably on the adjacent plasma membranes of stacked cells. Grains are excluded from the nuclei, the apical plasma membrane, and an apical zone about 2.5 µm wide. 1700X.

this feature will require re-examination with ultrastructural tracers. Electron microscopic observations also provide important information for interpreting light microscope autoradiographs. Namely the long columnar cells are rarely cut running the full width of the epithelium and what appears to be one single cell at the level of the light microscope may actually be profiles of two or more cells.

In tissue exposed to ^{3}H-ouabain from both the serosal and mucosal sides the radioactive label was limited to the mucosal epithelium (Figure 2). A zone about 2.5 µm wide at the apical end of the epithelium remains free of

grains. This corresponds to a zone where adjacent cells exhibit very little interdigitation. Nuclei are free of grains. In most areas the grains seem to follow basal and lateral cell borders. This is especially evident in cells that have been pulled apart slightly. Where grains do appear to be over cellular cytoplasm they are probably over cell interdigitations described above. Control binding studies (scintillation counting) were consistent with specific tissue binding of ^{3}H-ouabain, e.g., incubation medium high in potassium markedly reduced binding. Present morphological and autoradiographic observations provide direct evidence that the Na-K-ATPase is located on the basal and lateral plasma membrane in flounder urinary bladder. Thus as in many epithelia this enzyme is located at the cellular pole to which Na is transported.

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EFFECT OF pH ON OXYHEMOGLOBIN DISSOCIATION CURVES IN *Squalus acanthias*

BARBARA KENT, MARJORIE PEIRCE, AND E. CONVERSE PEIRCE II. DEPARTMENTS OF SURGERY, VETERAN'S ADMINISTRATION HOSPITAL, BRONX, NEW YORK, AND MT. SINAI SCHOOL OF MEDICINE, NEW YORK, NEW YORK

In earlier studies we reported a gill reflex response in *Squalus acanthias* in which a vagally mediated bradycardia and vasoconstriction of the gill vasculature are induced by an increase in sea water P_{CO_2}. Hypoxia is known to induce a similar response (Satchell, G.H., J. Exper. Biol. 39: 503, 1962). pH influences oxygen affinity of many marine blood pigments. The Bohr effect causes a shift to the left of the relationship between P_{O_2} and percent saturation with increasing pH or decreasing P_{CO_2}. The Root