TIME-COURSE OF THE ESTABLISHMENT OF UTERINE SEA WATER CONDITIONS IN PREGNANT DOGFISH (SQUALUS ACANTHIAS)

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Spiny dogfish (Squalus acanthias) possess the rather unspecialized form of viviparity termed 'lecithotrophy' (Wourms, Amer. Zool. 21:473-515, 1981). Fertilized eggs remain encapsulated for about four to six months in utero. These 'candles' then burst, and the pups, who possess yolk sacs, complete their period of gestation which lasts nearly two years (Nammack, Musick and Colvocoresses, Trans. Am. Fish. Soc. 114:367-376, 1985). In the last ten to fifteen months of gestation these pups reside in a solution derived from sea water and apparently ventilate while in utero (Kormanik, personal observations). While the major ion concentrations resemble normal sea water (Evans, Oikari, Kormanik and Mansberger, J. exp. Biol. 101:295-305, 1982) the uterine sea water is relatively acid, with a pH of about 6, the total CO_2 content is only a few tenths of a mM, PCO_2 is elevated and ammonia is extremely high in concentration, up to 22 mM (Kormanik and Evans, Bull. MDIBL 24:26-29, 1984). Thus it would appear that the uterus is not flushed with sea water as frequently as was previously suggested (Burger, In: Sharks, Skates and Rays, pp. 177-185, Johns Hopkins University Press, Balt., 1967). These pups would appear to be in an environment which would normally be considered toxic to water-breathing organisms yet they apparently thrive. This system provides a unique opportunity to study acid-base balance and respiration in fishes under extreme conditions, as well as gain some insight into the developmental physiology of elasmobranchs.

In order to examine the origin of these uterine sea water conditions, we monitored the changes in concentration with time of several uterine sea water constituents, as well as the gradients involved. Pregnant female spiny dogfish were caught by gill nets and held in live cars for several days to recuperate from the trauma of capture. Prior to use in experiments, an incision was made on the snout of the female, and the fish was pithed by the insertion of a wire (ca. 1.5 mm x 1 m) into the brain and down the length of the spinal column. This procedure was performed at the live car, and the fish was then immediately transferred to a holding tank where ventilation of the gills via the mouth and spiracles was commenced with fresh aerated sea water. The female was strapped to an operating board, dorsal side down and the head and body below the cloaca were immersed in sea water (15 + 1° C.), and running sea water was directed over the exposed portions of the body. The uterine horns were perfused with aerated sea water as well to temporarily maintain the pups. A blood catheter (P.E. 60) was installed in the dorsal aorta using a Tuohy needle. Tonus of the cloaca and uterine horns was reduced sufficiently after about 30 minutes so that the pups could be expelled alive and intact from the uteri. A waterinflated balloon was installed in the intestine to block any flow of intestinal contents into the cloaca. In some experiments, the urinary papilla was catheterized as well. In most experiments, spontaneous ventilation was reestablished. If ventilation was weak or absent, perfusion of the gills was maintained throughout the course of the experiment. At the start of the experiment, each uterine horn was filled with 120 ml of fresh sea water. Sea water samples (ca. 2 ml) were removed from the uterine horns

with time, and analyzed for total CO_2 (TCO_2; Capnicon II, Cameron Instr. Co.), pH (Orion 601A or Instr. Lab. Micro 13) and total ammonia (Solorzano, Limnol. Oceanogr. 14:799-801, 1969). Heparinized blood samples were removed via the aortal catheter with time, and all samples were analyzed as previously described (Kormanik and Evans, 1984). PCO_2 in the sea water samples was calculated using the Henderson-Hasselbalch equation and the appropriate values for solubility and 'apparent' pK (Boutilier, Heming and Iwama. In: Fish Physiology, vol. XA, pp. 403-430, Academic Press, Inc., New York, 1984). PCO_2 for blood was calculated from the pK and solubility for shark blood, determined from the expanded polynomials presented by Boutilier et al. (1984). The experimental period was commenced when the blood pH of the fish was rising and approaching normal levels (ca. 7.7), usually a few hours after surgery. Females with abnormally low initial blood pH were discarded.

Results are presented in Figures 1-3. Maternal blood pH increased slightly with time in the first 30 hours of the experiment and then stabilized (Fig. 1a). Uterine sea water pH declined from normal sea water pH (ca. 8.2) to about 6.2 in the first 20 hours, and remained at about that level for the duration of the experiment (Fig. 1a). Blood TCO_2 (not shown) was determined and blood PCO_2 was calculated. Sea water PCO_2 was calculated as well from uterine sea water pH (Fig. 1a) and TCO_2 (Fig. 2b). Values of PCO_2 for both blood and sea water are presented in Fig. 1b. Blood PCO_2 declined slowly from about three mm Hg to about two mm Hg during the course of the experiment, indicating that ventilation of the mothers' gills, whether spontaneous or aided by perfusion, was sufficient to alleviate build-up of respiratory CO_2. Thus a stable respiratory status was achieved. PCO_2 of the uterine sea water increased rapidly from values for sea water equilibrated with atmospheric PCO_2 (ca. 0.2 mm Hg) to nearly three mm Hg. Thus uterine sea water PCO_2 appeared to rapidly come to equilibrium with the PCO_2 of the blood. Uterine sea water PCO_2 then declined with time, thus
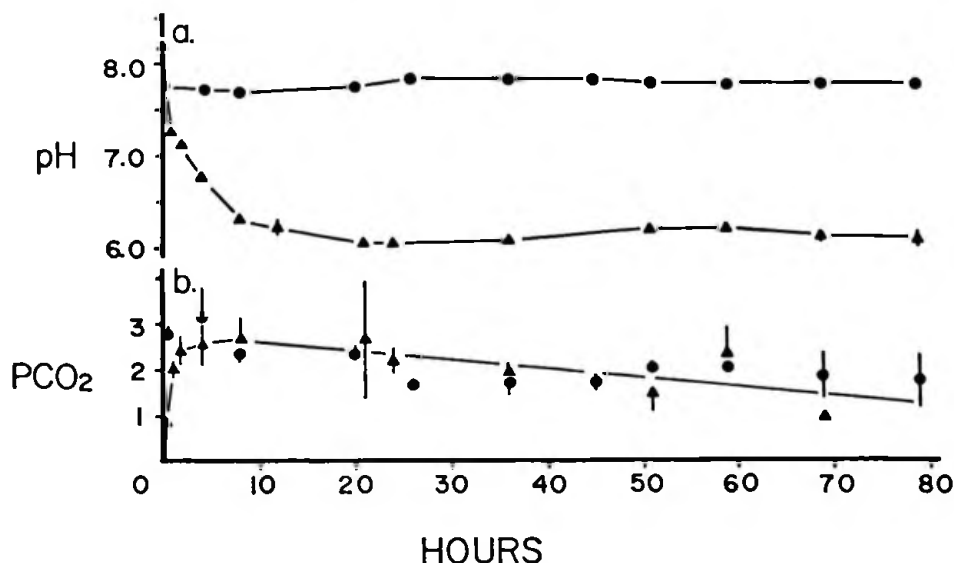

Figure 1   Changes in pH and PCO_2 of the blood (filled circles) and uterine sea water (filled triangles) of pregnant females.   Vertical bars indicate s.e. PCO_2 in mm Hg.   N = 2 to 12.

tracking the blood PCO_2. Uterine sea water total ammonia (Tam) and TCO_2 are shown in Fig. 2. The total ammonia concentration was highly variable, but increased with time, and, during the course of this investigation, approached values of over 10 mM. The values for TCO_2 however, declined with time, and within ten hours achieved a stable level of about 0.2 mM. Dissolved CO_2 (0.052 mM torr^{-1} * 2.5 torr = 0.13 mM) represents a substantial portion of the TCO_2, thus the final bicarbonate concentration of the uterine sea water was less than 0.1 mM.
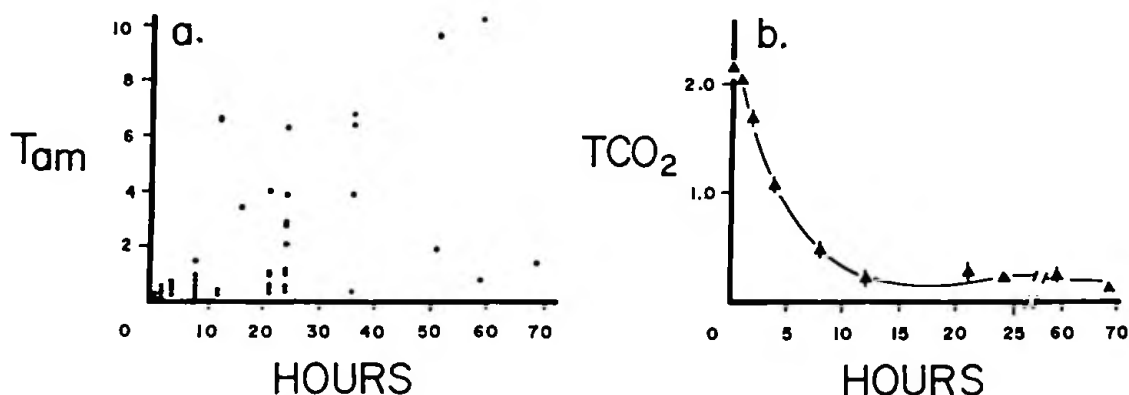
Figure 2   Changes in total ammonia (Tam) and Total CO_2 (TCO_2) in the uterine sea water of pregnant females. Tam and TCO_2 are in mM. Vertical bars indicate s.e. N = 2 to 12.
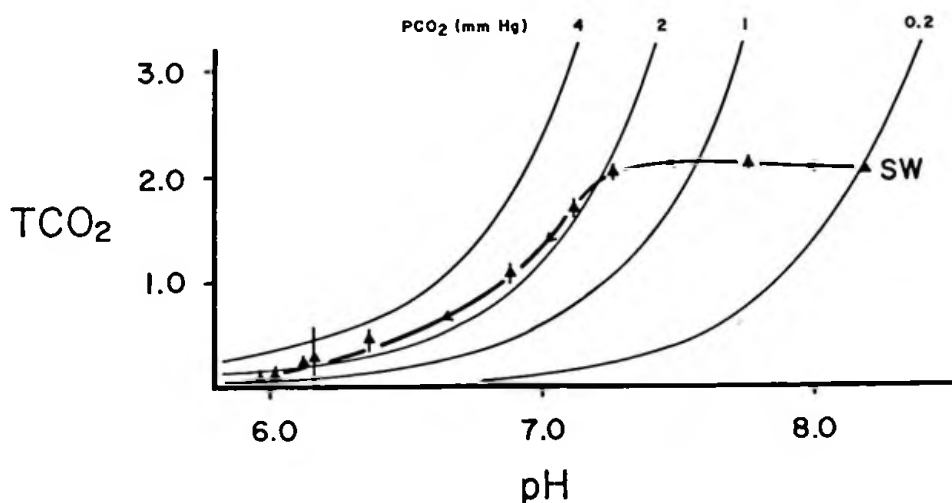
Figure 3 TCO_2 and pH diagram for uterine sea water (filled triangles), with PCO_2 isopleth for 4, 2, 1 and 0.2 mm Hg. Arrows indicate the direction of change with time. Vertical bars indicate s.e. TCO_2 is in mM. N = 2 to 12.

This rapid decline in TCO_2 is especially interesting since, for a passive buffer system, one would expect to see only a slight increase in TCO_2 along the sea water buffer curve with the increase in PCO_2. This aspect is further examined in Fig. 3, where a Davenport-type diagram was constructed. Sea water PCO_2 isopleths are presented ranging from atmospheric (ca. 0.2) to 4 mm Hg. Uterine sea water TCO_2 increased slightly as the PCO_2 increased and the pH decreased. The TCO_2 as well as the pH then decreased, following the PCO_2 isopleth for the blood.

These preliminary data provide some insight into the changes that occur in the uterine sea water of pregnant female dogfish in the absence of pups. Firstly, the CO_2 content of uterine sea water is actively reduced with time. The reduction of TCO_2 may be the result of active resorption of $HCO3^-$ (or OH^-) from the sea water in the uterine horns or excretion of protons into the uterine sea water, and the resorption of $HCO3^-$ occurring by the diffusion of CO_2 gas from the uterine horns to the blood. Given these data, it is impossible to distinguish among the alternatives (Cameron, Am. J. Physiol. 246:R452-R459, 1984). Nevertheless, the reduction observed in both the pH and TCO_2 would appear to be related. The increase in ammonia concentration seen in the uterine sea water may be secondary to the reduction in pH and the resultant large PNH_3 gradient established from the blood of the mother to the uterine sea water (Kormanik and Evans, 1984). It is not necessary to postulate any active process, such as NH_4^+ transport, for ammonia excretion into the uterine sea water, but such a process cannot be ruled out at this time. These data certainly indicate that the uterine sea water conditions we observed in fresh-caught pregnant females, that is low pH, low CO_2 content and high ammonia concentration (Kormanik and Evans, 1984), can occur in the absence of pups. The decline in pH and CO_2 content occurs rather rapidly, and ammonia build-up rather more slowly. The central question however, still remains: Why are these conditions established in the first place, when it would appear to be relatively easy for the mother to flush the uterine horns periodically with fresh sea water? The possible role of this uterine ammonia in nitrogen metabolism of these prenatal developing pups is currently under investigation.