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SHORT COMMUNICATION

NITROGEN BUDGET IN DEVELOPING EMBRYOS OF THE SPINY DOGFISH *SQUALUS ACANTHIAS*

BY GREGG A. KORMANIK

Department of Biology, University of North Carolina at Asheville, Asheville, NC 28814, USA and *Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672, USA*

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Embryos of the dogfish *Squalus acanthias* (L.) complete their development in a uterine environment that is quite unusual. During the latter portion of a gestation period which lasts nearly 2 years (Hisaw & Albert, 1947), the embryos are bathed by a uterine solution that resembles sea water with respect to the major ions, but the ammonia concentration may approach 22 mmol l^{-1} and the pH is about 6 (Kormanik & Evans, 1986).

The acidic uterine environment serves to protect the embryos from this potentially toxic high ammonia concentration by converting it to the relatively less permeable form, NH_4^+, and it also serves to promote ammonia accumulation in the uterine fluids. The primary source of this ammonia is the mother (Kormanik, 1988). Why, then, are the embryos exposed to such a high concentration of ammonia during the course of development? Nutrition in this species is considered to be lecithotrophic: nutrients are provided by the yolk stored in the yolk sac (see Wourms *et al.* 1988). Kormanik (1988) has suggested that uterine ammonia might act as a nitrogen source for the embryos developing *in utero*. This aspect of embryo nutrition was examined by measuring the total nitrogen in pups and yolks at different stages during the 2-year period of development. In addition, rates of ammonia and urea excretion were measured. In this way it is possible to describe and quantify the nitrogen budget of these developing embryos.

Embryos (= yolk+pup) of the spiny dogfish were collected from females that had been killed at different stages of the 2-year gestation period. Females were taken from Frenchman Bay at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, during the summers of 1985 to 1988.

For the total Kjeldahl nitrogen determinations, embryos were anesthetized on ice (early-term) and also by spinal transection (late-term). Yolk sacs were ligated with thread and then separated from the pups. Pups and yolks were weighed separately. The tissues were frozen, and returned to UNCA for subsequent analysis.

Pup and yolk samples were homogenized separately, and diluted to volume with

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distilled water. Sulfuric acid was added (5 % vol/vol) to preserve the samples. Total nitrogen in portions of these samples (containing about 1–2 mg total N) was determined after the methods of McKenzie & Wallace (1954), using a micro-Kjeldahl apparatus (Labconco). Digested samples were alkalinized and the ammonia contained in each was distilled into a sulfuric acid solution using a Kemmerer–Hallet distillation apparatus (Fisher Scientific). Ammonia in the distillates was determined using the phenolhypochlorite assay (Solorzano, 1969). Tryptophan standards (McKenzie & Wallace, 1954) were run concurrently with the unknowns, with complete recovery of nitrogen (97·8 ± 3·0 %; $N = 19$).

Total nitrogen excretion in embryos at different stages of development was determined as the sum of ammonia and urea excretion. Early-term embryos (stage A, pup length 1–1·5 cm; mass 0·1–0·2 g; age about 3–6 months) were collected in 'candles' (three to four embryos per candle) and allowed to acclimate to running sea water for 24 h to wash away any residual urea or ammonia remaining from the uterine fluids (Kormanik & Evans, 1986). The fragility of the yolks precluded separation of the embryos; ammonia and urea excretion rates were therefore determined on embryos residing in the intact candles. Candles were placed into 1-l plastic aquaria in 0·7 l of sterilized (by boiling) and aerated fresh sea water, and samples were removed for ammonia and urea analyses at hourly intervals over a 5-h period.

Late-term embryos (stage C, pup length 19·5 cm; embryo mass 55–75 g; age about 15–18 months) were collected as described above. Embryos were acclimated to fresh sea water for 1 h to wash away any residual ammonia or urea (Kormanik & Evans, 1986) and then transferred to 1-l plastic aquaria filled with 0·5 l of sterilized and aerated fresh sea water. Samples for the ammonia and urea assays were removed at hourly intervals over a 5-h period. Ammonia was analyzed using the phenolhypochlorite method (Solorzano, 1969), and urea by the diacetal monoxime method (Sigma kit no. 535-B). Embryos were weighed at the end of the experiments. Seawater temperature during the excretion experiments was 15 ± 1 °C.

All data are presented as mean ± standard error. Statistical comparisons noted below were made using Student's *t*-test, one-tailed, for unpaired data.

The results of the total Kjeldahl nitrogen recovered by digestion of the pup and yolk samples are reported in Table 1. The total nitrogen content of the pups increases during maturation and that of the yolks decreases. Thus nitrogen moves from the yolk stores into the pups as development proceeds and the pups increase in size. More instructive are the data for the sum of nitrogen in the pup and yolk pairs. The sum of total embryo nitrogen shows a 15 % decrease ($P < 0·001$) in the early-term *versus* late-term embryos. These data indicate that the nitrogen found in the yolk stores of the early-term embryos is more than sufficient to account for the amounts found in the late-term embryos.

Embryos can be expected to lose nitrogen *via* the gut, kidney and especially the gills, by the excretion of ammonia and urea. An estimate of this rate of loss was determined by the measurement of urea and ammonia excretion in these early

Table 1. *Pup mass, yolk mass and total Kjeldahl nitrogen at two stages of development in embryos of the dogfish* Squalus acanthias

	Early-term (stage A)	Late-term (stage C)
Mass (g)		
Pup	0·114 ± 0·011	41·32 ± 5·86
Yolk	47·44 ± 3·21	16·46 ± 1·13
Total nitrogen (mg)		
Pup	2·19 ± 0·036	1210 ± 140*
Yolk	2300 ± 130	730 ± 73*
Pup+yolk	2300 ± 40	1940 ± 130*

$P < 0·001$.
Data are presented as mean ± S.E., $N = 4–5$.
Statistical comparisons are made with the previous term.

Table 2. *Ammonia, urea and total nitrogen excretion at two stages of development of embryos of the dogfish* Squalus acanthias

	Ammonia (μmol 100 g^{-1} h^{-1})	Urea (μmol 100 g^{-1} h^{-1})	Total nitrogen (μg N 100 g^{-1} h^{-1})
Early-term (10)	0·038 ± 0·025	1·22 ± 0·48	34·6 ± 13·2
Late-term (15)	0·51 ± 0·16*	3·30 ± 0·21**	99·4 ± 7·1**

* $0·05 > P > 0·02$; ** $P < 0·001$.
Total nitrogen is the sum of ammonia and urea.
N, number of embryos.
Statistical comparisons were made with the previous term.

term and late-term embryos (Table 2). In the early-term embryos, urea represents over 98% of the total nitrogen excreted. In late-term embryos, ammonia excretion plays a greater role, but urea still accounts for 93% of the nitrogen excreted. Urea and ammonia excretion rates as well as the total nitrogen excretion rate are several-fold higher in late-term embryos than in early-term embryos. These data indicate that both early- and late-term embryos lose nitrogen when they are exposed to fresh sea water, which would occur when the uterine horns are flushed with sea water (Burger, 1967), and probably in uterine sea water as well, since the NH_3 and urea gradients are similar (Kormanik & Evans, 1986; Kormanik, 1988).

Squalus yolk sacs contain about 4·8% nitrogen by weight. This nitrogen passes to the pup and, at late-term the pup contains about 2·9% nitrogen by weight. This value is similar to total nitrogen amounts of 2·8–5·3% reported for adult *Squalus* and other elasmobranchs (see Vinogradov, 1953). These total Kjeldahl nitrogen

determinations represent the net changes that occur in embryo nitrogen under uterine conditions. A more intriguing question to ask, however, is how much nitrogen would be lost from these embryos if they completed their development in a solution lacking the high ammonia concentrations (i.e. in fresh sea water)? The amount of nitrogen excreted during the incubation period of embryo development was estimated in the following manner. Assuming, as a first approximation, that nitrogen excretion increases in a linear manner, then nitrogen excretion over a 1-year period (the difference in age between early- and late-term embryos, data from Table 1) is estimated as:

$$[(\text{early-term rate}) + 0{\cdot}5 \times (\text{late-term rate} - \text{early-term rate})] \times 1\,\text{year}\,.$$

This amounts to a total nitrogen excretion rate of 330 ± 40 mg N embryo^{-1} year^{-1} ($N = 10$), which is not significantly different ($P > 0{\cdot}1$) from the decrease in total Kjeldahl nitrogen found, on average, between these early- and late-term embryos (−360 mg, Table 1). Thus nitrogen excretion can account for all the nitrogen lost from developing embryos, based on net changes in total Kjeldahl nitrogen. It would appear that the uterine ammonia is not important to the nitrogen budget of these developing embryos; that is, there is no net influx of nitrogen from this high ammonia uterine environment. This latter observation fits the data on gradients for ammonia (as NH_3) and urea in developing embryos *in utero*, where both gradients are directed from embryo blood to the uterine sea water and would therefore favor nitrogen loss by diffusion (Kormanik & Evans, 1986). Thus, all the nitrogen that these embryos apparently require is invested in the egg before development proceeds.

However, two cautionary notes are in order. First, embryos in this investigation excrete both ammonia and urea at relatively low rates, and the estimates of nitrogen loss presented here are based on these low rates. Adult elasmobranchs exhibit urea effluxes of 20–60 μmol 100 g^{-1} h^{-1} (Evans, 1979). Evans & Kormanik (1985) presented resting rates of urea excretion by intact, late-term pups (= embryos) in sea water which may be up to 10-fold higher (i.e. 25 μmol 100 g^{-1} h^{-1}) than those presented here. A several-fold increase in urea excretion rates would increase nitrogen loss several-fold and would, therefore, represent a more substantial loss compared to the amount of nitrogen available in the yolk stores. Therefore, the estimates of nitrogen loss presented here may be rather conservative, at least for urea, the major form of excreted nitrogen in these embryos. Second, matrotrophy or lecithotrophy in these species was traditionally determined by comparing the organic content or dry weight of an egg and that of the full-term embryo (Gray, 1928). Although a net loss in weight of 25–35 % may indicate no nutrient transfer (*Squalus acanthias* loses 40 % of dry weight; Hisaw & Albert, 1947), a slight loss of 5–10 % could indicate nutrient transfer from the mother to the embryo (see Wourms *et al.* 1988, for a discussion). Thus, with respect to nitrogen, the loss observed in the present investigation of 16 % is intermediate between these values. The most promising answer to this question of nitrogen utilization lies in the examination of nitrogen budgets in embryos of

oviparous species, where maternal nutrient transfer is entirely eliminated after the eggs are laid. This aspect is currently under investigation.

In conclusion, in spite of the fact that late-term embryos of the dogfish *Squalus acanthias* reside in a high concentration of ammonia in the uterine environment, there is no need to suggest that this ammonia acts to augment embryo nitrogen. All the nitrogen required by the embryo during development comes from the yolk stores. Embryos lose some nitrogen during development by excretion of ammonia and urea, but very low excretion rates minimize this loss. Thus, at least with respect to nitrogen, the maternal contribution to embryonic nutrition appears to be completed before growth and development of the embryo begins.

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