EFFECT OF ACETAZOLAMIDE ON THE ESTABLISHMENT OF UTERINE SEA WATER CONDITIONS IN PREGNANT DOGFISH (*SQUALUS ACANTHIAS*)

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Spiny dogfish (*Squalus acanthias* L.) possess the rather unspecialized form of viviparity termed 'lecithotrophy' (Wourms, Am. Zool. 21:473-515, 1981). Fertilized eggs remain encapsulated in a thin egg case *in utero* for about four to six months. The egg case then bursts and the pups complete the 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 the gestation period, pups reside in a small volume of uterine fluid apparently derived from sea water (Evans, Oikari, Kormanik and Mansberger, J. Exp. Biol. 101:295-305, 1982) and ventilate (Kormanik, personal observations). While the major ions resemble sea water, this uterine fluid is relatively acidic, with a pH of about 6, the total CO_2 content (TCO_2) is only a few tenths of a mM, and the partial pressure of CO_2 (P_{CO_2}) is elevated to the levels found in the mother's blood. In addition, the ammonia concentration is extremely high, in some cases approaching 22 mM (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986). Burger (In: Sharks, Skates and Rays, pp. 177-185, Johns Hopkins University Press, Balt., 1967) suggested that the uterine horns were flushed with sea water, but our data suggest that if flushing does occur, it happens only infrequently. The uterine conditions reported above are established by the mother (Kormanik, Kremer and Patton, Bull. MDIBL 25:142-145, 1985) whose uterine tissues modify a sea water solution. The environment in which these pups reside is therefore quite remarkable. To further examine the role of the mother in elevating acidity and depressing the CO_2 content of this uterine solution, we examined the time-course of the establishment of these uterine sea water conditions in the absence of pups and in the presence of the drug acetazolamide, an inhibitor of carbonic anhydrase. This enzyme has been implicated in the movement of CO_2, HCO_3^- and protons across the gills of fish (see review by Perry, Can. J. Zool. 64:565-572, 1986) as well as many other epithelia.

To examine the origin of these uterine sea water conditions, we monitored the change with time of several of these uterine sea water constituents in the presence and absence of acetazolamide. Late-term pregnant female spiny dogfish (ca. 4 to 5 kg) were caught by local fisherman and held in live cars for several days prior to experiments to allow them time to recuperate from capture. Females, with the pups removed from the uteri, were prepared and the experiment set up as previously described (Kormanik et al., ibid.), except that in all these experiments, the urinary papilla was catheterized to prevent retrograde flow of urine into the uterine horns. In one series of experiments, sea water with acetazolamide (90 uM) was introduced into the uterine horns. The results were not different from those of the controls, that is, the uterine sea water was acidified and TCO_2 was reduced. These data are not reported. In the series of experiments reported below, the control blood sample was taken (t = 0), and then acetazolamide (Sigma Chemical Co.) was immediately injected into the bloodstream via the dorsal arterial cannula (20 mg kg^{-1}). The uterine horns were well flushed with sea water and at one hour after injection, were filled with 120 ml of fresh sea water and sampling was commenced. Samples were assayed and values for P_{CO_2} were calculated as previously reported (Kormanik et al., ibid.).

The results of the experiments are presented in Figures 1 and 2. In the controls (Fig. 1a.), the blood P_{CO_2} starts out at about 3 mm Hg, very slightly elevated probably due to the post-operative stress. Blood P_{CO_2} then decreased with time, indicating that the fish were well-ventilated under our experimental protocol, at least with respect to P_{CO_2}. In the acetazolamide-injected fish, maternal blood P_{CO_2} increased rapidly and reached values (ca. 6.5 mm Hg) more than double those of the controls, and remained at those levels for the duration of the experiments (24 hours).

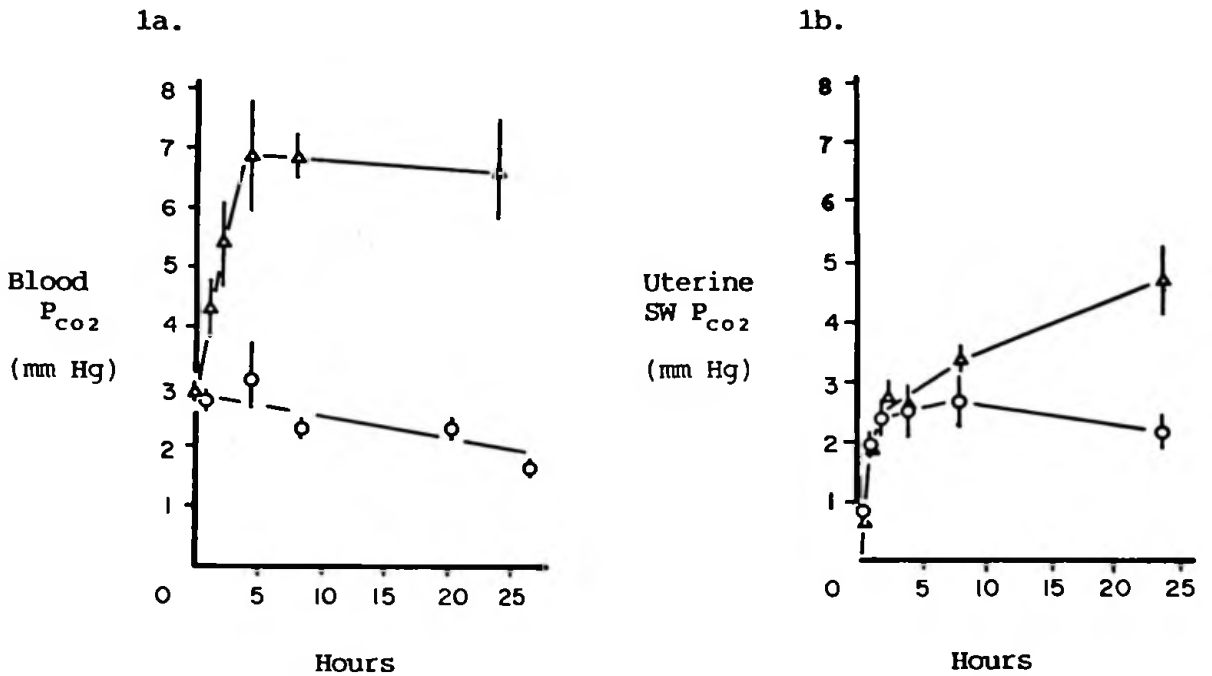

Figure 1. Blood P_{CO_2} (1a.) and uterine sea water P_{CO_2} (1b.) in acetazolamide-injected fish (open triangles) versus the controls (open circles). Bars indicate s.e., n = 4 to 12.

These observations confirm those of other investigators, that is, that inhibition of carbonic anhydrase reduces CO_2 excretion across the gills with a concomitant increase in blood P_{CO_2} (see Perry, Ibid.). The changes in P_{CO_2} of the uterine sea water are reported in Fig. 1b. In the controls, uterine sea water P_{CO_2} increased rapidly over the levels of the instilled sea water (ca. 0.2 mm Hg), and came to equilibrium with the blood P_{CO_2} (ca. 2.5 mm Hg) within five to ten hours. While the uterine sea water P_{CO_2} in the acetazolamide-injected fish also increased rapidly and exceeded that of the non-injected fish, even after 24 hours the P_{CO_2} (ca. 4.8 mm Hg) had not yet come to equilibrium with the elevated blood P_{CO_2} (ca. 6.5 mm Hg, Fig. 1a).

Uterine sea water pH and total CO_2 are shown in Figure 2. Uterine sea water pH (Fig. 2a) declined to about 7 (from a pH of 8.1 of the instilled sea water) and then stabilized in the acetazolamide-injected females, and thus never reached the pH of the controls. The changes in TCO_2 are shown in Fig. 2b. The uterine sea water TCO_2 of the injected fish, while variable,

increased with time compared to that of the controls which show a substantial decrease in TCO_2 from levels of the instilled sea water (ca. 2.1 mM). Thus acetazolamide inhibited the removal of CO_2 from the uterine sea water.

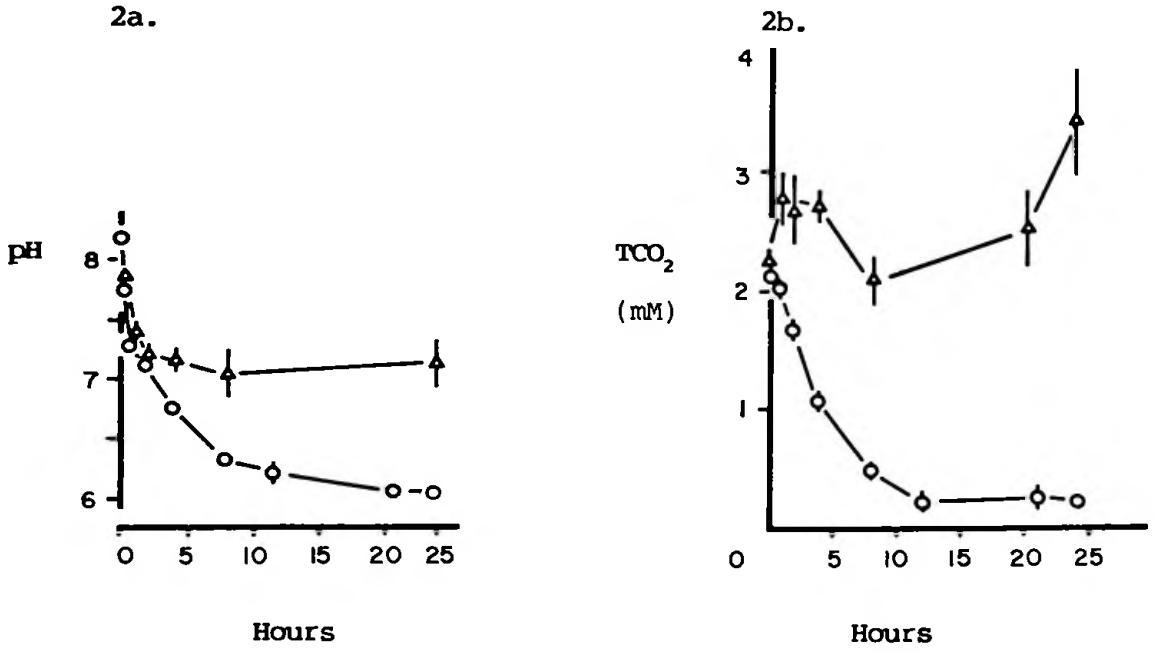

Figure 2. Uterine sea water pH (2a.) and total CO_2 content (TCO_2, 2b.) in fish injected with acetazolamide (open triangles) and controls (open circles). Bars indicate s.e., n = 4 to 12.

These preliminary data provide some insight into the manner by which the late-term mother modifies the uterine environment. In the presence of acetazolamide the P_{CO2} of the uterine sea water fails to come to equilibrium with that of the blood, since the conversion of blood HCO_3^- to CO_2 by red blood cells is limited during transit of the blood through the uterine epithelium and CO_2 excretion is limited, in a manner analogous to that which occurs in the gills. Acetazolamide also inhibits to some degree uterine sea water acidification and extraction of HCO_3^-. Most of the decline in pH we observed can be explained by the passive buffer properties of sea water coming to equilibrium with the elevated P_{CO2}, for as the P_{CO2} increases, the pH decreases, but HCO_3^- extraction from the uterine sea water was nevertheless inhibited. The role of carbonic anhydrase in uterine sea water acidification and HCO_3^- extraction is not clear, however, since carbonic anhydrase may supply protons for the acidification process and titration of the uterine sea water HCO_3^- with its subsequent removal in the form of CO_2. Alternatively, carbonic anhydrase may be responsible for the removal of HCO_3^- transported out of the uterine sea water by the epithelium. While the effect of acetazolamide is serosal, it is not known whether the effects are mediated by inhibition of carbonic anhydrase located in the red blood cell, or carbonic anhydrase possibly located in the uterine epithelium. As yet we have no evidence for the latter, but we plan to assay the uterine tissue for carbonic anhydrase in the near future. (Supported by NSF DCB-850251 to G.A.K.).