RHYTHMIC VARIATIONS IN HEPATIC AMMONIA METABOLISM OF TOAD, *BUFO VULGARIS*

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ABSTRACT

The activity levels of AMP-deaminase and glutamate dehydrogenase were assayed and the ammonia content was estimated over 24 h at 4 h interval, in the liver of toad, *Bufo vulgaris*. The enzyme activities exhibited rhythmicity with a synchronous increase in the ammonia content with a peak value at 20·00 h.

INTRODUCTION

THE majority of toad species is reported to be nocturnal, showing peak activity in the early part of the dark period[1]. Since the metabolic potential of the animal varies with physical activity, there could be corresponding variations in the various metabolic pathways mediated by several enzymes[2]. Hence this investigation is carried out with the primary aim to study the changes in nitrogen metabolism during the diel cycle. The present study reports the activities of hepatic AMP-deaminase, glutamate dehydrogenase (GDH) and ammonia content in toad *Bufo vulagris*. AMP-deaminase and GDH, catalyze the liberation of ammonia from AMP and glutamate respectively, and indicate the extent of operation of nitrogen metabolism in the tissue.

MATERIALS AND METHODS

Medium sized toads, *Bufo vulgaris* were collected in and around Tirupati. In the laboratory, they were maintained for one week in wooden boxes containing mud in natural conditions of 12 h light and 12 h dark phases of the day, with an ambient temperature around 25–30° C. The animals were fed with earthworms *ad libitum*. Animals of the same sex (male) and size (38–40 g) were selected and samples were taken at the following times during 24 h of the day, after starving the animal for one day before experimentation: 08·00, 12·00, 16·00, 20·00, 00·00, and 04·00 *h*. The activity levels of AMP-deaminase were estimated according to the method of Weil-Malherbe and Green[3] with modifications as suggested by Wagelin *et al.*[4]. The glutamate dehydrogenase activity was estimated following the method of Lee and Lardy[5] with modifications as suggested by Pramilamma *et al.*[6]. The ammonia content was estimated as given by Bergmeyer[7] and proteins by the method of Lowry *et al.*[8]. The activity of the AMP-deaminase was expressed as μ moles of ammonia/mg protein/h. The GDH activity was expressed in μ moles of formazan/mg protein/h and the ammonia content μ moles/gm wt. The data was analysed statistically following standard procedure[9].

RESULTS AND DISCUSSION

The results presented in Fig. 1 indicate that the activity levels of AMP-deaminase and GDH are maximal at 20·00 h and minimal at 08·00 h. The activity levels of AMP-deaminase and GDH were found to range from $0·0249 \pm 0·002$ to $0·079 \pm 0·005$ μ moles of ammonia/mg protein/h and from $0·4043 \pm 0·019$ to $1·1366 \pm 0·167$ μ moles of formazan/mg protein/h respectively. Besides this remarkable resemblance in exhibiting the maximal and minimal activity levels of these enzymes at 20·00 and 08·00 h respectively, they also showed a similar pattern of fluctuation in the rise and fall during the 24 h period. The peak activity of these enzymes at 20·00 h suggests high turnover of glutamate oxidation and deamination of adenine nucleotides, followed by a lesser turnover at 08·00 h. The Fig. 2. presents the ammonia content, which shows a similar rise and fall (with a maximal and minimal values of $3·795 \pm 0·237$ and $1·920 \pm 0·108$ μ moles of ammonia/g wt of tissue at 20·00 and 08·00 h respectively) corresponding to the activities of AMP-deaminase and GDH, indicating the direct role of these enzymes in the process of liberation of ammonia from AMP and glutamate. However, a contrast between the activities of these two enzymes shows that the reaction catalysed by the glutamate dehydrogenase is the major source of ammonia production in liver which is in accordance with the earlier reports[10].

The rhythmic variation in the activities of these enzymes, with a corresponding production of ammonia content as observed in the present investigation may have a correlation to the prey-hunting and feeding activity of the animal involving high locomotor activity. Synchronous to the elevated enzyme activities, ammonia production is also found to increase. Since, several animals are intolerant to modest concentrations of ammonia in their cellular moeity[11], the increased ammonia content in the present study at 20·00 h may

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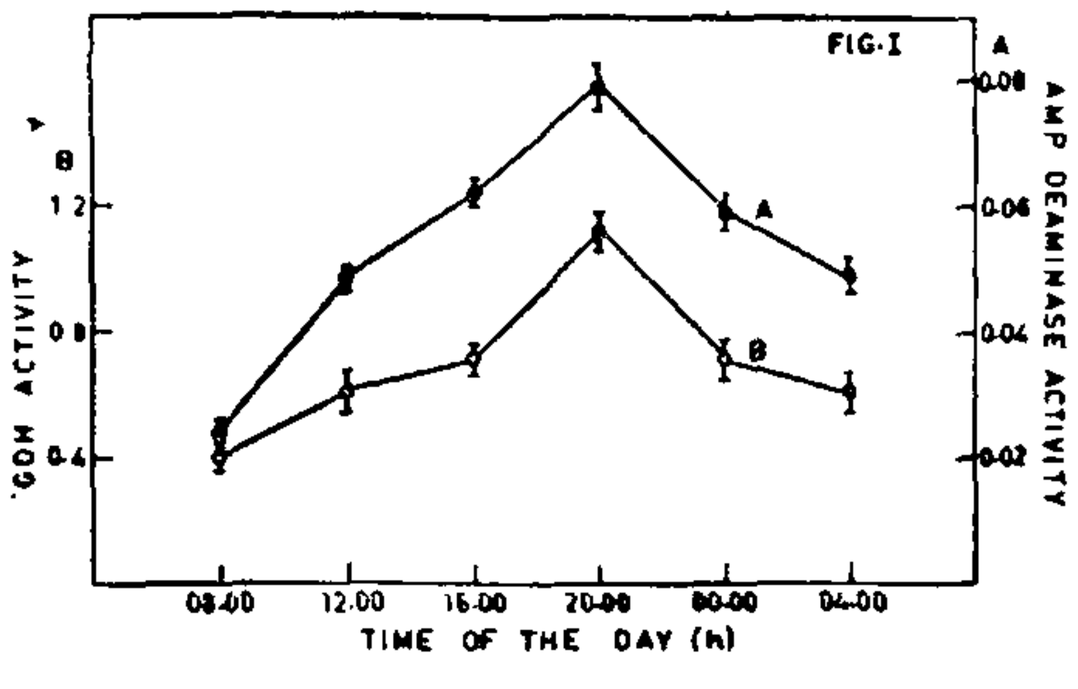

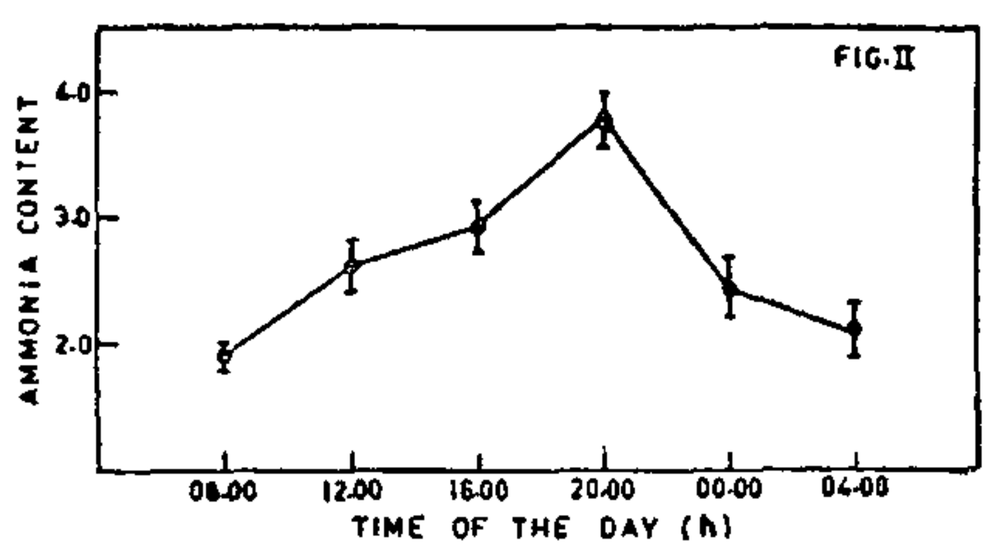

FIGS. 1–2. Fig. 1. AMP-deaminase activity (A), and Glutamate dehydrogenase activity (B) in the liver of toad, *Bufo vulgaris*, as a function of the time of day. Each point represents mean of 6 estimations (AMP-deaminase activity expressed as μ moles of ammonia/mg protein/h and GDH activity expressed as μ moles of formazan/mg protein/h). Fig. 2. Ammonia content in the liver of toad, *Bufo vulgaris*, as a function of the time of the day. Each point represents mean of 6 estimations (Ammonia content expressed as μ moles of ammonia/gm weight).

influence the general metabolism of the animal. The elevated levels of ammonia in liver, a metabolically active tissue, may retard the metabolic efficiency, leading to the decreased metabolic activity of the animal during the subsequent period of the day.

Thus, the hepatic ammonogenic enzymes of toad, AMP-deaminase, GDH and their product ammonia showed cyclic variations during the diel cycle corresponding to the general activity of the animal.

1. Higginbotham, A. C., *Ecology*, 1939, **20**, 58.
2. Gearluce, G., *Biological Rhythms in Human and Animal Physiology*, Dover Publications, Inc., New York, 1971.
3. Weil-Malherbe, H. and Green, R. H., *Biochem. J.*, 1955, **61**, 218.
4. Wagelin, I., Manzoli, P. A. and Fane, G., *Comp. Biochem. Physiol.*, 1978, **59**, 55.
5. Lee, Y. L. and Lardy, H A., *J. Biol. Chem.*, 1957, **240**, 1427.
6. Pramilamma, Y., Kesava Rao, K. V. and Swami, K. S., *Ind. J. Exp. Biol.*, 1975, **13**, 177.
7. Bergmeyer, H. A., *Methods of Enzymatic Analysis*, Academic Press, New York, 1965.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
9. Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd Ltd., Edinburgh, 1970.
10. Tarver, H., *The Liver*, Vol. I, Academic Press, New York, 1963.
11. Lowenstein, J. M., *Physiol. Rev.*, 1972, **52**, 382.

RESEARCH AWARDS

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