CHARACTERISTICS OF THE UTERINE ENVIRONMENT FOR LATE GESTATION PUPS OF THE DOGFISH, SQUALUS ACANTHIAS.

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The spiny dogfish, Squalus acanthias, possesses the rather unspecialized form of viviparity termed lecithotropy (Wourms, J. P., Amer. Zool. 21:473-515, 1981). Fertilized eggs "hatch" in utero and the pups, which possess yolk sacs, complete the period of gestation which lasts nearly two years (Woodhead, A. D., Bull. MDIBL 16:103-106, 1976). In the last months of gestation, pups can be removed from the uterus and will survive indefinitely in sea water (Evans, D. H. et al., J. Exp. Biol. 101:295-305, 1982; and unpublished data). In the non-gravid female the uterine lining is thin, but in the gravid female the uterine lining becomes highly vascularized (Jollie, W. P. & L. G. Jollie, J. Ultrastruct. Res. 20:161-178, 1967), and the uterine artery increases in size to allow for increased blood flow (Fuller, E. O. et al., Bull. MDIBL 23:19-22, 1983). This high degree of vascularization and extensive blood flow has led some authors (Burger, J. W., In: Sharks, Skates and Rays, pp. 177-185, Johns Hopkins Univ. Press, Balt., 1967; Jollie & Jollie, 1967; see also Wourms, 1981) to suggest that the uterus (and therefore the mother) might play some role in supplying O_2 and removing waste products such as CO_2, thus aiding the pup in respiration. Also, Burger (1967) has reported that the mother flushes the uterus with sea water which also may play a role in respiration and the removal of waste products for the pups. Thus the role of the mother in care for the pups in utero is not clear. To examine these aspects of maternal care for the pups we compared the blood of the mother and pups as well as the uterine sea water and Frenchman's Bay sea water with respect to various parameters associated with respiration, acid-base balance and nitrogenous waste excretion.

Pregnant female S. acanthias were caught by gill nets from Frenchman's Bay, ME, and held in live cars for several days prior to use. Blood of females was sampled by puncture of the caudal dorsal aorta, usually within less than 30 seconds of removal from the live car. The female was sacrificed, and pups removed from the uterus. Pup blood was sampled within a few minutes of removal from the uterus, by caudal puncture. Uterine sea water samples were collected through the uterine wall with syringe and needle, or by blunt rubber catheter inserted into the uterus. Samples were stored on ice, and assayed immediately for hematocrit, CO_2 content (TCO_2) and pH. Blood was then centrifuged, and the plasma and sea water samples stored frozen for later assay. Ammonia was assayed within 24 hours. Blood and sea water pH were determined with an IL Mod. 213 Blood/gas analyzer. TCO_2 was determined with a Capnicon Total CO_2 Analyzer (Cameron Instr. Co.). Ammonia in sea water was determined by the Solorzano method (Limnol. Oceanogr. 14:799-801, 1969) and in blood plasma, with Sigma Kit #170-UV. Urea in plasma and sea water was analyzed with the monoxime assay (Sigma Kit #535). In another series of experiments we measured the rate of ammonia excretion of pups transferred through a series of sea water baths (see Evans, D. H. & C. Hooks, Bull. MDIBL 23:59-61, 1983, for methods). All values are reported as mean ± standard error. Comparisons were made on unpaired samples, using Student's t-test, two-tailed.

In the first series of experiments (Table 1), we compared the blood of the mothers to that of the pups. We have also presented values for pCO_2, calculated

Table 1. Selected values for the blood of S. acanthias pups compared to the pregnant females.

	pH	TCO_2 (mM)	Urea (mM)	Ammonia (mM)	Na (mM)	H'crit (%)	pCO_2 (calc.) (mm Hg or Torr)
Mothers	7.811 ± 0.014	8.55 ± 0.04	336 ± 8	334 ± 125	251 ± 4	20.9 ± 2.9	2.12 ± 0.18
(n)	(5)	(3)	(6)	(3)	(4)	(3)	(3)
pups	7.855 ± 0.044	9.56 ± 1.40	342 ± 5	1160 ± 95	250 ± 5	15.9 ± 0.9	1.77 ± 0.10
(n)	(14)	(7)	(20)	(18)	(14)	(10)	(7)
sig.	p > 0.1	p > 0.1	p > 0.1	p < 0.005	p > 0.1	p < 0.05	p < 0.05

Table 2. A comparison of uterine sea water removed from pregnant, late gestation S. acanthias to Frenchman's Bay sea water (where n = number of uteri sampled).

	pH	TCO_2 (mM)	Urea (mM)	Ammonia (mM)	Na (mM)	Vol (ml)	pCO_2 (calc.) (mm Hg or Torr)
Uterine sea water	5.889 ± 0.084	0.20 ± 0.02	0.50 ± 0.32	9.74 ± 2.17	418 ± 4	103 ± 16	2.30 ± 0.35
range	5.361- 6.080	0.160- 0.290	0.00- 1.65	3.40- 22.3	402- 428	57- 187	1.76- 3.14
(n)	(9)	(5)	(6)	(9)	(5)	(5)	(4)
Bay sea water	8.17 ± 0.02	1.79 ± 0.11	0.00 ---	0.00070 ± 0.00028	405 ± 0	---	0.189 ± 0.012
(n)	(2)	(3)	(2)	(4)	(2)	---	(3)
sig.	p ± 0.001	p ± 0.001	p > 0.1	p ± 0.001	p > 0.05		p < 0.005

Table 3. Summary of the ammonia gradients (for NH_3 and NH_4^+) developed in the various body compartments of S. acanthias pups in utero (where pNH_3 is in μTorr, and $[NH_4^+]$ in μM).

	pup blood	uterine sea water	mother's blood	Bay sea water
pNH_3 (n)	333 ± 28 (18)	30.7 ± 6.8 (9)	87.8 ± 31.9 (3)	0.41 ± 0.16 (4)
NH_4^+ (n)	1140 ± 93 (18)	9470 ± 2170 (9)	330 ± 124 (3)	0.67 ± 0.28 (4)

from the pH and TCO_2 (pK' and CO_2 solubility from Randall, D. J. et al., Am. J. Physiol. 230(3):590-594, 1976). Blood values for pH, pCO_2 and TCO_2 are similar to those reported in the literature for rested sharks at this temperature (15°C, see Heisler, N., Resp. Physiol. 33:145-160, 1978). Blood urea, Na, pH and TCO_2 were not significantly different in the mother and pup; however, hematocrit and pCO_2 in the pups were significantly lower, and ammonia concentration was significantly higher, by a factor of three.

We also examined the uterine environment, and compared values for the uterine sea water and the sea water from Frenchman's Bay (Table 2). The Na concentration of both sea waters is not significantly different, corroborating the data presented by Evans et al. (J. Exp. Biol. 101:295-305, 1982) which demonstrated that uterine sea water and ambient sea water are relatively similar, at least with respect to the concentration of the major ions. However, the uterine sea water pH is over two pH units more acid than ambient Bay sea water and the TCO_2 is far lower than that of normal sea water ($p < 0.001$). The pCO_2 of the uterine sea water (Table 2) is not significantly different from that of the blood of the mother. However, in these preliminary observations, the pCO_2 of the pup blood is slightly lower, but not significantly different from ($p > 0.05$) the uterine sea water. Conditions are thus different from the typical pattern of CO_2 excretion observed in aquatic fishes, where a gradient of one to two mm Hg usually develops from the blood of the fish to the sea water environment. However, these observations are not paired, that is, pup with ambient uterine sea water. Nevertheless, the pCO_2 of the uterine sea water is substantially elevated above that found in Bay sea water and would appear to be in equilibrium with the pCO_2 of the blood of the mother.

Uterine sea water has a relatively low concentration of urea (compared to that of the blood), which was highly variable. Nearly all of the values for urea in uterine sea water were higher than those for ambient sea water, where in all samples, no urea was detectable (Table 2). Our observations of small quantities of urea in uterine sea water corroborate observations made earlier by Price and Daiber (Physiol. Zool. 40:248-260, 1967) on *S. acanthias*.

Of far greater interest were the values we determined for uterine ammonia concentration, which ranged upwards to 22 mM, far higher than Bay sea water, which measured in the fractional μM range, typical of environmental waters. In all our determinations of uterine sea water, the pups were living in a very high concentration of ammonia and acidity *in utero*.

From these data, then, two obvious questions present themselves. Firstly, how could such a concentration of ammonia and acid arise; and secondly, why would such a condition be permitted to occur? The high uterine ammonia concentration could arise for several reasons. The build-up of such a high concentration of ammonia and acid might indicate that uterine flushing with sea water, previously reported in the literature (Burger, 1967) is minimal. Additionally, the source of ammonia and acid could be either the pups or the mother. To examine the first possibility, one can calculate how long it might take the pups to elevate the ammonia concentration to this level. The average rate of ammonia excretion we measured was about $0.5 \pm \mu mol \cdot 100^{-1} \cdot h^{-1}$ ($n = 8$). With an average of six pups per horn of the uterus, and about 100 ml of sea water per horn (Table 2), it would take about 23 days to reach this ammonia concentration. Is the sea water held in the uterus for this period of time? If the sea water weren't flushed, one can also calculate the urea concentration that would build up after 23 days, given the same conditions and an average rested urea excretion

rate of 15 $\mu mol \cdot 100^{-1} g \cdot hr^{-1}$ (Evans & Hooks, 1983). After 23 days, the urea concentration in the uterine sea water would be about 300 mM, and it certainly is not. Therefore, either the mother is removing urea to prevent its build-up in the uterine sea water, while not removing ammonia over the long period of time that the water is held in utero, or the water may be held for a relatively shorter period of time, and the mother might elevate the ammonia concentration by contributing to it, that is, by excreting ammonia into the uterus. To examine this latter possibility we have examined the ammonia gradients involved for both the ionic form, NH_4, and the gaseous form, NH_3 since ammonia can move across the tissues in either form (see Kormanik, G. A. & J. N. Cameron, Mar. Biol. Lett. 2:11-23, 1981). Values for pK' and solubility for sea water and blood were obtained from the data of Cameron and Heisler (J. Exp. Biol. 105:107-125, 1983), and NH_3 and NH_4 were calculated from the Henderson-Hasselbalch equation (see Kormanik & Cameron, 1981). The gradients for the pup blood, uterine sea water, mother's blood and Bay sea water (raw data from Tables 1 and 2) are presented in Table 3. From these data then, it is apparent that the pNH_3 gradient is directed toward the uterine sea water from the blood of both the mother and the pups, while the gradient for NH_4 is directed away from the uterine sea water toward the blood of the mother and pups. The sum of the net gradients for both NH_3 and NH_4 is directed toward Bay sea water for both the mother and the pups. The opposing gradients for NH_3 and NH_4 in the uterine sea water, along with the accumulation of ammonia, would support the idea that ammonia tends to move predominantly down the partial pressure gradient of pNH_3 (Kormanik & Cameron, 1981).

Interestingly, the low pH of the uterine sea water we determined might serve to 'trap' ammonia in the form of the rather less diffusible NH_4 and therefore aid its accumulation in the uterus. Additionally, NH_3 is the far more toxic form of ammonia (see review by Colt, J. E. & D. A. Armstrong, In: Bioengineering Symposium for Fish Culture (FCS Publ. 1):34-47, 1981). The acidity of the uterine sea water would tend to protect the pups from the toxic form of NH_3, by lowering the partial pressure. Thus the mother appears to contribute to the build-up of uterine sea water ammonia by providing a favorable pH gradient for the movement of ammonia, which at the same time might protect the pups from the toxic effects.

But why accumulate a toxic waste product like ammonia in the uterine sea water in the first place? It would appear to be a simple matter to flush the uterus with sea water and eliminate the build-up of ammonia (as well as acid), but this apparently does not occur. Since both ammonia and acid build up, but not urea, it would appear to be a selective process. The only rationale we can suggest, given these preliminary data, is that ammonia is present to act as a nitrogen source for the developing pup, and the concomitant build-up of acidity would serve to not only trap ammonia in the uterine sea water, but also detoxify it. One piece of evidence we have to support this hypothesis is that the pups do not excrete ammonia to any great extent (see above), and in several instances pups actually take up ammonia from the surrounding sea water solutions (unpublished results). The use of an ordinarily toxic nitrogenous waste product as a source of nitrogen for a developing vertebrate embryo would be of extreme interest. This aspect is currently under investigation in our laboratory. (Funded by NSF PCM 83-02621 to DHE and NSF-ROA and UNC-A intramural grant to GAK).