

4. The ciliary folds in the three teleosts and the dogfish have a high activity enzyme, susceptible to sulfonamide and anion inhibition. Thus far there is nothing to distinguish these enzymes from the carbonic anhydrase in ciliary processes of mammals, including primates. Our earlier physiological work shows that the chemistry of aqueous humor formation in fish is similar to that of mammals (Maren, Wistrand, Swenson and Talalay, Invest. Ophthalmol. 14:662, 1975).

5. We could not detect carbonic anhydrase in lens or corneal endothelia in the sea-going fish of any of the species tested. In these structures the enzyme appears in low concentration in fresh water fish. It is hoped that these differences will be useful in revealing the function of lens carbonic anhydrase, which is presently unknown (Friedland and Maren, this Bulletin). Supported by NIH Grant HL 22258.

PILOT STUDIES ON ION MOVEMENT IN LENS

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Although lens was one of the first tissues to be recognized for its high carbonic anhydrase content through most of the vertebrate phyla, this structure remains one of the few in which the function of carbonic anhydrase is entirely unknown. Nearly twenty years ago, it was found that elasmobranch lens lacked carbonic anhydrase (Maren, Comp. Biochem. Physiol. 5:201, 1962). This summer (this Bulletin) we found carbonic anhydrase also was absent from lens of saltwater teleosts.

We surmise that these differences may be a useful investigative key in the search for the role of carbonic anhydrase in lens. We report here the beginning of work in which we shall try to discover how movements of anion and cation are linked in vertebrate lens and the possible dependence of this on carbonic anhydrase. Both mammalian and amphibian lens have high K^+ (120 mM) and low Na^+ (17 mM), and efflux of Na^+ is an active process. Cl^- and HCO_3^- are present at about 20 mM, and Cl^- efflux appears to be passive. Thus there is a very large anion gap; presumably negative charges on large molecules and/or PO_4 , or other anions. Relatively little attention has been given to HCO_3^- efflux (Duncan, G. in *The Eye*, vol. 5, ed. by H. Davson and L.T. Graham, Academic Press, New York, 1974; Kinsey, V.E., *Documenta Ophthalmologica, Proceeding Series*, vol. 8, p. 310, 1976).

We describe pilot experiments on the efflux of labeled Cl^- and HCO_3^- from dogfish lens. The procedure was to soak the freshly dissected lens in shark-Ringers solution containing $Na^{36}Cl$ or $NaH^{14}CO_3$ for one to two hours, then place it in a chamber arranged so that it is supported on a plate of perforated plastic immersed in 17 ml fresh shark-Ringers solution at 12°C. We measured the rate of appearance of isotope in the external fluid at early periods, well before equilibrium was reached. As controls for the free diffusion of ions in this situation, dialysis bags of about the same volume as lens were filled with shark-Ringers solutions containing the isotopes and placed in the chamber. We define a rate constant, k_{out} , for this specific system as

$$\frac{\text{total counts appearing in medium per min}}{\text{total counts in lens or bag}}$$

For efflux of each isotope, four lenses and four bags were studied. In a few cases the use of paired lenses enabled a rough decay rate to be calculated from differences in isotope concentrations in lens at several time intervals, also yielding k_{out} .

The chloride efflux from dogfish lens was rapid and the same from both lens and bag, $k_{out} \cong 0.12 \text{ min}^{-1}$. These rates are faster than efflux from mammalian and amphibian lens. Data indicate that Cl^- movement is passive as in mammalian and amphibian lens (Duncan, vide supra).

Movement of $\text{NaH}^{14}\text{CO}_3$ from the bag also yielded $k_{\text{out}} \cong 0.12 \text{ min}^{-1}$. Efflux from lens was much slower, however, with $k_{\text{out}} \cong 0.023 \text{ min}^{-1}$. This is close to the value obtained for rabbit lens by Kinsey (vide supra).

These very preliminary data suggest experiments to find whether HCO_3^- efflux may be linked to the active efflux of sodium. In lenses containing carbonic anhydrase, the enzyme may speed formation of HCO_3^- from CO_2 to subserve Na transport. It is of particular interest that the pentose phosphate pathway in lens provides a continuous source of CO_2 . (Kinoshita and Wachtel, J. Biol. Chem. 233:5, 1958.) Supported by NIH Grant EY 02227.

ULTRASTRUCTURAL STUDIES ON THE HEART OF *Boltenia ovifera* (SEA POTATO)

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In the past few years we have examined and confirmed that the heart of *Boltenia ovifera* is composed of single cell-layered myoepithelium. Tight junctions separate the luminal and junctional surfaces from the surface facing the pericardial cavity. The luminal membrane generates action potential and seems to be the site of E-C coupling (Weiss, Goldman and Morad, J. Gen. Physiol. 68:503-518, 1976). The extraluminal membrane is insensitive to ionic or drug variations and does not seem to play a prominent role in generation of excitation or excitation-contraction coupling. It would be of interest to examine whether the functional differences in these two membranes are represented by corresponding structural differences. In this report we have carried out an ultrastructural analysis to examine this possibility. Freeze-fracture, transmission and scanning electron microscopy were used to examine the cell surface topography as well as the molecular architecture of the luminal and extraluminal membranes.

Methods

The tubular hearts were dissected free of pericardium and fixed in 1% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4 with added H_2O_2 (Peracchia and Mittler, J. Cell Biol. 53:236-238, 1972). NaCl

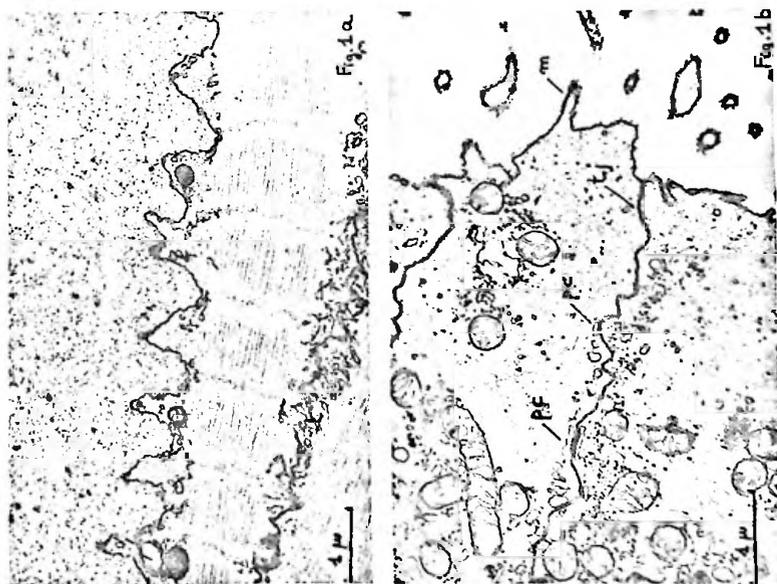


Figure 1a,b. Thin sections illustrating the different features of myocardial cells, at the level of the heart lumen and pericardial cavity, respectively. m = microvilli; pc = pericardial cavity; tj = tight junction; scale marker = 1 μm .