BLOOD AMMONIA IN PRENATAL PUPS OF THE DOGFISH, SQUALUS ACANTHIAS EXPOSED TO DIFFERENT SEA WATER AMMONIA GRADIENTS

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Pups of the dogfish, Squalus acanthias spend the last several months of the intrauterine gestation period in a solution resembling sea water at least with respect to the major ions, but the pH is about 2 units lower than normal sea water, and the ammonia concentration may be as high as 22 mM (Kormanik and Evans, Bull. MDIBL 24:26-29, 1984). The relationship of this low pH and high ammonia concentration is not known. The low pH may help to detoxify the ammonia by reducing the partial pressure of ammonia gas (PNH_3), the relatively toxic form of ammonia, while at the same time preserving the gradient of PNH_3 for ammonia excretion by pups in utero (see Kormanik, Kremer and Patton, this volume; Kormanik and Evans, 1984). This hypothesis was tested in the following experiments.

Late-term prenatal pups (Squalus acanthias) were collected from sacrificed females, and acclimated to fresh sea water for about one day prior to experiments. In the first series of experiments, five pups (ca. 60 g each) were placed in two liters of well aerated sea water (15 ± 1^{o} C.) adjusted to a total ammonia concentration of 10 mM (as Cl salt), about the concentration found in uterine sea water (Kormanik and Evans, 1984). The pH was 8.14 at the start of the experiment, about that of normal sea water. Heparinized blood samples (100 ul) were taken from the pups by ventral puncture of the dorsal aorta in the tail region at 3, 16 and 42 hours. After the 16 hour sample, the sea water was replaced with a fresh solution at 10 mM total ammonia, and a pH of 8.2. Blood and sea water pH, and ammonia in sea water and blood plasma were determined as previously described (Kormanik, Kremer and Patton, this volume). NH_4^+ and PNH_3 were calculated from total ammonia and pH for the blood and sea water as previously described (Kormanik and Evans, 1984). Since blood pH could not be measured in all pups in all time periods, an average value, 7.8, was used in the gradient calculations.

The results of the first series of experiments are presented in Table 1. To minimize the amount of blood removed from these small pups, we did not sample pup blood at t = 0. Blood ammonia in these pups increased rapidly throughout the course of the experiment and approached toxic levels even before the last sampling period. In all cases, substantial gradients for both NH_4^+ and PNH_3 were directed from sea water to the blood of the pups.

In a second series of experiments, pups were set up as before, but were exposed to sea water with ammonia at 10 mM with the pH adjusted to 4.1 with HCl. It was necessary to add more acid every few hours to keep the pH low. Over one night the pH increased to 5.5 and was then maintained at that level for the duration of the experiment. The results are presented in Table 2.

Maintenance of a stable low pH by hand was rather difficult in these experiments, nevertheless, the pH was kept below levels normally seen in the uterine sea water (ca. 6, Kormanik et al., this volume) during the course of this experiment. With the decreased sea water pH, the PNH_3 gradient remained directed from blood to sea water throughout the course of this series of experiments. Thus in spite of the fact that the total ammonia gradient (predominantly in the form of NH_4^+) was directed from sea water to blood by

Table 1 The effect of high ammonia, normal pH (8.1 to 8.2) sea water on blood ammonia in prenatal dogfish pups. Significance denotes a comparison to the previous time period for paired data (mean $\pm$ s.e.).

	3 hrs	16 hrs	42 hrs
Blood Ammonia (in mM) (n)	1.25 $\pm$ 0.33 (5)	2.32 $\pm$ 0.28 (5)	5.17 $\pm$ 0.29 (3)*
significance		$p < 0.001$	$p < 0.001$
Ammonia gradients (sea water - blood)			
ΔNH_4^+ (in mM)	8.51 $\pm$ 0.32	7.39 $\pm$ 0.28	4.58 $\pm$ 0.29
ΔPNH_3 (in uTorr)	4860 $\pm$ 100	5850 $\pm$ 80	5000 $\pm$ 90

* 2 pups died during the night

Table 2 The effect of high ammonia, low pH (4.1 to 5.5) sea water on blood ammonia in prenatal dogfish pups. Significance denotes a comparison to the previous time period for paired data (mean $\pm$ s.e.). N = 5 to 6.

	3 hrs	16 hrs	42 hrs
Blood ammonia (in mM)	0.38 $\pm$ 0.14	0.20 $\pm$ 0.15	0.43 $\pm$ 0.22
significance		$p > 0.1$	$p > 0.1$
sea water pH	I----- 4.1 $\pm$ 0.1 ------I---- 5.5 $\pm$ 0.2 --------I		
Ammonia gradients (sea water - blood)			
ΔNH_4^+ (in mM)	9.23 $\pm$ 0.13	9.80 $\pm$ 0.14	9.58 $\pm$ 0.22
ΔPNH_3 (in uTorr)	-111 $\pm$ 40	-48 $\pm$ 43	-114 $\pm$ 65

nearly 10 mM, the blood ammonia concentration remained at concentrations typical of pups adapted to fresh sea water. These observations are in sharp contrast to those of the first series of experiments (Table 1). In both series, the total ammonia concentration of sea water was 10 mM, but the direction of the PNH_3 gradient was varied by adjusting the pH. The data are instructive on two points. Firstly, ammonia movement appears to follow the PNH_3 gradient. Thus ammonia excretion by the pups can be maintained if the PNH_3 gradient is directed from blood to sea water, regardless of the NH_4^+ gradient. These data support the suggestion that the gills of these prenatal pups are relatively impermeable to the ion NH_4^+. Secondly, high blood levels of ammonia appear to be toxic to the pups, presumably due to the elevated PNH_3 of the blood. Thus the low pH observed in the uterine sea water would appear to help protect the pups from the build-up of toxic levels of NH_3 in the blood as well as maintain a PNH_3 gradient from pup blood to uterine sea water. (Supported by NSF DCB-850251 to GAK).