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Ultracytochemical localization of Na^+, K^+-activated ATPase in the oocytes of *Heterandria formosa* Agassiz, 1853 (Pisces, Poeciliidae)

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Summary. Using ultracytochemical methods, this paper studies the presence of Na^+, K^+-activated ATPase during oocyte development in *Heterandria formosa*. ATPase was discovered in all four oocyte stages, but its level was highest in stages II and III in which yolk formation took place. Na^+, K^+-activated ATPase was found on the membranes of both the yolk vesicles and the cortical granules and on the oocyte and follicular microvilli and the primary oocyte membrane, indicating that the microvilli were the sites of substance exchange between the follicle and the oocyte.

Introduction.

The oocytes and eggs of teleosts are always enveloped in membranes which vary in thickness depending on the ecological conditions existing after they are laid (Riehl, 1978). This thickness ranges from 3 μm in viviparous fish to 70 μm in surf-spawning fish.

A layer of accessory cells, the follicular epithelium, surrounds the oocyte egg-membrane, and these cells have a function in oocyte nutrition (Götting, 1966 ; Flügel, 1967*a* ; Gupta and Yamamoto, 1971 ; Wegmann and Götting, 1971 ; Hirose, 1972 ; Azevedo, 1974 ; Riehl, 1977 ; Riehl and Schulte, 1977 ; Shackley and King, 1977). When the oocytes of the swordtail, *Xiphophorus helleri*, were labelled with myofer, it was discovered that the substances necessary for nourishment or yolk formation were transported to the oocytes from the mother's body in a low molecular, soluble form by way of the follicular epithelium (Wegmann and Götting, 1971).

Thus, the egg-membrane, which otherwise seems to be a strong barrier, is perforated with numerous radial canals filled with oocyte and follicular cell microvilli. These microvilli are considered to be the actual site of substance exchange between the follicle and the oocyte (Flügel, 1967*b* ; Azevedo, 1974 ; Riehl, 1977 ; Riehl and

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Schulte, 1977 ; Shackley and King, 1977), although this assumption has not been fully proven. The goal of the present study was to localize, by ultracytochemical means, the sites of Na^+, K^+-activated ATPase in which exchange occurred and thereby to test the validity of the above hypothesis.

Material and methods.

Adult females of the Poeciliid, *Heterandria formosa*, were used. After the fish were anesthetized (MS 222, Sandoz), the ovaries were removed and placed immediately in freshly prepared 3 p. 100 paraformaldehyde in 0.1 M cacodylate buffer (pH 7.5 ; 4 °C) for 1 hr. They were then cut in 1 mm pieces and washed several times with ice-cold 0.1 M cacodylate buffer (pH 7.5) and 0.1 M Tris buffer (pH 7).

Incubation was carried out at 25 °C in Ernst's medium (Ernst, 1972) for 30 to 60 min. 5 mM p-Nitrophenylphosphate-disodium-salt (Na_2NPP), 10 mM $MgCl_2$, 10 mM KCl, 20 mM $SrCl_2$ and 100 mM tris-HCl buffer (pH 9.0) were added to the medium. After incubation, the tissue pieces were treated according to Ernst's method (1972). The following controls were used :

— addition of 10 mM ouabain to the complete medium,
— medium without substrate (Na_2NPP),
— medium without K^+,
— medium without Mg^{2+},
— medium with 5 mM β-glycerophosphate as substrate instead of 5 mM Na_2NPP.

The tissue pieces were postfixed with 1 p. 100 osmium tetroxide in 0.1 M cacodylate buffer (pH 7.5), then dehydrated in ethanol and embedded in epon. A Reichert ultramicrotome OM U 3 was used to cut the ultrathin sections. Some of these were contrasted with lead citrate (Reynolds, 1963) and studied with a Zeiss EM 9S at 60 kV.

Results.

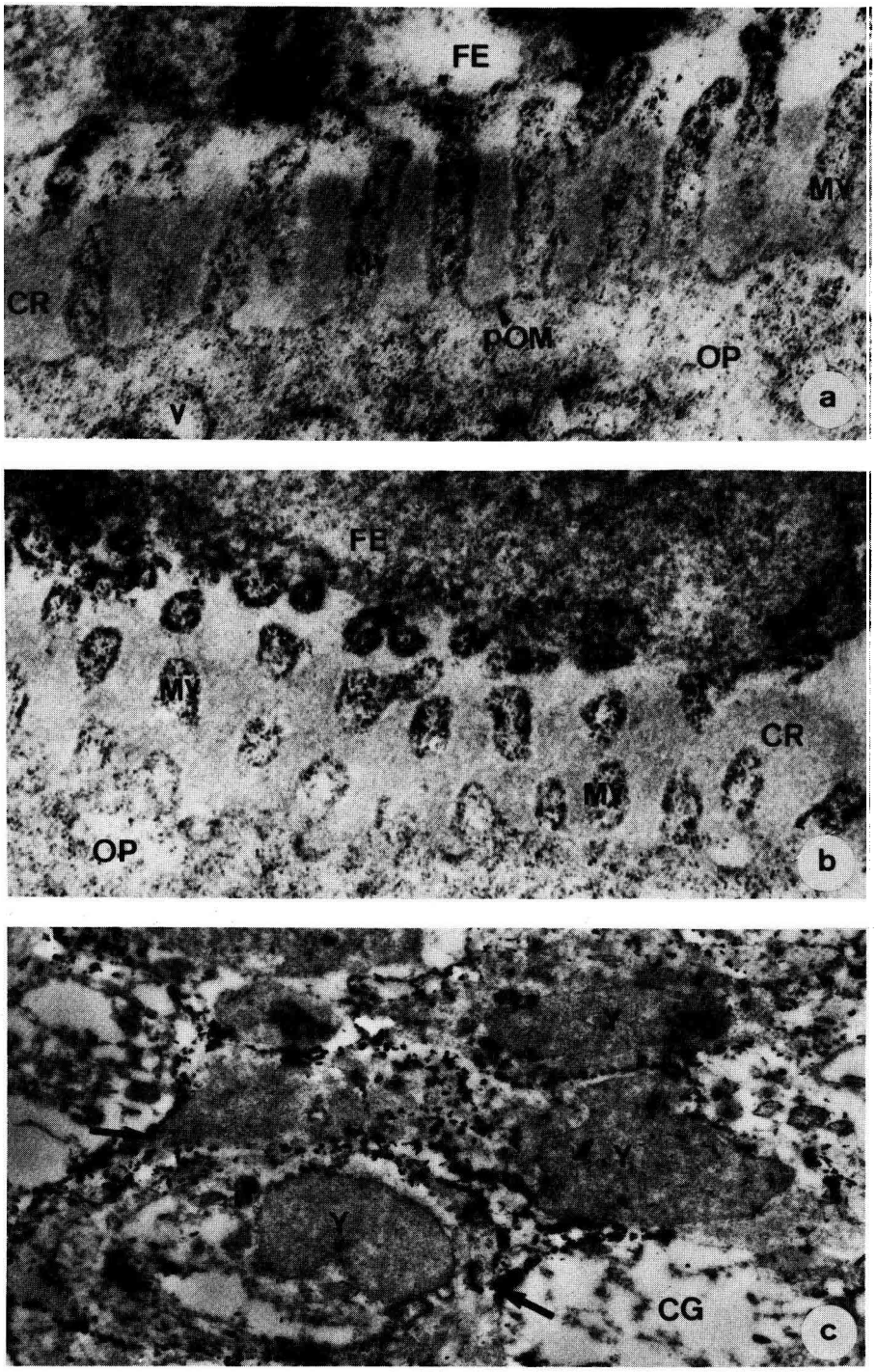
ATPase was present in all four stages (stage division according to Arndt, 1956). It was lowest in stage IV, when the oocytes reached final size and were completely filled with yolk, and highest in stages II and III. In both these stages, the oocytes increased many times over in volume and weight.

FIG. 1. — a) *Cross-section through the cortex radiatus in stage II.* Na^+, K^+-activated ATPase is located on the microvilli (intracellular) and the primary oocyte-membrane. × 72 400.

b) *Tangential section through the cortex radiatus in stage II.* Precipitates are seen on the microvillus membranes. That part of the microvilli close to the follicular epithelium reacts more strongly × 72 400.

c) *Proof of Na^+, K^+-activated ATPasein the oocytoplasm (stage II).* Yolk vesicle and cortical granule membranes react positively. × 12 700.

CG = cortical granule, CR = cortex radiatus (egg-membrane), FE = follicular epithelium, MV = microvilli, OP = oocytoplasm, pOM = primary oocyte-membrane (oolemma), V = vesicle, Y = yolk elements.


FE
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CR
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In stage I the oocytes were surrounded only by a primary oocyte-membrane (oolemma). In the early part of stage II the egg-membrane (cortex radiatus) began to form. An electron-opaque substance, representing the cortex radiatus, was deposited between the oocyte microvilli. The oocyte and follicle microvilli then formed the radial canals.

There was positive proof of Na^+, K^+-activated ATPase. Many precipitates were located intracellularly on the oocyte microvilli and the primary oocyte-membrane (fig. 1*a*) ; some precipitate was also found on the vesicle membranes. Tangential sections through the cortex radiatus showed precipitates on the microvillus membranes (fig. 1*b*). However, there were fewer precipitates on that part of the microvilli close to the oocyte than on the part toward the follicular epithelium (fig. 1*b*).

A high level of ATPase was also determined inside the oocytes. Many precipitates were found on the membranes surrounding the yolk elements and the cortical granules (fig. 1*c*, arrows). Furthermore, the dictyosomes and the membranes of various vesicles reacted positively to Na^+, K^+-activated ATPase. The results of controls b-e were negative, whereas control-a gave a very weak reaction.

Discussion.

Na^+, K^+-activated ATPase has already been ultracytochemically located in certain tissues and organs of fish. It was found in the gills and chloride cells (Epstein *et al.*, 1967 ; Utida *et al.*, 1971 ; Kamiya, 1972 ; Forrest *et al.*, 1973 ; Sargent and Thomson, 1974 ; Sargent *et al.*, 1975 ; Karnaky *et al.*, 1976*a*, *b* ; Thomson and Sargent, 1977 ; Hootman and Philpott, 1979), as well as in the pseudobranches (Dendy *et al.*, 1973) and the muscular system (Dahl and Nicolaysen, 1971 ; Nag, 1972 ; Johnston *et al.*, 1972, 1974). Fish oocytes, however, have not been tested before for the presence of Na^+, K^+-activated ATPase.

The highest ATPase content in the oocytes of *Heterandria formosa* was found in stages II and III, while there was clearly less in stages I and IV. These findings could be correlated with the process of oocyte development. In stage II, large amounts of yolk begin to be stored, and at the same time, many cortical granules are formed. At the end of stage III, vitellogenesis is complete.

The localization of Na^+, K^+-activated ATPase on the membranes of yolk vesicles and cortical granules indicated that nutriments were probably actively transported into these organelles. This active transport could be the result of a higher ATPase level in stages II and III than in stages I and IV. In stage I there was no extensive transport, while in stage IV it was mostly finished, and the yolk elements and cortical granules in the oocytes were merely re-arranged. One of these events was the combination of small yolk vesicles (lipid yolk) into one large one (Arndt, 1956, 1960 ; Erhardt, 1976 ; Riehl and Schulte, 1977).

The localization of Na^+, K^+-activated ATPase on the microvilli and the primary oocyte-membrane confirms that the microvilli are the sites of substance exchange. These findings are further supported by the ultracytochemical localization of neutral lipids in the microvilli (Riehl, unpublished data).

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Résumé. L'ATPase Na^+, K^+ dépendante est présente aux 4 stades de développement de l'ovocyte, mais son niveau est plus élevé aux stades II et III quand se forme le vitellus. L'ATPase Na^+, K^+ dépendante est localisée sur les membranes des vésicules de vitellus et des granules corticaux, et sur la membrane des microvillosités d'origine ovocytaire et folliculaire, ce qui démontre le rôle des microvillosités dans l'échange de matériel entre le follicule et l'ovocyte.

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