# EFFECT OF ELEVATED PLASMA AMMONIA ON TISSUE LEVELS OF GLUTAMINE AND GLUTAMATE IN EMBRYOS OF THE DOGFISH (SQUALUS ACANTHIAS)

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During the latter half of a 22 month gestation period, late-term embryos of the viviparous dogfish (Squalus acanthias) are incubated in uterine seawater with a high (average 10mM) ammonia concentration (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986; Kormanik, J. Exp. Biol. 137:443-456, 1988). This high uterine seawater ammonia elevates blood ammonia of the embryos by several-fold. Ammonia is usually toxic, and represents a challenge which must be met. Elasmobranchs use glutamine synthetase to trap ammonia as glutamine and feed it to urea synthesis pathways via carbamoyl phosphate synthetase (CPS) III (Anderson, Science 208:291-293, 1980). Glutamine may also be an important energy source for mitochondria in elasmobranch red muscle (Ballantyne et al., J. Exp. Zool. 261:110-114,1992; Chamberlin and Ballantyne, J. Exp. Zool. 264:267-272, 1992) as well as a precursor for amino acid, adenylate and protein synthesis. However, glutamine synthetase has a very low Km (15uM) for ammonia (Shankar and Anderson, Arch. Biochem. Biophys. 239:248-259. 1985). One could argue that glutamine synthesis (and ammonia incorporation) is unaffected by elevated blood ammonia, since glutamine synthetase is probably saturated at normal (ca. 200uM) blood ammonia levels. To test this hypothesis, we examined the effect of elevated blood ammonia on glutamine (GLN) and glutamate (GLU) levels in plasma and several tissues of these late-term embryos.

Pregnant female Squalus acanthias were collected by commercial fishermen from Frenchman Bay, ME and stored in livecars until use. Embryos were collected as previously described (Kormanik, ibid.). One group of embryos was assayed immediately upon removal from the mothers. One group was acclimated to fresh sea water (SW) for 24 hours, and one group was acclimated to fresh SW (for 24 h), and then transferred to fresh SW with 1mM total ammonia (1mM Tamm) added (as chloride), for 24 h. At the end of the experimental periods embryos were immediately killed by decapitation or destruction of the brain with a scalpel. Blood was collected by caudal puncture in Na heparinized syringes and centrifuged. Tissues were immediately assayed, or immediately frozen on dry ice and assayed, or stored at -100° C. until assay. No differences in results due to procedure were apparent. Tissues (<100mg) were homogenized with a motor-driven teflon pestle in 250ul of 0.35 M perchloric acid, neutralized with to pH 7 with 4 M KOH/0.4 M KCl (after Wright et al., J. Exp. Biol. 188:143-157, 1994) and centrifuged at 3000xg. Plasma was similarly deproteinized. Glutamine and glutamate were assayed using the GLN-1 assay (Sigma) and ammonia, using an enzymatic assay (Sigma 170-UV). Results were analyzed using Student's t-test for unpaired data (one-tailed). All comparisons were made to the 24 h seawater-acclimated fish (24 h SW). Data are expressed as mean ± 1 SEM.

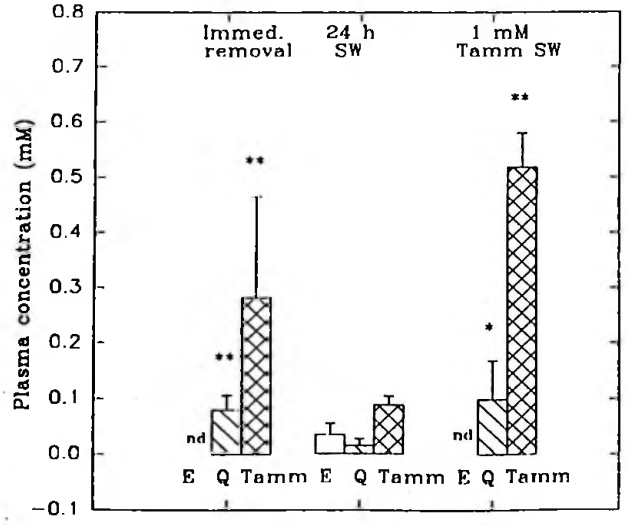


**Figure 1.** Plasma concentrations of GLU (E), GLN (Q) and Tamm in embryos exposed to experimental conditions. P<0.05 (*), P<0.01 (**), nd = not detected, n = 3 to 7.

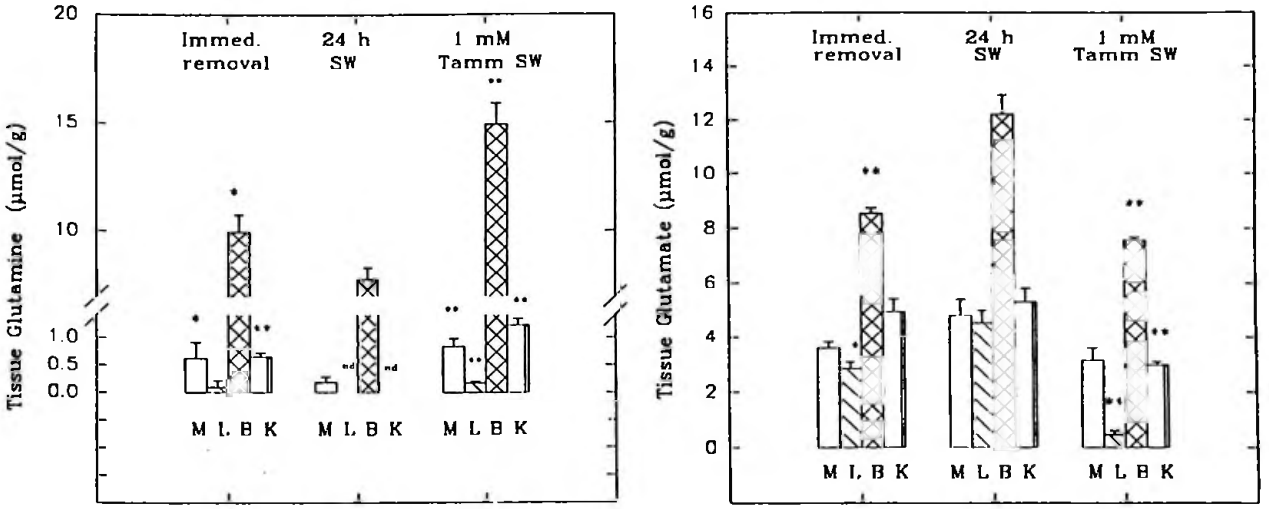


Figure 2. Tissue levels of glutamine and glutamate in muscle (M), liver (L), brain (B) and kidney (K) under experimental conditions. P<0.05 (*), P<0.01 (**), nd = not detectable. N = 3 to 7.

Exposure of embryos to 1mM Tamm SW elevated plasma ammonia to levels comparable to those seen in fish removed straight from the uterus (Figure 1). Tissue ammonia levels (not shown) were also significantly elevated. Tissue levels of GLN and GLU are shown in Figure 2. Immediately upon removal from the uterus, embryos had easily detectable levels of GLN, with highest levels in brain and lowest levels in liver. After SW acclimation, liver and kidney GLN were undetectable. After exposure (24 h) to elevated 1 mM Tamm SW, all tissues showed significantly elevated GLN levels. GLU levels of most tissues are elevated in SW-acclimated animals, compared to embryos acclimated to either uterine conditions or SW with high ammonia levels (Fig. 2). Plasma concentrations of GLN and GLU are also shown in Figure 1. Both GLN and GLU are detectable in plasma of SW-acclimated animals. Exposure to high ammonia <u>in utero</u> or in SW eliminated plasma GLU but significantly elevated plasma GLN.

Previous studies failed to detect glutamine in elasmobranch plasma (Boyd et al., J. Exp. Zool. 199:435-442, 1977; Leech et al., J. Exp. Zool. 207:73-80, 1979) and muscle (Boyd et al., 1977). Due to the absence of circulating GLN, and the mitochondrial location of both glutamine synthetase and CPSIII, Anderson (Biochem. J. 261:523-529, 1989) suggested that GLN synthesis is used only for urea synthesis by the liver. Chamberlin and Ballantyne (1992), using techniques that preserve this labile amino acid, did find GLN in muscle and plasma. Values we present here are comparable to those of Chamberlin and Ballantyne (1992). The highest levels of GLN we found were in tissues (liver, brain, kidney) with the highest activity of glutamine synthetase (see Chamberlin and Ballantyne, 1992).

Our data demonstrate that GLN does appear in the plasma, its level increases with ammonia challenge, and therefore GLN can be mobilized for potential use in tissues other than the site of synthesis, as suggested by Chamberlin and Ballantyne (1992). The liver is the most likely candidate for export of GLN to the plasma, since it is more massive than either the brain or kidneys (20 to 45 times wet weight, respectively) and exhibited the greatest percentage decrease (-84%) in GLU content. If GLN was simply deaminated, and the ammonia passed to CPSIII of the urea cycle with GLU regenerated, tissue levels of GLU would not be expected to decrease more than GLN increased. It is possible, however, that GLN is metabolized in the liver. GLN synthesis by the liver appears to be sufficient not only to supply that required for urea synthesis but also to export GLN for use by other tissues (Chamberlin and Ballantyne, 1992). (Supported by NSF DCB-8904429 to GAK and a Burrows-Wellcome Fellowship to RV).