UREA CYCLE ENZYMES IN THE ROCK GUNNEL, *PHOLIS GUNNELLUS*

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The rock gunnel (*Pholis gunnellus*) is an intertidal inhabitant of the rocky shores of the Northern Atlantic. It is found emersed under rocks, seaweed and debris at the low tide line, which may compromise its ability to excrete ammonia. Previous experiments demonstrated that emersion for 24 hours results in a doubling of blood urea levels (Kormanik and Evans, Bull. MDIBL 27:34-35, 1988) as well as a doubling in total body content of urea (Kormanik et al., unpublished results). The metabolic origin of this accumulated urea is not known. Arginase or uricolysis may be involved. Since some teleosts possess ornithine urea cycle (OUC) enzymes (Mommsen and Walsh, Science 243:72-75, 1989), an investigation was begun to see if these enzymes are present in the rock gunnel.

Animals were collected from the shores of Frenchman Bay, ME, adjacent to MDIBL, kept in running seawater (12-15° C.) and fed chopped mussels until they were used in experiments. Fish (3-7 g.) were killed by decapitation, the spine was transected and the liver or brain removed and placed into the appropriate ice-cold homogenization medium. Tissue was homogenized for seven strokes (1300 rpm) using a Potter-Elvehjem homogenizer. Five enzymes are important in the OUC: glutamine(GLN)/n-acetyl glutamate(NAG)-dependent carbamoyl phosphate synthetase (CPSIII), ornithine transcarbamylase (OTC), arginase (ARGase), argininosuccinate lyase (ASL) and argininosuccinate synthetase (ASS). Time permitted examination of only the first three, plus glutamine synthetase (GSase), since glutamine is a substrate for CPSIII. ARGase was assayed using the technique of Huggins et al., Comp. Bioch. Physiol. 28:587-602 (1969); OTCase, Wright et al., J. Exp. Biol. 198:127-135 (1995); CPSIII, Huggins et al., (1969) $\pm$ NAG and GLN; GSase, Chamberlin et al., Am. J. Physiol. 260:R159-R166 (1991). Tissue mass permitted only one or two assays (at 25° C.) per fish. Data are expressed as mean $\pm$ 1 SEM, n=6-7.

CPSIII activity was determined in the presence and absence of GLN and NAG, a positive modulator; there was no significant activity detectable above that for controls (-NAG) in the liver of *Pholis*. Liver from *Squalus acanthias* yielded CPSIII activities of 4.5-6.3 micromol citrulline g tissue^{-1} hr^{-1} in our assay, typical for elasmobranchs (Anderson, Science 208:291-293, 1980). Comparable levels have been detected in a urea-synthesizing teleost, *Opsanus beta* (Mommsen and Walsh, 1989). Arginase activity was high (327 $\pm$ 25 micromol urea min^{-1} g tissue^{-1}), comparable to that found in some elasmobranchs. Arginolysis does yield urea, but since arginine is an essential amino acid, net arginolysis for urea production doesn't make much sense. OTCase was easily detectable (2.49 $\pm$ 0.13 micromol product min^{-1} g tissue^{-1}), at levels typical for non-urea synthesizing teleosts, but 2-20 times lower than that for urea-synthesizing teleosts. GSase activity was determined for brain and liver (65.3 $\pm$ 7.9 and 5.09 $\pm$ 1.76 micromol product min^{-1} g tiss^{-1}). Activity was substantial, especially in the brain. GSase is involved in the detoxification of ammonia. These preliminary data suggest that *Pholis* lacks a complete OUC cycle, especially since no CPSIII was detectable. Urea likely arises from other catabolic pathway(s). Supported by NSF IBN 9507456 (GAK) and NSF DBI 9531348 to MDIBL (YC).