NITROGEN BUDGET IN DEVELOPING ELASMOBRANCH EMBRYOS

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We have shown that late-term embryos of the dogfish Squalus acanthias are retained in a uterine environment relatively high in ammonia, and tentatively suggested that this ammonia may act as a nitrogen source for the developing embryos (Kormanik & Evans, J. Exp. Biol. 125;173-179, 1986). A subsequent investigation showed that total nitrogen declines by about 16% when stage 'A' embryos are compared to stage 'C' embryos (Kormanik, J. Exp. Biol. 144;583-587, 1989). While this decline is significant, the intriguing aspect is that the decline in nitrogen is not as great as the total decline in dry weight (ca. 40%, Hisaw & Albert, Biol. Bull. 92;187-199, 1947). This rather modest loss of nitrogen may in fact represent some nitrogen transfer from mother to embryo. To extend these previous observations, we investigated the nitrogen budget in an oviparous species, Raja erinacea, where there is no maternal contribution.

Dogfish (Squalus acanthias) and skate (Raja erinacea) embryos were collected as previously described (Kormanik & Evans, ibid.; Kormanik et al., this issue). Eggs were opened, the egg cell (= yolk) and jelly were separated, preserved with sulfuric acid (final conc. 10%) and frozen. Remaining eggs were labelled, kept in sea water and returned to University of North Carolina at Asheville where they were incubated at about 25° C. until hatching (ca. 7 mo.). Hatchlings were killed by decapitation and homogenized. Total nitrogen (N_{tot}) was determined in embryos and egg contents using a Hach Digesdahl, according to standard procedures (Hach).

In another series of experiments we determined the egg and embryo content of urea and trimethylamine oxide (TMAO), the major nitrogenous osmolytes in the elasmobranchs. Fresh eggs were opened, portions of the yolks were homogenized on ice with distilled H_2O (1:9), precipitated with trichloroacetic acid (final conc. 3.5%) and centrifuged. Embryos were killed by decapitation, homogenized on ice and otherwise treated as the egg homogenates. Samples were analyzed for urea (Sigma Kit # 535). TMAO was assayed using a procedure modified from Forster et al. (J. Gen. Physiol. 42:2;319-327, 1958). Samples of the yolk were dried to constant weight at 60° C. to determine water content. Results are expressed as mean ± 1 S.E.M.

The results of the N_{tot} determinations are presented in Table 1. In preliminary experiments, we found that about 98% of the N_{tot} was in the egg cell; little was found in the jelly. The sum of both components is included in Table 1. N_{tot} decreased by nearly 40%. The loss of nitrogen in this oviparous species is more than twice as great as that observed for S. acanthias. N_{tot} per unit wet weight decreased as the embryos increased in weight.

The results of the urea and TMAO determinations are presented in Table 2. Urea and TMAO concentrations in the egg cells of the skate are higher than those of adult plasma (340 and 50 mM, respectively; this investigation). The distribution and concentrations are similar to those seen in muscle cells of this species (Forster & Goldstein, Am. J. Physiol.

Table 1. Nitrogen in eggs and hatchlings of the skate, Raja erinacea.

	wet wt. (g)	N_{tot} (mg)	N_{tot}/g (mg/g wet wt)
Egg (n = 10-11)	4.32 ± 0.29	283 ± 17	66.0 ± 1.5
Hatchlings (n = 5)	5.97 ± 0.49	171 ± 14	26.9 ± 4.6
Difference (Hatchlings/eggs)	138%	60.4%	40.8%
Signif. (p <) (2-tail)	0.01	0.001	0.001

Table 2. Urea and TMAO in egg cells of the skate, Raja erinacea (n = 5-6).

	Urea	TMAO	H_2O (in %)
Amount [umol*g^{-1}]	230 ± 10	96.4 ± 2.7	57.8 ± 0.7
Concentration [mmol*(kg H_2O^{-1})]	398 ± 21	167. ± 5	
% egg N_{tot}	9.8	2.0	

230(4):925-931, 1976). The amount of nitrogen as urea and TMAO in the eggs represents less than 12% of the total nitrogen stores.

In both species, losses of urea, ammonia and TMAO via the gills and kidney probably represent the major routes of nitrogen loss. Rates of ammonia and urea excretion are similar in these two species (Evans & Kormanik, J. Exp. Biol. 119:375-380, 1985; Payan et al., Am. J. Physiol. 224;2,367-372, 1973). Certainly urea (Mommsen and Walsh, Science 243;72-75, 1989) and possibly TMAO (Read, Biol. Bull. 135:3;537-547, 1968) are synthesized to replace that lost by excretion and diffusion. However, Goldstein et al. (Comp. Biochem. Physiol. 21;719-722, 1967) were unable to demonstrate TMAO synthesis in S. acanthias. We examined the changes that occur in these pools in S. acanthias, since R. erinacea hatchlings were unavailable for this series of experiments. The results are presented in Table 3.

Urea and TMAO contents of early-term S. acanthias embryos are similar to those of R. erinacea. Since eggs and embryos vary in size (see below), and we are unable to track specific eggs as they develop, the best we can do is to compare the ranges, and assume the smallest or largest eggs give rise to the smallest and largest of embryos. As the embryos develop, then, the wet weight increases, total TMAO decreases, and total urea increases. Total nitrogen, treated in the same manner, decreases only 3 to 14 %. Since the uterine environment for these late-term dogfish embryos contains little urea

Table 3. Urea and TMAO content in early and late-term embryos of the dogfish, Squalus acanthias. Early-term and late-term are stages 'A' and 'C' of Hisaw & Albert (ibid.), about one year apart in development.

Content	wet wt. (g)	TMAO	Urea	N_{tot}[1]
		(umol g^{-1})		
early-term	30.6 ± 1.8 (18)	110 ± 8 (3)	202 ± 3 (17)	3450
late-term	39.2 ± 2.8 (6)	67.8 ± 1.4 (6)	272 ± 19 (5)	2400

Total Content	wt. range[2] (g)	TMAO range	Urea range	N_{tot} range
		(mmol)		
early-term	21 - 43	2.3 - 4.7	4.2 - 8.6	72 - 148
late-term	26 - 60	1.8 - 4.1	7.1 - 16.3	62 - 144
(late-/ early-term)	(124 - 140%)	(78 - 87%)	(169 - 189%)	(86 - 97%)

1 - N_{tot} from Kormanik, J. Exp. Biol. 144;583-587, 1989.
2 - from Kormanik et al., unpubl. and Hisaw and Albert (ibid.)

or TMAO, these data demonstrate that urea is synthesized from nitrogen stores (endogenous or exogenous) as the embryo develops, but TMAO, stored in the egg, declines and is not replaced by synthesis (Goldstein et al., ibid.).

These data show that embryos of the skate, R. erinacea, an oviparous species, lose more nitrogen during development than do embryos of the primitively viviparous dogfish, S. acanthias. This piece of evidence, albeit indirect, supports a role for uterine ammonia in the nitrogen budget of S. acanthias. The dogfish embryo must synthesize urea, since the urea pool increases through development, as the pool of total nitrogen decreases. Dogfish embryos lose 23 mmol N in about 1 year (Kormanik, ibid.), while the urea pool increases by 4.5 mmol (from Table 3). If all of this N is considered as urea (16 mmol), embryos need to synthesize at most about 2 umol urea $embryo^{-1}$ $hour^{-1}$ to account for the urea accumulated and/or lost by diffusion, a number well within the synthetic capabilities of elasmobranch tissues (Anderson, Science 208;291-293, 1980; Mommsen and Walsh, ibid.). Glutamine is the donor of $-NH_2$ groups to CPS III of the ornithine-urea cycle, which may also accept ammonia albeit at a lower rate (Anderson, ibid.; Mommsen and Walsh, ibid.). Blood ammonia in late-term uterine incubated embryos is 3-4 fold higher than fish in fresh sea water (Kormanik, J. Exp. Biol. 137;453-456, 1988). The potential effect of this elevated ammonia on urea or glutamine synthesis should be considered. (Supported by NSF DCB-8904429 to GAK and a Hearst Foundation, Inc. scholarship to NOL).