that the A23187-dependent K<sup>+</sup> conductance seen in the present studies is 2 orders of magnitude less than that encountered in mitochondria strongly supports our previous contention that it is the removal of divalent cations from the matrix by the ionophore which activates an endogenous  $K^+/H^+$  exchanger<sup>2-4, 10</sup>. For most of the studies in which A23187 was used to activate mitochondrial K<sup>+</sup>/H<sup>+</sup> exchange, less than 1 nmole A23187 was added per mg protein<sup>3,4,15</sup>. This corresponds to about 4 nmoles mg<sup>-1</sup> mitochondrial phospholipid and is therefore well below the

- threshold for direct K+ transport found in the present study. The chances of A23187 contributing directly to Na<sup>+</sup>/ H<sup>+</sup> exchange in biological membranes are considerably greater than those for K<sup>+</sup>/H<sup>+</sup> exchange. The transport of Ca<sup>2+</sup> on A23187 is inhibited by Na<sup>+16</sup> and it has been reported recently<sup>17</sup> that A23187 can transfer both Na<sup>+</sup> and K<sup>+</sup> across the chloroplast envelope, but only Na<sup>+</sup> through thylakoid membranes. The present studies suggest that such a discrepancy could be explained by the presence of an endogenous  $K^+$  exchanger in one of the membranes.
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## Changes in activity levels of AMP deaminase and adenosine deaminase in the Indian apple snail, Pila globosa (Swainson) during starvation and aestivation stress

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Summary. Activity levels of AMP deaminase and adenosine deaminase have been studied in hepatopancreas, foot and mantle tissues of Pila globosa with reference to starvation and aestivation. The activity levels of both enzymes were decreased in all the tissues of aestivating snails while in starved animals AMP deaminase activity was increased, in contrast to the decreased adenosine deaminase activity.

Aestivation is an adaptation of animals to water scarcity in the environment during which significant changes in the pattern of nitrogenous excretary products can be expected<sup>2</sup>. The successful survival of Pila globosa during aestivation is primarily due to the excretion of nitrogenous waste products in the form of uric acid<sup>3</sup>, though this snail is undoubtedly ammonotelic in its active life. Problems relating to different aspects of aestivation have received considerable attention in former years<sup>4-6</sup>. A decrease in ammonia and

urea levels and accumulation of uric acid in the tissues and body fluid during aestivation was reported<sup>7-9</sup>. In the present study the activities of AMP deaminase and adenosine deaminase, the main enzymes responsible for the production of ammonia were estimated during starvation and aestivation, in order to elucidate their role in the diversion of ammonotelism to urecotelism.

Collection and maintenance of Pila globosa and the method for introducing aestivation has been discussed

Activity levels of AMP deaminase and adenosine deaminase in selected tissues of normal, starved and aestivated Pila globosa

Sample	Tissue	AMP-deaminase			Adenosine deaminase		
		Normal	Starved	Aestivated	Normal	Starved	Aestivated
1	Hepatopancreas % change p-values	$45.3 \pm 3.21$	$51.6 \pm 3.9$ + 12.1 p < 0.05	$11.4 \pm 1.0$ - 75.3 p < 0.001	18.2 ± 1.7	$14.3 \pm 1.3$ -21.3 p<0.001	$6.8 \pm 0.9$ $-62.6$ p<0.001
2	Foot % change p-values	$55.6 \pm 3.5$	$62.3 \pm 4.7$ + 10.7 p < 0.005	$23.5 \pm 1.3$ -57.7 p<0.001	$17.3 \pm 1.3$	$15.1 \pm 1.1$ - 13.6 p < 0.005	$7.2 \pm 0.7$ - 58.4 p < 0.001
3	Mantle % change p-Values	$40.2 \pm 3.0 + 11.3$	$45.3 \pm 4.0$ + 11.3 p < 0.01	$20.9 \pm 2.1$ - 48.1 p < 0.001	13.1 ± 1.1	$   \begin{array}{r}     11.7 \pm 0.9 \\     -10.7 \\     p < 0.025   \end{array} $	$6.9 \pm 0.6$ - 47.3 p < 0.001

Values expressed in μmoles of ammonia formed per mg protein/h. p-Values indicate levels of significance. Values are means ± SD of 6 observations.

elsewhere 10. One set of animals was starved for a month and another set was aestivated for 1 year. Freshly-collected snails acclimated to laboratory conditions for a week were used as controls. The tissues viz. hepatopancreas, foot and mantle were isolated in the cold. 10% tissue homogenates were prepared in cold 0.2 M succinate Butter, centrifuged at 1000 x g for 15 min and the supernatants were used as enzyme source. AMP deaminase activity was estimated by the method of Weil-Malherbe<sup>11</sup> with slight modifications<sup>12</sup> and adenosine deaminase by the modified method of Glanti and Ginseppe<sup>13</sup>.

The activity levels of AMP deaminase and adenosine deaminase were significantly decreased in all the tissues of snails during aestivation, but during starvation the AMP deaminase activity was increased and the activity of adenosine deaminase was decreased. The changes were relatively larger in the hepatopancreas as might be expected, being the metabolic centre in these animals<sup>14</sup>. The decreased activity of the 2 enzymes during aestivation might suggest decreased ammonia production which is in good agreement with previous reports<sup>15</sup>. The decrease in AMP deaminase and adenosine deaminase might also be attributed 1. to the decreased levels of adenosine nucleotides during aestivation<sup>16</sup> and 2. to the diminished energy state of the animals<sup>17</sup>, since AMP deaminase requires ATP as an activator. The decrease in AMP deaminase during aestivation has an adaptive value for conserving precious energy which can be stored for revival of the animal when the environment turns favorable. As aestivation is a process for escaping drought, the aestivating animals either has to develop methods for detoxifying ammonia, or somehow restrict its production. There was indeed a decrease in the activity of glutamate dehydrogenase, which produces ammonia by the oxidative deamination of glutamate during aestivation8. Thus Pila globosa, which is ammonotelic during active life shifted to urecotelism during aestivation by lowering the ammoniagenesis and enhancing uric acid synthesis<sup>18</sup>. The increased activity of AMP deaminase during starvation suggests that the animal was still producing ammonia. Under these conditions its elimination is not a problem for the animal, since water is not a limiting factor during starvation.

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## Lactate dehydrogenase isozymes of two new *Xenopus* species<sup>1</sup>

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Summary. The lactate dehydrogenase (LDH) isozyme patterns of Xenopus epitropicalis and Xenopus amieti have been characterized by means of polyacrylamide gel electrophoresis and isoelectric focusing and compared with those of their closely related species. The LDH-A and B genes differ in X. epitropicalis and X. tropicalis but are the same in X. amieti and X. ruwenzoriensis.

Lactate dehydrogenase (LDH), a tetrameric enzyme, is in most vertebrates composed of the 2 subunits A and B which are coded by separate genes<sup>2,3</sup>. When both subunits are synthesized 5 isozymes are built by random aggregation. Although in amphibians various isozyme bands ranging from 2 to 25<sup>4-12</sup> have been reported, there exist in amphibians only the 2 genes which code for the A and B subunits<sup>13</sup>. Patterns of more than 5 bands are either the result of allelic polymorphism<sup>13-19</sup>, gene duplication<sup>19</sup> or the existence of secondary isozymes<sup>19</sup>. The occurrence of only 2 bands has been clarified at least in 1 case; in Triturus vulgaris they have been identified as A<sub>4</sub> and B<sub>4</sub> polymers respectively<sup>20</sup>. No heteropolymers are formed in vivo.

The genus *Xenopus* contains diploid and polyploid species<sup>21,22</sup>. Except for *X. fraseri* the electrophoretic mobility of the  $A_4$  polymer is the same for all diploid species (2n = 36 chromosomes) and X. tropicalis  $(2n = 20)^{19}$ . In contrast, the gene which codes for the B subunit differs in all but 1 case. As a result, species-specific isozyme patterns are formed. There exist simple patterns with 5 isozymes, and more complex ones as well. One main cause for a complex pattern is the occurrence of secondary isozymes.

Recently, new Xenopus species have been discovered<sup>23,24</sup>. In the present paper the LDH isozyme patterns of 2 new species are characterized.

Materials and methods. The LDH isozymes were separated