OSMOTIC AND IONIC RELATIONSHIPS BETWEEN EMBRYONIC AND UTERINE FLUIDS DURING GESTATION OF SQUALUS ACANTHIAS

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The gestation period of Squalus acanthias appears to be of the order of two years (Woodhead, Bull. MDIBL 16, 103-106, 1976) with embryos available in the summer months either enclosed in egg cases, termed "candles" (4-8 embryos from 1 mm to approximately 3 cm per candle) or free-swimming with attached yolk sacs ("pups" of 40-70 g). Burger (in "Sharks, Skates and Rays", p. 178, 1967) suggested that during late gestation (pup stage) the uterine fluids were flushed with sea water and our preliminary analyses (Kormanik and Evans, Bull. MDIBL 18, 65-69, 1978) supported this conclusion. We have found that pups are able to osmoregulate in sea water and display the major hallmarks of elasmobranch salt and water balance (Kormanik and Evans, Ibid; Evans and Mansberger, Bull. MDIBL 19, 101-103, 1979), but no detailed analysis of the ionic and osmotic relationships between pup plasma, uterine fluids and maternal plasma has been published. In addition, the osmotic and ionic relationships between early embryos, candle fluids, uterine fluids and maternal plasma are unknown. The present study attempted to fill these data gaps.

Pups and candles were removed from sacrificed females which had been maintained in live cars. At the same time samples of uterine fluids were taken by syringe through the uterine wall and frozen for subsequent analysis. Samples of pup blood were taken (within 30 minutes of removal from the female) by heparinized syringe from caudal vessels. The plasma was separated by centrifugation and frozen for analysis. Samples of candle fluid were either taken immediately or after 4-6 days of incubation of the candles in running sea water (13-15°C). To sample embryonic fluids, embryos (2.5-3 cm total length) were gently removed from the candle (either immediately or after incubation in sea water), the yolk sac artery cut and the blood collected into a heparinized hematocrit tube. Samples from 6 embryos from a single candle were pooled for analysis, centrifuged, and the plasma frozen. In other experiments embryos were dissected free and frozen. All frozen samples were shipped to Miami for analysis. Total body determinations were made after homogenation of a 1.0-1.7 g pool of embryos in 10 ml distilled water with a Potter-Elvehjelm glass-teflon homogenizer. The homogenate was allowed to sit (with occasional mixing) at room temperature for 2.5 hr., then centrifuged and the supernatant analyzed. Total osmolarity of thawed samples was determined on a Wescor vapour pressure Osmometer, urea by a modified urease reaction (Sigma Kit 640-A), Na and K by flame photometry (IL Model 143), and Cl by ampermetric tritration (Aminco Cotlove).

Table 1 compares the major constituents of "pup" plasma, surrounding uterine fluids and maternal plasma with sea water. It is clear that uterine fluids at this stage of development are essentially sea water, with decidedly more Na and Cl than either maternal or pup plasma and very little urea. Interestingly, pup plasma appears to contain significantly ($p < 0.001$) more potassium than adult plasma, but in the range of the K concentrations found in the surrounding uterine fluids/sea water. These ionic gradients between the uterine fluids and the maternal blood stream may present a net influx of unwanted monovalent ions, but presumably the adult rectal gland and branchial epithelium can excrete the excess salt (Evans, in "Comparative Physiol. of Osmoregulation in Animals, ed. by B.M.O. Maloiy, pp. 305-390, 1979). In addition, we have found that the pup can also excrete excess salt via functional rectal glands (Kormanik and Evans, op. cit.) and possibly branchial mechanism (Evans and Mansberger op. cit.).

Table 2 compares the major constituents of candle-stage uterine fluids (12 month earlier than the pup stage), intra-candle fluids, and the embryo plasma (2.5-3 cm in length). At this stage uterine fluids are distinctly

Table 1.--Major Constituents of Uterine Fluids, Pup and Maternal Plasma, compared to MDIBL Sea Water

Solute	Sea Water	Maternal Plasma	Uterine Fluids	Pup Plasma
Total	945	1007*	952 ± 8 (12)	946 ± 2 (17)
Na	450	234 ± 7 (4)**	445 ± 4 (12)	249 ± 0.7 (17)
Cl	534	221 ± 9 (4)**	520 ± 4 (12)	231 ± 0.4 (17)
K	7.0	3.3 ± 0.2 (4)**	7.3 ± 0.5 (12)	7.9 ± 0.7 (16)**
Urea	--	357*	8.5 ± 3.3 (12)	342 ± 3 (17)

Total concentration in mOsm/kg, solute concentrations in mM/l, X ± S.E. (N)

*Data from Murdaugh and Robin (in "Sharks, Skates and Rays," ed. by Gilbert, Mathewson and Rall, pp. 249-264, 1967).

**Data from Kormanik and Evans (Op. Cit., 1978).

Table 2.--Major Constituents of Uterine Fluids, Candle Fluids and Embryo Plasma

Solute	Uterine Fluid (8)	Candle Fluid (5)	Embryonic Plasma (1)
Total	952 ± 9	943 ± 9	not determined
Na	333 ± 9	317 ± 5	300
Cl	345 ± 6	345 ± 6	353
K	16.4 ± 1	17.6 ± 0.8	10.3
Urea	310 ± 11	308 ± 13	307

Same units as Table 1.

The data from the embryonic plasma is a single pooled sample of 6 embryos.

different from sea water with reduced concentrations of Na and Cl, but increased concentrations of both K and urea. However,

uterine fluids have greater concentrations of Na, Cl and K, but lower urea concentrations, than the maternal plasma (Table 1). The candle fluids and the embryonic plasma are essentially identical to the uterine fluids (with the exception of a reduced K concentration in embryonic plasma). The passive role of the candle membrane in this distribution of solutes is indicated by our finding that incubation of candles for 4-6 days in running sea water results in substantial changes in the content of the intra-candle fluids. In these experiments (4) the Na and Cl concentrations of the fluids rose to 420 ± 4.2 mM and 496 ± 1.3 mM respectively, while the K concentration fell to 8.2 ± 0.2 mM and the urea levels were reduced to near zero (0.4 ± 0.2 mM). One might conclude from the distribution of solutes in Table 2 and the inability of the candle membrane to regulate ion and urea concentrations against sea water that the developing embryo is bathed in candle fluids whose composition is produced and controlled by active transport steps at the level of the uterine lining. Earlier investigations of the transepithelial electrical potential across early uteri indicated that the TEP was essentially zero (Kormanik and Evans, op. cit.) so the Na, Cl and K gradients between the maternal blood stream and the early uterine fluids must be produced by active transport. One could propose further that urea merely leaks into the uterine fluids from the maternal blood stream. Since the candle is permeable to these ions and urea the solutes merely diffuse into the candle fluids and produce an incubation medium isotonic and iso-ionic to the body fluids of the developing embryo.

However, we found that even after 4-6 days of incubation in sea water, during which the intra-candle fluids are moving toward ionic equilibrium with sea water (and losing their urea) the embryos were alive and apparently regulating both their body ionic and urea content. Table 3 presents the data for both the total body content and plasma

Table 3.--Effect of Seawater Incubation on Total Body and Plasma Constituents of *S. acanthias* embryos (2.5-3 cm)

Solute	Total Body Level (mM/100g)		Plasma Concentration (mM/l)	
	Control (3)	4-6 days SW (2)	Control (1)	4-6 days SW (2)
Na	14.5 ± 1.5	14.8 ± 0.2	300	271 ± 0
Cl	15.1 ± 1.5	15.9 ± 0.2	353	323 ± 9
K	4.6 ± 0.1	4.8 ± 0.04	10.3	7.8 ± 1.0
Urea	32.9 ± 1.2	32.3 ± 1.0	307	306 ± 62

Data are from pooled samples of 6 embryos.

concentrations of Na, Cl, K and urea of embryos incubated within candles for 4-6 days in sea water. It is quite evident that these 2.5-3 cm embryos are able to maintain body ionic and urea contents constant in the face of rather substantial gradients across their permeable membranes. One must propose therefore that mechanisms of ionic and urea regulation are resident very early in the development of *Squalus acanthias* embryos despite the fact that an incubation medium is probably produced by active and passive mechanisms of transport in the maternal uterine lining.

Finally, it is interesting to note that both young embryos and pups maintain plasma K levels distinctly (2-3 times) above those found in the adult. The mechanisms or reasons for this regulation are unknown. This research was supported by NSF PCM80-08366 to DHE.