THE ONTOGENY OF MITOCHONDRIA-RICH CELLS IN EMBRYOS OF THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The dogfish, Squalus acanthias L., represents an example of primitive (aplacental) viviparity (Wourms, Am. Zool. 21:473-515, 1981). Eggs are enclosed in a thin-walled egg capsule and hatch internally. The embryos develop during a gestation period of about 22 months (Hisaw & Albert, Biol. Bull. 92;3:187-199, 1947). The nature of the uterine environment and the ability of these embryos to osmoregulate has been studied by several investigators (Evans et al., J. Exp. Biol. 101:295-305, 1982; Kormanik & Evans, J. Exp. Biol. 125:173-179, 1986). Early Squalus embryos (less than 25 - 30 mm) are bathed in uterine fluids with Na and Cl concentrations that are intermediate to maternal plasma and sea water, with urea concentrations approaching maternal blood values (Evans et al., ibid.). Later in development, the uterine solution resembles sea water with respect to the major ions, and urea is low in concentration (Evans et al., ibid; Kormanik & Evans, ibid.). Evans et al. (ibid.) concluded that early (25 - 30 mm) embryos could iono- and osmoregulation when exposed to sea water for four to six days, since total body levels of Na, Cl, and K did not increase, and urea did not decrease during this period. We have found that earlier stages, that is, encapsulated Squalus eggs with neural folds (3 mm), begin to fail at 3 to 6 days incubation in sea water (Kormanik et al., unpubl.).

The dye DASPEI was used to visualize mitochondria-rich cells to determine the ontogeny of some of the potential osmoregulatory organs in these developing embryos. Similar techniques have been used to visualize chloride cells in the opercular epithelium of Fundulus heteroclitus (Karnaky et al., Am. J. Physiol. 246:R770-775, 1984; Zadunaisky, Fish Physiol., Vol. XB, 129-176, 1984) and mitochondria-rich cells of the rectal gland (Smith, Bull MDIBL 29:14-17, 1990).

First-year (3 - 30 mm, n= 3-6) or second-year (19 - 20 cm, n= 4-7) embryos of the dogfish Squalus acanthias were collected during the summer of 1990 from recently killed females. Embryos were anesthetized in MS-222 (1:2000). Small embryos (3 mm neural fold to 20 mm with umbilicus) were removed from the remainder of the egg and stained whole. Gill filaments and slices of rectal gland (ca. 1 mm) were dissected from large embryos (19 - 20 cm). Tissues were placed into Elasmobranch Ringer's Solution (after Forster et al., Comp. Biochem. Physiol. 42A;3-12, 1972, with addition of TMAO; in mM, 280 Na, 6 K, 3 Mg, 5 Ca, 350 Urea, 72 TMAO, 300 Cl) with 50 uM DASPEI (2-(4-dimethylaminostyryl)-N-ethylpyridinium iodide, ICN Pharm., Inc.; Karnaky, pers. comm.) for 15 minutes, removed and rinsed with three changes of fresh ERS. Fundulus heteroclitus gill tissue was processed as above, using Teleost Ringer's solution modified after Degnan et al., J. Physiol. 271;155-191, 1977, with Na elevated and HCO_3 decreased to more closely approach blood values (in mM, 165 Na, 1.3 Ca, 1.2 Mg, 2.6 K, 165 Cl, 5 HCO_3). Tissues were examined whole and photographed using an inverted epifluorescence microscope (Olympus) with the appropriate filter sets for DASPEI (>515 nm, same as for fluorescein isothiocyanate). Control tissues also were examined under the

same conditions but without DASPEI, in order to look for interfering autofluorescence.

The results are shown in Figure 1. Control tissues with no DASPEI (not shown) were dark, and showed minimal overall autofluorescence. Rectal gland slices from adult (Figure 1A) and 19 cm embryo (Figure 1B) Squalus stained equally well with DASPEI. Tubule morphology is indistinguishable in the two preparations, suggesting that the rectal gland in 19 cm embryos is probably functional. Rectal glands in 19 cm embryos weigh 16.4 ± 2.3 mg (n = 7), or 0.0405 ± 0.0008 % of body weight (less yolk sac). Adult rectal gland weights (1 - 2 g) are comparable at 0.043 to 0.046 % of adult body weight (calculated from Conte, Fish Physiology Vol. 1, pp. 241-292, 1969). We were unable to see any evidence of staining in the rectal gland region of either whole or sliced small embryos (20 - 30 mm). Certainly more precise histological technique is required to determine if mitochondria-rich cells are present in the latter.

For comparison, a gill filament from seawater-adapted Fundulus heteroclitus is included in Figure 1C. Mitochondria-rich chloride cells are abundant, between the respiratory lamellae and especially on the side of the afferent filamental artery (top of photo). A gill filament from a 19 cm Squalus embryo is shown in Figure 1D. Mitochondria-rich cells are abundant between the respiratory lamellae, and on the side of the afferent filamental artery (top of photo). Distribution of cells appears similar to that of the teleost gill. While individual cells are distinct, more "background" staining appears here than with the teleost. The reason is unknown. Figure 1E shows the earliest appearance of mitochondria-rich cells we found, as two parallel streaks on the gill arch of a 20 mm Squalus embryo. In the earliest intact Squalus embryos (8 mm) we examined (not shown), no distinct staining for mitochondria-rich cells was evident in the gill arches, nor were respiratory filaments present.

The results of this investigation show that mitochondria-rich cells begin to appear on the gill arches of Squalus embryos when the embryo is between 8 to 20 mm in length. Unfortunately, we were unable to determine more definitively the time of their first appearance, since no intermediate stages were available for study this summer. Evans et al. (ibid.) have demonstrated that embryos can osmoregulate at a later stage (25 - 30 mm). It is at this length that the kidneys might begin to function (Witschi 1956, after Price and Daiber, Physiol. Zool. 40;3:248-260, 1967) and the anlage of the rectal gland is present (Chan and Phillips, J. Anat. 100;4:899-903, 1966). Thus the iono- and osmoregulatory capabilities of the developing effectors might now be sufficient to meet the needs of the embryo in the reduced gradients they experience. When S. acanthias embryos reach about 50 mm in length, they are exposed to the ionic concentrations of full-strength sea water, with minimal urea in utero for the duration of the developmental period, which lasts about 12 months longer (Kormanik, unpubl., Kormanik and Evans, ibid.). They must ionoregulate during this time. Older embryos (19-20 cm) have many mitochondria-rich cells on the gill filament, and the gross morphology and DASPEI staining properties of the rectal gland tubules appear identical with that of the adult. In addition, the rectal gland of 20 cm embryos is the same percentage of body weight and the Na-K-ATPase activity is similar to that of the adult (Kormanik, Lofton and Vibbard, unpubl.).

These data suggest that both the gills and the rectal gland of late-term embryos are active, confirming ion balance studies on whole embryos made by Evans et al. (ibid.). While elegant studies performed with seawater teleost gills and especially opercular preparations of Fundulus have demonstrated that mitochondria-rich cells are truly chloride cells and involved in ion transport (see Zadunaisky, ibid., for a review) the same cannot yet be said for the mitochondria-rich cells of the elasmobranchs. More definitive studies on the function of mitochondria-rich cells in the gills of elasmobranchs are required.

Figure 1.

DASPEI staining of mitochondria-rich cells. Panel A shows a rectal gland from an adult (4 kg) at the inner region of the loosely packed tubular layer. A tubule lumen (arrow) is visible (200x). Panel B shows a rectal gland from a 19 cm embryo (200x), an arrow indicates a tubule lumen. Panel C shows a gill filament from seawater-adapted Fundulus heteroclitus for comparison; chloride cells (arrows) are distinct (100x). Panel D shows a gill filament from a 19 cm embryo. Individual fluorescing cells are distinct (arrow), occupying the space between the respiratory lamellae (100x). Panel E shows a gill arch from a 20 mm embryo. This photo is slightly out of focus due to the instability of the scope and an extremely long (55 sec) exposure time. DASPEI-stained cells are visible as two streaks (arrows) parallel to the arch (100x). Panel F is the same arch as E under darkfield illumination (100x); loops growing perpendicular to the arch (arrow) are respiratory filaments.

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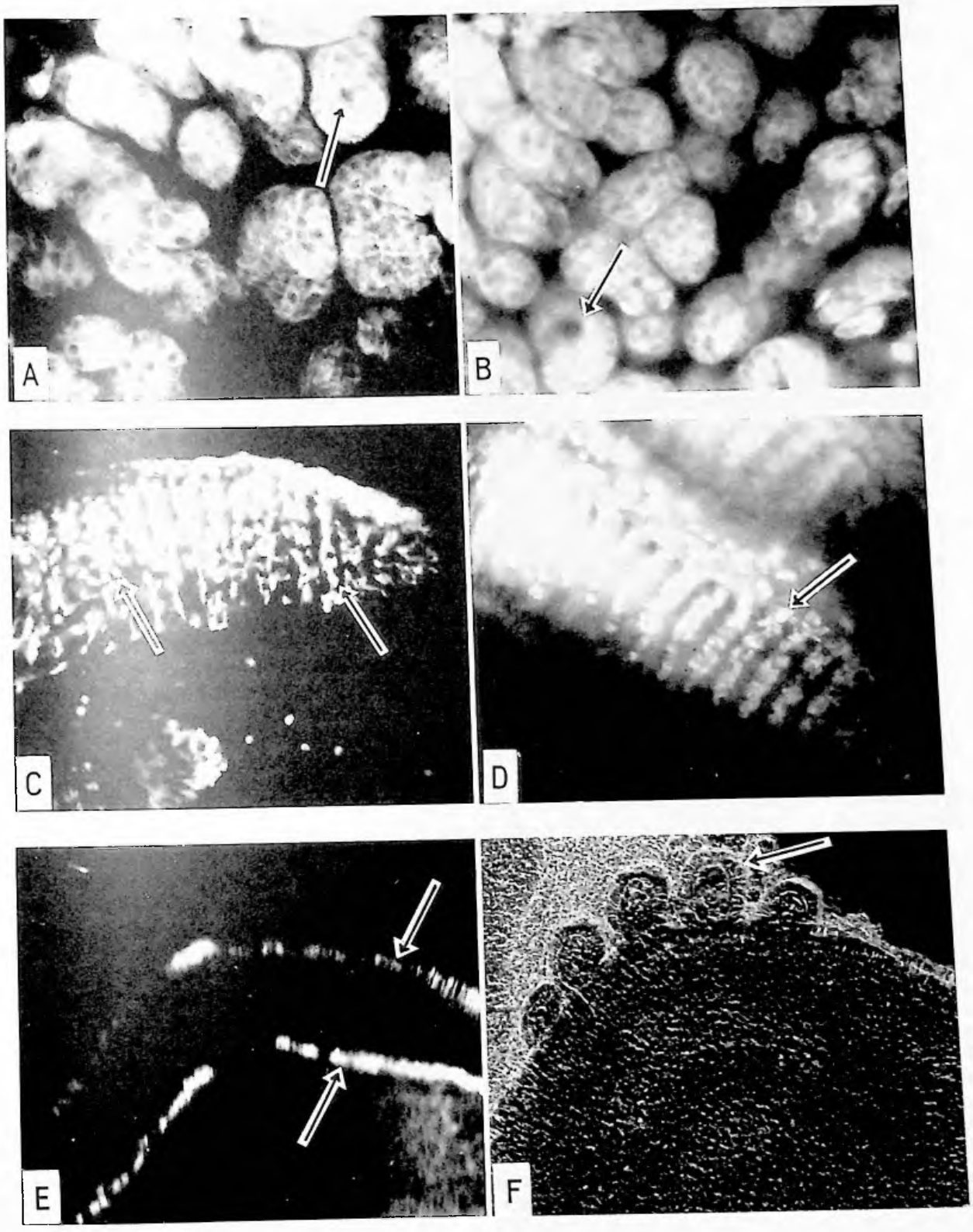
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