AMMONIA DISTRIBUTION BETWEEN BLOOD AND TISSUES IN LATE-TERM EMBRYOS OF THE DOGFISH, SQUALUS ACANTHIAS

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Ammonia is a highly reduced form of nitrogen excreted by many aquatic fish (Wood, in Fish Physiology, CRC Press, ed. D.H. Evans, pp. 379-425, 1993). Ammonia occurs in two forms: as NH_3, which behaves as a respiratory gas, and as NH_4^+, a charged ion. The relative concentration of each depends on the pH. Since the pK'_a of NH_4^+ is about 9.25, at physiological and environmental aquatic pH (ca. 7.5-8.0), the NH_4^+ form predominates. The distribution of ammonia across membranes depends on the membrane permeability to each form, and the electrical and chemical gradients. Wood (1993) reviews the factors that affect ammonia distribution. If $P_{NH3} >> P_{NH4+}$, then the pH gradient determines the distribution (e.g. mammals, where the ratio of intra- to extracellular ammonia is about 3). If P_{NH4+} is "significant" (see below), then the transmembrane potential dominates the steady-state distribution (Wood, 1993). Data for teleost fish suggest a much higher ratio for intra- to extracellular ammonia (e.g. approaching 35, Wright et al., J. Exp. Biol. 136, 149, 1988a; Wright et al., J. Exp. Biol. 134, 123, 1988b; Wright and Wood, Fish Physiol. Biochem. 5, 159, 1988), and a trend toward the predominance of P_{NH3} during the evolution of fish to amphibians to mammals (Wood, 1993). However, in shark gill epithelium, previous data indicate that NH_3 permeability predominates, since $P_{NH3}:P_{NH4+}$ is 1000:1 (Evans and More, J. Exp. Biol. 138, 375, 1988). We examined the distribution of ammonia between blood and tissues in late-term dogfish embryos, to determine whether dogfish more closely resemble the teleost or mammalian model.

Embryos were collected from pregnant dogfish (Squalus acanthias) as previously described (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986). Fish were acclimated to fresh seawater (15° C.), or were exposed to seawater with ammonia concentrations adjusted from 0 to 3 mM (with NH_4Cl), about the highest they could tolerate (3 of 5 died). Fish were acclimated for 24 to 48 hours. Blood ammonia reached maximum levels and stabilized by 24 hours (not shown). At the end of the experimental periods, animals were killed and blood and tissue samples taken and processed as previously described (Kormanik and Verity, Bull. MDIBL 34:92-93, 1995). Ammonia was determined on the deproteinized extracts of tissues and plasma using an enzymatic assay (Sigma 170-UV). Water content of the tissues was determined by drying samples at 70° C. to constant weight (ca. 24 hrs). Tissue ammonia concentrations were calculated using ICF Tamm = (wet Tamm - ECFV * plasma [Tamm])/ICFV, where I- and E- refer to intra- and extracellular and FV refers to fluid volume. ECFV was taken as 12.7% (Robertson, Biol. Bull. 148, 303. 1975), and ICFV = Water content - ECFV. Tamm refers to total ammonia (NH_3 + NH_4^+).

The results of the experiment are presented in Figure 1, where tissue Tamm is plotted against plasma Tamm for liver, muscle and brain. Relevant data for these curves are included in Table 1. Liver tissue exhibited the greatest slope, and the highest concentrations of Tamm. Liver tissue also had the lowest water content (24.2 ± 0.98 %, n=5) compared to brain (79.7 ± 0.88 %, n=3), muscle (70.5 ± 0.71 %, n=5) and plasma (88.8 ± 0.32 %, n=3). The latter were comparable to data for adult fish. Liver water content reported for these embryos is low, which may be attributable to the high lipid content of liver in this lecithotrophic species. Slopes ($Tamm_i/Tamm_e$) for these tissues ranged from 4.01 to 7.33. All slopes were highly correlated ($p < 0.01$).

Roos and Boron (1981; in Wood, 1993) present an equation which describes the steady-state distribution of Tamm between intra- and extracellular compartments, relating $Tamm_i/Tamm_e$ to the transmembrane potential, pH, electrochemical gradient and ratio of the permeabilities of NH_3 and NH_4^+. This equation was used to calculate P_{NH3}/P_{NH4+} ratios presented in Table 1. Assumptions included $pH_e = 7.9$, $pH_i = 7.4$, transmembrane voltage = -90mV, T = 15° C., and $pK'_a = 9.25$, which are

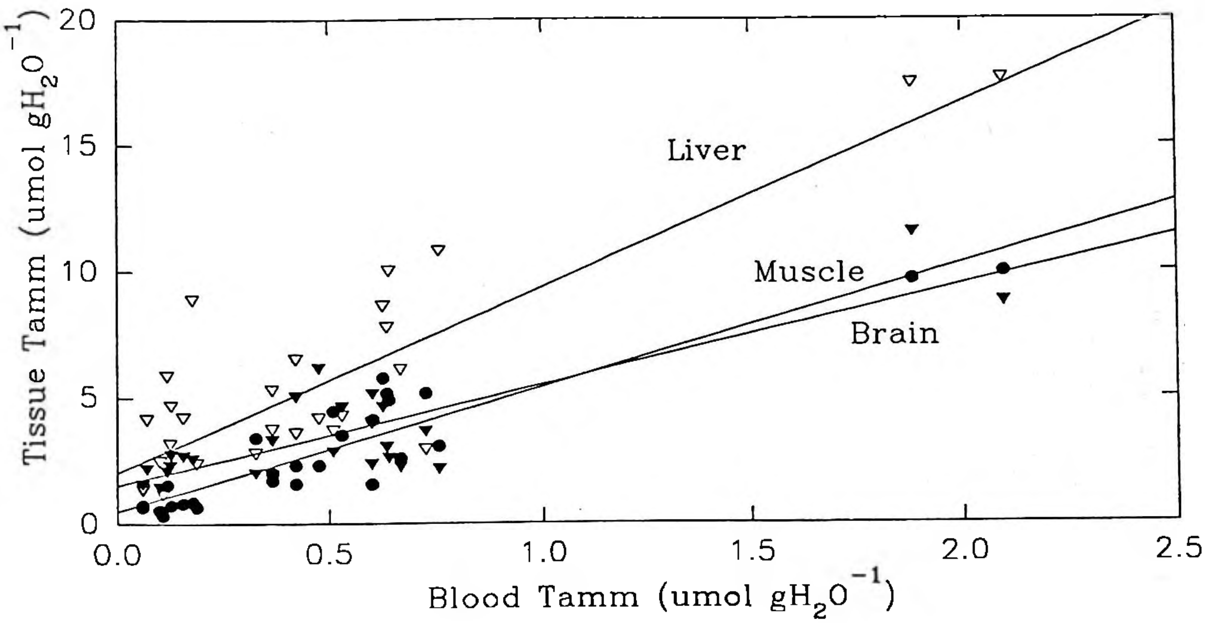

Figure 1. Tissue total ammonia (Tamm) versus plasma total ammonia (Tamm) in liver, muscle and brain of Squalus acanthias embryos acclimated to elevated ambient ammonia concentrations. Open triangles, liver; closed triangles, muscle; closed circles, brain.

Table 1. Relationship between $Tamm_i/Tamm_e$ and the permeability of NH_3 and NH_4^+. Correlation coefficients (r) and significance (p) are presented for the data in Figure 1.

Tissue	$Tamm_i/Tamm_e$	P_{NH3}/P_{NH4+}	n (pairs)	r	p <
Brain	4.01	242	27	0.738	0.01
Muscle	4.90	121	30	0.923	0.01
Liver	7.33	48	30	0.846	0.01

reasonable values for these fish. When P_{NH3}/P_{NH4+} approaches 0.1, $Tamm_i/Tamm_e$ asymptotically approaches 35. When P_{NH3}/P_{NH4+} approaches 100, $Tamm_i/Tamm_e$ asymptotically approaches 3 (Wood, 1993). In elasmobranch brain, muscle and liver, P_{NH3}/P_{NH4+} ranges from 242 to 48, indicating that the permeability of NH_3 predominates in the distribution of ammonia in these tissues.

These data contrast with those of Wright et al. 1988a; 1988b and Wright and Wood, 1988) who report $Tamm_i/Tamm_e$ ratios of 30-35 in white muscle at rest for lemon sole and trout. These $Tamm_i/Tamm_e$ ratios correspond to P_{NH3}/P_{NH4+} ratios of 1.7-1. Thus for Tamm to be distributed according to transmembrane potential, rather than to transmembrane pH, P_{NH4+} must be "significant", but not necessarily larger than P_{NH3} (Wood, 1993). Our data, however, indicate that the permeability to NH_3 predominates in these elasmobranch tissues, and dogfish resemble the mammalian model, with ammonia distributed according to transmembrane pH. Liver had the highest $Tamm_i/Tamm_e$ ratio, indicating a relatively higher permeability to NH_4^+. This higher Tamm content in liver tissue may be indicative of ammonia "trapping", resulting from a lower P_{NH3}/P_{NH4+} ratio (Table 1) that would facilitate movement of blood ammonia nitrogen into the liver for urea synthesis. (Supported by NSF IBN-9507456 to GAK; NSF REU 9322221 for CH).