GLUTAMINE SYNTHETASE AND GLUTAMINASE IN SEVERAL TISSUES FROM LATE-TERM EMBRYOS OF THE DOGFISH, *SQUALUS ACANTHIAS*

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Embryos of the ovoviviparous dogfish, *Squalus acanthias* have a gestation period of nearly 22 months. During the latter part of this period they are incubated in a uterine seawater solution with a high concentration of ammonia (Kormanik and Evans, J. Exp. Biol. 125, 173-179, 1986). A potential role for this ammonia nitrogen in urea metabolism of embryos is under investigation. Urea synthesis in elasmobranchs uses glutamine synthesized from ammonia via glutamine synthetase (Anderson, Science 208:291-293, 1980). Glutamine is also consumed in various fish tissues via glutaminase (Chamberlin et al., Am. J. Physiol. 260:R159-R166, 1991; Chamberlin and Ballantyne, J. Exp. Zool. 264:267-272, 1992). The ratio of these enzymes in various tissues can indicate the balance between glutamine synthesis and degradation, and thus indicate sites for net production of glutamine. The activities of these two enzymes were compared in several tissues of late-term pups of the dogfish, *Squalus acanthias*.

Embryos were collected from pregnant dogfish (*Squalus acanthias*) as previously described (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986) and maintained in running seawater (for ca. 24 hrs), if not used immediately in the assays. Embryos were quickly killed by cervical transection of the spine followed by immediate destruction of the brain with a scalpel. Tissues (brain, liver, white muscle, kidney and gill dissected from arches) were removed, weighed and placed into iced homogenization medium (1:9; 50 mM imidazole, pH 8.2). Tissues were homogenized for 30 sec (high speed) using a tissue homogenizer (Biospec Prod., Inc.). Tissues were immediately assayed (at 25° C.) for glutamine synthetase and glutaminase using the procedures of Chamberlin et al. (1991).

The results of these assays are reported in Table 1. Tissues levels of glutamine synthetase rank in order: brain, liver, kidney, gill and muscle. Of particular interest are the ratios of glutamine synthetase:glutaminase. The highest ratio appears in the liver and kidney, known sites for substantial glutamine synthesis. The gill ratio is just as high as that of the brain, suggesting that the gill may also be a site of net glutamine synthesis in these embryos. Muscle ratio of activity is about 1.

The ratios of glutamine synthetase:glutaminase give some indication of the ability of the tissue for net synthesis of glutamine. Glutaminase activities, when corrected for temperature of assay (10° vs 25° C. for the present assay), are comparable to those reported for the bowfin or lake char, both ammonotelic species (Chamberlin et al., 1992). Glutamine synthetase levels, however, are higher, especially for the liver (160x) and kidney (60x), which is typical for a ureotelic elasmobranch. Brain glutamine synthetase is typically high in fish, to protect this tissue from ammonia. For a more comparable elasmobranch species (*Raja erinacea*), Chamberlin and Ballantyne (1992) report the following glutamine synthetase:glutaminase ratios (liver, 12.5; kidney, 11.1; brain, 0.92; gill, no data; white muscle, 0.10). Thus these dogfish embryos appear

Table 1. Glutaminase and glutamine synthetase activity in several tissues of late-term embryos of the dogfish (*Squalus acanthias*). Activities are expressed in micromol min^{-1} g wet $mass^{-1}$, as mean ± 1 SEM, n = 7 animals. The ratio is expressed as glutamine synthetase/glutaminase activity, thus a ratio greater than one suggests net synthesis capability.

	Glutaminase	Glutamine synthetase	Ratio
Liver	0.15 ± 0.050	12.6 ± 2.3	82.8
Kidney	0.81 ± 0.120	8.7 ± 1.6	10.7
Brain	4.06 ± 0.64	17.9 ± 1.9	4.4
Gill	0.71 ± 0.137	3.1 ± 0.53	4.4
White muscle	0.19 ± 0.086	0.22 ± 0.04	1.1

to possess a greater capacity for net synthesis of glutamine in all tissues examined here, and especially for the liver and brain, compared to *Raja erinacea*. Enzyme activities of adult *S. acanthias* for liver, kidney and brain (not shown) were all lower as well, compared to embryos. Net glutamine synthesis from ammonia with subsequent export to the liver by these tissues as well as synthesis of glutamine by the liver are likely important in urea synthesis and retention by these embryos. Efficient trapping of ammonia can also help explain the net gain of total body urea seen during development (Kormanik, Lofton and O'Leary-Liu, Bull. MDIBL 31:44-46, 1992). Relatively low levels of ammonia excretion are also seen in these embryos (Kormanik, J. Exp. Biol. 144:583-587, 1989). Thus net synthesis of glutamine in the gill may be responsible for some nitrogen retention during development, since ammonia is likely far more permeable than the relatively larger, charged glutamine molecule. Several tissues of these embryonic dogfish appear poised to trap ammonia as glutamine, the nitrogen donor for urea synthesis.

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