Arginase activity of *Bufo arenarum* embryos is sensitive to external osmotic pressure

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We investigated the impact of environmental osmotic stress on the arginase activity in ***Bufo arenarum*** *embryos. The activity at the first developmental stages was not sensitive to extreme variations of osmotic pressure of the environment. Later, at gill circulation, opercular fold and right operculum stages, the enzyme activity of embryos developed in concentrated solutions decreased significantly with respect to control. At complete operculum stage, the arginase activity increased 1.3-2.5 fold in all conditions, and was significantly higher in embryos grown in distilled water than in control animals.*

Key terms: *anuran embryos,* ***Bufo arenarum****, embryonic arginase, osmotic stress.*

Among vertebrates, Amphibia is a transition class in the colonization of land. At present, all of them remain dependent on free water availability to a greater or lesser degree (Duellman & Trueb, 1986; Shoemaker *et al*, 1992). The possibility of a relationship between water availability and the nature and pattern of the excreted nitrogen metabolism end products, has stimulated much work on amphibians, that have to withstand, at some stage of their life cycle, changes in the accessibility of water.

Adaptive responses of organisms to changes in their environment can occur at several organization levels (Hochachka & Somero, 1984). At the molecular level, changes can be considered in the function of the enzyme systems. Thus, the knowledge of the ontogenetic changes in the function of the urea cycle associated with water-land transition appears to be particularly important to the study of the biochemical adaptation of anurans to habitats characterized by different water availability.

It is well known that arginase (L-arginine ureohydrolase; EC 3.5.3.1) is a prominent enzyme in the liver and kidney of ureotelic amphibians; we have used it as an indicator of the urea cycle function. In a previous paper (Rovedatti *et al*, 1995), we studied the evolution of the arginase specific activity during the embryonic development of *Bufo arenarum* in standard conditions, observing a gradual increase in the enzyme activity along the developmental stages. Similar findings have been recently reported by Callery and Elinson (1996) in embryos of *Eleutherodactylus coqui*, an species that does not metamorphose.

In this study we have extended our research investigating how the environmental osmotic stress affects the activity of arginase. We chose some representative stages in order to compare the impact of incubation in different media on the enzyme activity. The capacity of *Bufo arenarum* embryos to develop in distilled water has been shown in our laboratory (Castañé *et al*, 1987).

Bufo arenarum embryos were obtained by fertilization *in vitro*, as described previously (Castañé *et al*, 1987).

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From fertilization on, embryos were allowed to develop in glass Petri dishes containing three incubation media: one group was developed in 10% Holtfreter's solution (control), a second group was placed in distilled water and the third group was incubated in a concentrated Holtfreter's solution (115 mOsm). The solutions were replaced daily. Throughout experiments, dishes were kept in a Ghilon TC 120 incubation chamber with the temperature set constant at 20 ± 1 °C and 12 h light-dark cycle. The experiments were carried out irrespective of the season. Developmental stages were determined according to the tables of Del Conte and Sirlin (1951).

Groups of 150-300 embryos morphologically normal of each selected stage were homogenized on ice and the arginase activity and protein content were determined following the already described techniques (Rovedatti *et al*; 1995). Enzymatic activity was expressed as nmoles urea/min/mg protein. All measurements were carried out in duplicate or triplicate. Chemicals used were of analytical grade. Results are presented in Table I.

Normality in the distribution of the values was checked by Kolmogorov-Smirnov test. Enzyme data were transformed as 1000 x log specific activity. Standard least-squares linear regression analysis of arginase specific activity on time (hours post fertilization) was done. Analysis of covariance for the regression slopes was carried out (Draper & Smith, 1981). For each stage, comparisons between the three developmental conditions were performed using one way analysis of variance (ANOVA), and when significant variation was found, pairwise comparisons of means were carried out using the Scheffé procedure (Zar, 1996). For all tests, the significance level was $p < 0.05$.

Table I shows that arginase specific activity is not sensitive to extreme variations of osmotic pressure of the environment at the early stages of development. However, the activity of the group developed in concentrated solution decreased significantly with respect to control in gill circulation, opercular fold

Table I

Arginase specific activity of embryos of *Bufo arenarum* developed in media of different osmolarity (nmol urea • min^{-1} • mg $protein^{-1}$). In parentheses, number of assayed homogenates; means ± SEMs.

Stage	Incubation media		
	Control (11.5 mOsm)	Concentrated Holtfreter (115 mOsm)	Distilled water
NF to gastrula	ND (28)	NM	NM
Tail bud	1.2 ± 0.2 (28)	1.0 ± 0.1 (3)	1.5 ± 0.1 (5)
Muscular response	2.0 ± 0.4 (10)	1.4 ± 0.4 (4)	1.2 ± 0.2 (4)
Gill circulation	3.9 ± 0.6 (6)	1.5 ± 0.2 (3)	2.6 ± 0.3 (5)
Opercular fold	5.7 ± 1.8 (3)	1.8 ± 0.1 (3) a	2.3 ± 0.4 (3)
Right operculum	6.8 ± 0.7 (9)	3.0 ± 0.8 (3) a	4.7 ± 1.2 (3)
Complete operculum	8.5 ± 1.5 (12)	7.6 ± 2.3 (2) b	11.9 ± 2.0 (6) a,b

NF: non fertilized oocytes; ND: not detectable; NM: not measured.
a: Significant differences with respect to control at $p < 0.05$.
b: Significant differences with respect to the above stage of the same group.

and right operculum stages. The beginning of this reduction corresponds to the moment at which the gills are exposed to the external medium and epithelial ion exchange processes are established. Thus, the Na^+/NH_4^+ exchange could be a mechanism complementary to the urea cycle for the elimination of ammonia.

The interpretation of the lower specific activity found in embryos of concentrated Holtfreter group is not easy. It is not known if embryos have the capacity to overcome external increases in osmotic pressure by means of urea accumulation in their body fluids, as it has been shown in adults of other species (Balinsky, 1981; Shoemaker & McClanahan, 1982; Shoemaker *et al*, 1992).

We may suggest that embryos developed in concentrated Holtfreter (a condition simulating reduced water availability) may be induced to change their catabolism of nitrogen products towards the uric acid synthesis pathway, favored by an increase in the synthesis of nucleic acids (Bretos *et al*, 1967).

It is worthwhile mentioning that Balinsky *et al* (1976) postulated that the ability of amphibians for colonizing new habitats characterized by a shortage of environmental water requires the development of adaptive biochemical mechanisms, achieved not only through the acquisition of new enzymes, but by means of changes in the activities of pre-existing ones.

Arginase activity increased along the stages of development in all three groups. The covariance analysis for the comparison of the slopes of linear regression did not show significant differences (Table II).

In addition, the ANOVA comparing the stages of each group revealed significant differences between right operculum and complete operculum stages in the embryos grown in both concentrated Holtfreter's solution and distilled water (Table I).

In animals grown in concentrated solutions to the complete operculum stage, arginase activity reached a value similar to that found in control embryos, while the activity was significantly higher in those incubated in distilled water than in controls. This phenomenon suggests a biochemical compensation of the embryonic arginase activity when the gills are reabsorbed.

Table II

Linear regression analysis parameters for arginase activity of *Bufo arenarum* embryos developed in media of different osmolarities, and statistics of the covariance analysis for the comparisons of the slopes.

Parameters	Incubation media		
	Control	Concentrated Holtfreter	Distilled water
Slope	3.90×10^{-3}	3.75×10^{-3}	4.48×10^{-3}
Intercept	2.88	2.71	2.75
Standard error of slope	4.62×10^{-4}	6.78×10^{-4}	4.80×10^{-4}
Correlation coefficient	0.79	0.81	0.88
R-squared (%)	61.93	65.74	78.4
Standard error of estimation	0.230	0.189	0.178

F (comparison of slopes): 0.10
F table: 2.84

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