Proc. Indian Acad. Sci. (Ani. Sci.), Vol. 89, Number 1, February 1980, pp. 47–52.

Natural variation in the levels of ammonia, urea and some selected enzymes in hepatic tissue of *Rana hexadactyla*, *Rana tigrina* and *Rana cyanophlyctis*

V LAKSHMIPATHI* and K V RAMANA RAO
Department of Zoology, S V University, Tirupati 517 502
* Present address : Department of Zoology, CKM Arts and Science College, Warangal 506 006

MS received 30 April 1979; revised 20 October 1979

Abstract. Natural variations existing in the levels of ammonia, urea, succinate dehydrogenase, glutamate dehydrogenase and arginase activities in the liver tissue of three species of genus *Rana* were studied. The variations existing in the levels of these factors are discussed in the light of the habitat preferences exhibited by these three species.

Keywords. *Rana hexadactyla*; *Rana tigrina*; *Rana cyanophlyctis*; natural variations.

1. Introduction

Amphibians occupy one of the important positions in vertebrate phylogeny. They demonstrate a wide spectrum of habitat preferences, with fully aquatic species at one extreme to the fully terrestrial species at the other. The biochemical differences existing in the amphibian species, therefore, assume importance since they offer clues for the molecular mechanisms involved in the transition from aquatic to terrestrial mode of life.

The pattern of nitrogen excretion in an animal depends upon its environment (Campbell 1973) and a correlation is reported to exist between the degree of terrestriality of an animal and its dependence of ureotelism (Hochachka and Somero 1973).

The three species under investigation exhibit variations in their habitat preferences, breeding habits and distribution. Of the three species, *Rana tigrina* can tolerate the terrestrial environment more than the other two species (Daniel 1972).

Since, it has been suggested that the production of excretory ammonia and the synthesis of urea are confined to the liver in amphibians (Brown and Cohen 1960; Cohen and Brown 1960) the levels of ammonia, urea and those of selected enzymes were undertaken in this tissue to understand the possible correlation between the urea production and the habitat preference of these three species.

2. Materials and methods

Frogs, *Rana hexadactyla*, *Rana tigrina* and *Rana cyanophlyctis*, were collected from ponds around Tirupati. After acclimatisation for about a week in laboratory conditions they were killed and the tissues were removed quickly to the ice-jacketed containers for further processing.

Ammonia levels were estimated by the method described by Bergmeyer (1965), glutamine levels were estimated by the method described by Colowick and Kaplan (1967) and the urea levels were estimated by the method described by Natelson (1971). The activity levels of dehydrogenases were estimated by the modified methods of Nachlas *et al* (1960) (for SDH) and Lee and Lardy (1965) (for GDH as described by Reddanna and Govindappa (1978). Arginase activity was estimated by the method of Campbell (1961).

3. Results

3.1. *Ammonia and urea levels*

Table 1 presents the relative levels of ammonia and urea found in the liver of the three species. The levels of ammonia are high in the order of *R. cyanophlyctis* > *R. hexadactyla* > *R. tigrina*, while those of urea are high in order of *R. tigrina* > *R. hexadactyla* > *R. cyanophlyctis*. Glutamine levels are high in the order of *R. tigrina* > *R. hexadactyla* > *R. cyanophlyctis*.

The relative ratio of urea to ammonia, glutamine to ammonia and urea to glutamine are high in the order of *R. tigrina* > *R. hexadactyla* > *R. cyanophlyctis*.

Within the species, the ratio of urea to ammonia is high in *R. tigrina*, while the ratio of glutamine to ammonia is high in *R. hexadactyla* and *R. cyanophlyctis*.

3.2. *Enzyme levels*

3.2a. *Arginase* : The levels of arginase (EC 3.5.3.1) are high in the order of *R. tigrina* > *R. hexadactyla* > *R. cyanophlyctis* (table 2).

The enzyme level is highest in *R. tigrina* and the difference is significant at 0·1% level when compared to the other two species. The difference in arginase levels is not significant between *R. hexadactyla* and *R. cyanophlyctis*.

3.2b. *Glutamate dehydrogenase* : The levels of glutamate dehydrogenase (GDH) (EC 1.4.1.3) are high in the order of *R. hexadactyla* > *R. cyanophlyctis* > *R. tigrina*. It is highest in *R. hexadactyla* and the difference between the enzyme level of this species and that of *R. tigrina* and *R. cyanophlyctis* is significant at 0·1%.

3.2c. *Succinate dehydrogenase* : The levels of succinate dehydrogenase (SDH) (EC 1.3.99.1) are high in the order of *R. tigrina* > *R. hexadactyla* > *R. cyanophlyctis*. While the difference is not significant between *R. hexadactyla* and *R. tigrina* the enzyme level in *R. cyanophlyctis* differs at 1% and 2% levels from *R. hexadactyla* and *R. tigrina* respectively.

3.2d. *SDH/GDH ratio* : The relative ratio of SDH to GDH is high in the order of *R. tigrina* > *R. cyanophlyctis* > *R. hexadactyla* (table 2).

4. Discussion

Urea nitrogen and ammonia nitrogen together constitute the bulk of nitrogen excreted by amphibia. The relative amounts of the two compounds are said to vary from species to species. The relative ratios of ammonia, urea and glutamine in the three species indicate that the synthesis of urea is favoured more in *R. tigrina*. The levels of glutamine and ammonia are high in *R. hexadactyla* and *R. cyanophlyctis*. In these two species, probably, ammonia excretion is preferred. Therefore, it can be presumed that while *R. tigrina* is ureotelic, *R. hexadactyla* and *R. cyanophlyctis* are relatively ammonotelic. The generalisation that fresh water animals excrete ammonia in the form of NH_4^+ while the terrestrial animals, because of the restricted water availability, detoxify this compound to urea (Campbell 1973; Hochachka and Somero 1973) is also applicable to amphibians (Balinsky 1970). Since the production of urea is metabolically expensive aquatic species in general have low activities of ornithine-urea cycle enzymes when there is plenty of water. However, during adverse conditions, these animals increase the activities of these enzymes (Boernke 1973a, b). The initial stimulus for this increase is attributed to the reduced water turnover (Balinsky 1970).

The high urea synthesis in *R. tigrina*, which for the most part lives at the edge of water can, therefore, be attributed to the adaptation of preferred terrestrial habitat. Nevertheless, the high levels of glutamine and ammonia (table 1) observed in this species also suggest that it can excrete both ammonia and urea with a shift being more towards the urea. This is further evident by high ratio of urea to ammonia over that of glutamine to ammonia (table 1). *R. hexadactyla* and *R. cyanophlyctis*, on the other hand, being aquatic species, prefer the more economic excretory pattern by excreting ammonia. This is indicated by the high levels of ammonia, and high glutamine-to-ammonia ratio over that of urea levels (table 1).

4.1. *Urea synthesis and arginase levels*

The arginase levels (table 2) in *R. tigrina* are significantly high over those of *R. hexadactyla* and *R. cyanophlyctis*. This high level of activity also agrees with the high urea content found in liver of this species.

It has been suggested that terrestrial amphibians can increase the arginase levels under dehydration stress and this increase is attributed to have an adaptational significance in maintaining the osmotic balance and in reducing the ammonia toxicity (Boernke 1973a). The high levels of arginase in *R. tigrina* which is relatively terrestrial and is exposed to fluctuating environmental conditions can, thus, be attributed to have an adaptational value in meeting the demands of osmotic stress.

4.2. *Glutamate dehydrogenase and succinate dehydrogenase levels*

The levels of GDH and SDH presented in table 2 suggest that the level of GDH is high in *R. hexadactyla* and *R. cyanophlyctis* over that of *R. tigrina*, while the SDH levels are high in *R. tigrina* over *R. hexadactyla* and *R. cyanophlyctis*. The ratio of SDH to GDH is high in the order of *R. tigrina* > *R. cyanophlyctis* > *R. hexadactyla*.

Table 1. Levels of ammonia, urea and glutamine (μM/g wet wt of tissue)

	R. hexadactyla	*R. tigrina*	*R. cyanophlyctis*
Ammonia	3·0738 ±0·2367 (10)	2·9695 ±0·2991 (10)	3·7269 ±0·3329 (10)
Urea	4·9646 ±0·1976 (10)	7·8198 ±0·3382 (10)	2·0354 ±0·0965 (10)
Glutamine	6·521 ±0·1742 (6)	7·575 ±0·4435 (6)	3·856 ±0·2261 (6)
Urea/ammonia	1·615	2·633	0·546
Glutamine/ammonia	2·121	2·551	1·035
Urea/glutamine	0·761	1·032	0·528

(Values are mean ± S.E. No. of observations are given in parentheses.)

Test of significance

Species	P. value		
	Ammonia	Urea	Glutamine
R. hexadactyla vs. *R. tigrina*	NS	< 0·001	< 0·05
R. hexadactyla vs. *R. cyanophlyctis*	< 0·05	< 0·001	< 0·001
R. tigrina vs. *R. cyanophlyctis*	NS	< 0·001	< 0·001

NS = Not significant at 0·05 level.

The exact metabolic role of GDH is open to speculation. However, *in vivo*, this enzyme is reported to function as glutamate deaminase (Campbell 1973). Hochachka and Somero (1973) recognised two important functions of GDH—to collect the amino groups from various amino acids by the transamination and to deliver the α-ketoglutarate to the Kreb's citric acid cycle. The second function of GDH is reported to be regulated by the energy status of the cell (Hochachka and Somero 1973). With the high levels of GDH activity in *R. hexadactyla*, one would expect a greater delivery of α-ketoglutarate to the Kreb's cycle and consequently the levels of SDH to be high. The low levels of SDH-to-GDH ratio in this species, however, suggest that α-ketoglutarate instead of entering into Kreb's cycle is, probably, recycled to the transamination reactions.

In *R. cyanophlyctis* the SDH-to-GDH ratio approaches near unity. This indicates that most of the α-ketoglutarate produced by the glutamate oxidation in this species, is, probably, fed into Kreb's cycle. In *R. tigrina* the SDH level is about three times that of GDH. This indicates that the energy yielding enzymes of Kreb's cycle are more active in this species. This may be attributed to the

Table 2. Levels of arginase (μM urea/mg protein/heart), glutamate dehydrogenase and succinate dehydrogenase (μM/forma/mg protein/hr)

	R. hexadactyla	*R. tigrina*	*R. cyanophlyctis*
Arginase	35·381 ± 1·0202 (6)	177·248 8·138 (6)	31·435 ± 2·8154 (6)
GDH	0·9864 ± 0·0647 (6)	0·222 ± 0·0585 (6)	0·3864 ± 0·044 (6)
SDH	0·5454 ± 0·0377 (6)	0·7889 0·0439 (6)	0·3134 0·0286 (6)
SDH/GDH	0·553	3·5554	0·811

(Values are mean ± S.E. No. of observations are given in parentheses.)

Test of significance

Species	P. value		
	Arginase	GDH	SDH
R. hexadactyla vs. *R. tigrina*	$< 0·001$	$< 0·001$	$< 0·05$
R. hexadactyla vs. *R. cyanophlyctis*	NS	$< 0·001$	$< 0·01$
R. tigrina vs. *R. cyanophlyctis*	$< 0·001$	$< 0·05$	$< 0·001$

increased demands for the energy imposed by terrestrial habitat preference. The low levels of GDH in this species can be attributed to the non-competitive inhibition by the GTP produced during the oxidation of citric acid cycle intermediates (Hochacka and Somero 1973; Harper *et al* 1977).

5. Conclusions

The correlation between the environment and the nitrogen excretion common to most amphibians is also applicable to the three species under discussion. The aquatic species, *R. hexadactyla* and *R. cyanophlyctis* are, thus, ammoniogenic while *R. tigrina* is ureogenic. The high energy demand imposed by the terrestrial habitat preference in *R. tigrina* is probably met by the oxidation of more of citric acid cycle intermediates.

Acknowledgements

The authors thank Prof. K S Swami for facilities and encouragement and to the University Grants Commission, New Delhi, for a fellowship to the first author.

References

Balinsky J B 1970 Nitrogen metabolism in amphibians in *Comparative biochemistry of nitrogen metabolism* (ed. J W Campbell) (London : Academic Press) pp 519–637

Bergmeyer U H 1965 *Methods in enzymatic analysis* (New York : Academic Press) p. 401

Boernke W E 1973a Adaptation of amphibian arginase. I. Response to dehydration ; *Comp. Biochem. Physiol.* **B44** 647–655

Boernke W E 1973b Adaptations of amphibian arginase. II. Response to temperature ; *Comp. Biochem. Physiol.* **B44** 1035–1042

Brown G W Jr and Cohen P P 1960 Comparative biochemistry of urea synthesis 3, activities of urea cycle enzyme in various higher and lower vertebrates ; *J. Biol. Chem.* **214** 1775–1780

Campbell J W 1961 Studies on tissue arginase and ureogenesis in the elasmobranch *Mustelus canis* ; *Arch. Biochem. Biophys.* **93** 448–455

Campbell J W 1973 Nitrogen excretion in *Comparative animal physiology* (ed. C L Prosser) W B Saunders Company (Philadelphia, London, Toronto : W B Saunders) pp 279–316

Cohen P P and Brown G W Jr 1960 Ammonia metabolism and urea biosynthesis in *Comparative biochemistry* (ed. M Florkin and H S Mason) (New York : Academic Press) pp 161–244

Colowick S P and Kaplan N O 1967 in *Methods in Enzymology* (New York: Academic Press) Vol. 2 p. 63

Daniel J C 1972 Field guide to the amphibians of western India ; *J. Bombay Nat. Hist. Soc.* pp. 506–522

Harper H A, Rodwell V W, Mayer P W 1977 in *Review of physiological chemistry* (16th ed.) (California : Lange Medical Publications) pp. 337–368

Hochachka P W and Somero G N 1973 in *Strategies of biochemical adaptation* (Philadelphia : W B Saunders) pp. 144–163

Lee Y L and Lardy H A 1965 Influence of thyroid hormones on L-glycerophosphate dehydrogenase and other dehydrogenase in various organs of the rat ; *J. Biochem.* **240** 1427

Nachlas N M, Margulius S P and Seligman A M 1960 A colorimetric method for the estimation of succinate dehydrogenase ; *J. Biol. Chem.* **235** 499–503

Natelson 1971 *Techniques in clinical chemistry* (Illinois : Thomas C G Publishers) p. 146

Reddanna P and Govindappa S 1978 Effect of *in vivo* muscular stimulations. III. Some aspects of carbohydrate metabolism of cardiac tissue ; *Curr. Sci.* **47** 531–533