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Storage excretion in the Indian apple snail, *Pila globosa* (Swainson), during aestivation

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Uric acid accumulates in several tissues of the Indian apple snail, *P. globosa*, during aestivation. This accumulation is particularly high in the foot muscle and reproductive organs. Since the kidney, a tiny organ in the snail, has only a limited capacity to store uric acid, extrarenal tissues are used as storage depots of uric acid during aestivation. It is suggested that the aestivating snail, faced with a cleidoic situation, resorts to storage excretion, a phenomenon well documented in insects.

Uricotelism is an adaptation in the excretory nitrogen metabolism of terrestrial animals for water conservation. An extreme case of uricotelism is practiced by many insects, where uric acid, instead of being excreted, is stored either in specialized fat body cells (urocytes) or underneath the cuticle. When cockroaches are fed on a high protein diet, the fat body becomes enlarged and is filled with white deposits of uric acid. Because it is non-toxic and barely soluble in water, uric acid is deposited as urate at innocuous sites in the body so that it is sequestered from the animal's active metabolic pool and water is spared from being used for nitrogen excretion. The stored uric acid serves as a source of nitrogen for the insect during starvation and moulting. This interesting phenomenon in insects is designated as 'Storage Excretion'[1].

The Indian apple snail, *P. globosa*, aestivates in summer when the ponds, streams and paddy fields which it inhabits dry up[2]. Faced with the drought conditions, the snail retires into the deeper layers of the mud, tightly closes the shell opening with operculum and goes into a state of dormancy till the advent of rains. Totally cut off from its surroundings in the shell, the snail becomes a cleidoic animal. It is known to aestivate for three to five years under dry conditions imposed in the laboratory[3]. The snail switches over to uricotelism and stores uric acid in the kidney during aestivation[4,5]. Uric acid levels also increase in many other tissues of the aestivating snail[6]. Based on a critical appraisal of the conceptual framework of the phenomenon in insects, it is concluded here that nitrotelism in the aestivating *P. globosa* is a clear case of storage excretion.

Active snails (*P. globosa*) were maintained in an aquarium containing vegetation like *Hydrilla* on which the snails feed. Aestivation was induced in the snails by embedding them in dry sand kept in a large trough[7]. Tissues from active snails and those aestivating for two months were excised and weighed. Uric acid was extracted from tissues with 0.6% (w/v) lithium carbonate according to Cochran[8]. After centrifugation of the extracts, the supernatants were saved and the residues were re-extracted. The combined supernatants were treated with 5 volumes of 0.5 *M* $HClO_4$. The precipitated protein was removed by centrifugation and uric acid in the supernatants was determined by the method of Steel[9]. To test the reliability of the analytical method, uric acid was estimated in blood samples from four human subjects. The values determined varied between 1.9 and 3.9 mg (2.6 ± 0.7 mg)/ dl, in excellent agreement with those reported in the literature[10]. Significance of the changes in the uric acid levels during aestivation was assessed by paired comparison "t" test.

The results on the quantitative distribution of uric acid in different tissues of *P. globosa* are furnished in Table 1. The uric acid levels reported by Saxena[5] as well as Reddy and Swami[6] for active and aestivating snail tissues (converted to mg/g wt based on a tissue water content of 70%) are substantially lower than those determined here (Table 1). While freshly excised tissues were used in the present study, Saxena[5] and Reddy and Swami[6] employed snail tissues dried

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to a constant weight in an oven at 110°C for uric acid analyses. The well known decomposition of uric acid by heat (see Merck Index) may have contributed to the lower values reported by these workers.

The increase in the uric acid content in the tissues of aestivated snails (Table 1) is not due to a possible reduction in the tissue water content caused by dehydration during aestivation. The aestivating snails retain a pool of water in the mantle cavity to prevent dehydration of tissues and ensure that tissue water contents of active and aestivating snails are not appreciably different[2]. Moreover, elevation in the tissue uric acid levels of aestivating snails is noticeable even when the results are expressed on the dry weight basis[5,6]. Uric acid has also been shown to increase during aestivation in other snails, in the kidney of *Otala lactea*[11] and in the lungs as well as kidney of *Pomacea*[12].

The uric acid content increased 2 to 10-fold in the different tissues of *P. globosa* during aestivation suggesting storage of this compound (Table 1). The increase is more pronounced in tissues like foot, vagina and uterus. Further, tissues like intestine, stomach, hepatopancreas, mantle, gill, foot, vagina and uterus are larger in size and hence can accommodate larger stores of uric acid than the kidney which is a tiny organ. Thus, faced with a steady, but extremely slow, accumulation of uric acid in a cleidoic situation, the aestivating snail perhaps found the need for extrarenal sites of uric acid storage. With the various physiological activities either totally suspended or going on at a very low ebb, the digestive, locomotor, respiratory and reproductive organs served as convenient storage depots of uric acid. The storage of uric acid in the gill is consistent with the observation that aestivating *Pila* is largely anaerobic[13]. Similarly, there is a significant reduction in the activities of respiratory enzymes in the hepatopancreas and mantle of the snail during aestivation suggesting a hypometabolic state [14].

While the uric acid stored in the kidney is excreted during revival of the snail from aestivation[5,12], the fate of that in extrarenal depots is not clear. Nolfi[15] suggested that stored uric acid functions as a reserve source of carbon and nitrogen to meet the subsequent metabolic needs of the animal. To that end, there is evidence for the presence of the entire sequence of enzymes necessary for the conversion of uric acid into ammonia and carbon dioxide in a gastropod mollusc[16].

The evidence for storage excretion outside Insecta has largely been inconclusive so far. The occurrence of uric acid granules and concretions in the tissues of gastropods[12,17,18], the land crab *Cardisoma guanhumi*[19,20] and ascidians[15,21,22] prompted Campbell[23] to consider these animals as possible cases of storage excretion. Of these, aestivating *Pomacea*, like *P. globosa*, is uricotelic and resorts to storage excretion for conservation of water under drought conditions. The crab, *Cardisoma guanhumi*[19,20], though an ammonotele, encounters dehydrating conditions in its habitat and hence storage of uric acid in its tissues may well be an adaptation for terrestrial living. On the other hand, it is difficult to envisage an excretory or storage role for urate in marine gastropods[17,18] and ascidians[21,22] which are ammonotelic and always live in an abundance of water. These animals can readily eliminate ammonia without ever allowing it to build up to toxic levels and hence it appears paradoxical that they convert ammonia to uric acid at considerable metabolic cost[24]. It is entirely probable that aquatic animals which store uric acid and other purines in tissues have their own physiological compulsions, which we do not yet understand, for doing so.

Presence of uric acid or other purines in tissues at high concentrations, by itself, is not reason enough to conclude the incidence of storage excretion, unless it is linked to purinotelism and regulation of water balance by the animal. By these criteria, the accumula-

Table 1 — Uric acid levels (mg/g wet wt) in the tissues of active and aestivating snails

[Values are mean ± SD of 5 estimations. Figures in parentheses are ranges. For all the tissues except kidney, the P values were < 0.05 suggesting that the observed differences are significant]

Tissue	Active life	Aestivation	Ratio (Aestivation/ active life)
Kidney	69.1 ± 33.9 (32.9 – 109.8)	143.3 ± 102.3 (67.6 – 321.1)	2.1
Intestine	38.9 ± 12.7 (26.9 – 60.4)	119.8 ± 27.1 (89.9 – 152.3)	3.1
Stomach	35.5 ± 18.9 (24.1 – 68.7)	103.4 ± 16.5 (77.9 – 118.6)	2.9
Hepatopancreas	33.0 ± 12.0 (21.7 – 50.2)	94.0 ± 5.8 (89.6 – 104.2)	2.8
Mantle	27.2 ± 8.4 (20.7 – 41.4)	93.9 ± 12.4 (78.9 – 111.2)	3.5
Gill	22.8 ± 5.8 (17.0 – 32.0)	90.7 ± 14.9 (71.3 – 107.4)	4.0
Foot	20.1 ± 5.0 (14.2 – 26.2)	137.1 ± 52.0 (76.0 – 199.9)	6.8
Vagina	12.8 ± 2.0 (10.4 – 15.5)	64.0 ± 3.4 (58.9 – 66.7)	5.0
Uterus	6.5 ± 2.7 (4.9 – 11.3)	66.1 ± 7.1 (58.7 – 75.9)	10.2

tion of uric acid at extrarenal sites in *P. globosa* and other snails during aestivation definitely represents storage excretion. Uricotelism associated with the development of squamate and avian embryos in the cleidoic egg[25] also qualifies as a case of storage excretion. Insects therefore are not the exclusive practitioners of storage excretion in the animal kingdom. It appears that storage excretion is an inherent attribute of purinotelism.

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