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Levels of inorganic and organic constituents in the blood of the slug *Laevicaulis alte* (Ferussac 1821) in response to starvation stress

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Abstract. Elevation in the levels of the blood inorganic ions and depletion in the levels of the blood organic reserves, except TNPS, noticed during 'starvation regime' of the slug are discussed in view of their specific roles in energy metabolism.

Keywords. Inorganic constituents; organic constituents; blood; *Laevicaulis alte*; starvation.

1. Introduction

Starvation is a phenomenon of considerable ecological importance during which the animals utilize the food reserves for their very sustenance and reinforce them when normal feeding is restored. Excepting a few studies on the effects of humidity of the environment, season, aestivation, hibernation and starvation on the biochemical composition of some snails (Burton 1965, 1971 ; Matsumoto *et al* 1974 ; Colin Little 1968 ; Vijayabrahmanandam and Krishnamoorthi 1973 ; Meincke 1975 ; Chandrasekharam 1977) very little is known of the blood inorganic and organic constituents during starvation. Since variations in the inorganic and organic reserves of the blood, a transporting medium, furnish important information regarding the physiological state of the animal, it is felt worthwhile to examine the levels of inorganic and organic constituents in the blood of a garden slug, *L. alte* in relation to starvation.

2. Materials and methods

Collection and maintenance of slugs was described elsewhere (Sowjanya and Padmanabha Naidu 1979). The slugs were divided into four batches of which one served as the control and each of the remaining three was starved for one, two and three weeks respectively providing *ad libitum* water. Blood was collected into small ice-cold test tubes by making an incision with a sharp blade along the

mid-dorsal line of the body wall of the slug and centrifuged at 3000 rpm for 10 min to remove mucous and cell debris, if any. The supernatant was used for biochemical analysis.

Sodium, potassium and calcium were estimated by flame photometric method (Oser 1965) ; magnesium by the method of Hesagawa and Tohuke as given in Milton and Waters (1955) and chloride by the method of Schales and Schales (1941). The total anthrone positive substances (TAPS) representing total carbohydrate were estimated by the method of Carroll *et al* (1956), glucose by the method of Mendel *et al* (1954), total protein by the method of Lowry *et al* (1951), total ninhydrin positive substances (TNPS) by the method of Moore and Stein (1954) and total lipid by the method of Folch *et al* (1957).

3. Results and discussion

Results presented in table 1 indicate that starvation induced a stepwise increase in the blood inorganic ion pool with increase in time. A similar increase in the blood inorganic ion pool has been reported in fresh water snails (Meenakshi 1956a; Raghupatirami Reddy 1965 ; Vijayabrahmanandam and Krishnamoorthi 1973) and in the garden snail, *Cryptozona ligulata* (Krupanidhi 1978). The elevation in the inorganic ion pool in the present study may be attributed to dehydration as reported for some land snails (Burton 1971 ; Matsumoto *et al* 1974) and also to the release of different ions from the tissues into the blood in response to starvation stress. The augmented levels of calcium and magnesium upon starvation might be advantageous to the slug to develop greater oxygen affinity of the blood pigment, haemocyanin, since the divalent cations control the degree of aggregation of haemocyanin sub-units and raise the oxygen affinity facilitating the dissociation of oxyhaemocyanin at much lower oxygen tensions (Chantler *et al* 1973). The higher levels of magnesium in the blood of the starved slug may bring about a reduction in its metabolic rate as in aestivation (Meenakshi 1956b ; Vijayabrahmanandam and Krishnamoorthi 1973) so as to conserve energy yielding nutrients for longer periods.

In general the concentration of inorganic ions in the blood of the terrestrial snails and slugs is higher when compared to the freshwater gastropods. Though the garden slug, *L. alte*, is a terrestrial form it contains low amounts of potassium in the blood. Robertson (1953) observed a correlation between the concentration of the blood potassium and the activity of the crabs. The low concentration of potassium in the present study might be due to the inactive nature of the slug. Observations of Robertson (1953) also indicate that high concentrations of magnesium in the blood could be attributed to their inactive state. The results of the present investigation with regard to magnesium content appears justifiable since starvation is known to cause inactivation.

From the results presented in table 1 it is also possible to pinpoint the degree of utilization of the organic reserves at each starvation point, except TNPS, suggesting their utilization. Blood glucose was found to be drastically reduced with increase in starvation time. It is well known that glucose is the first metabolite to be used up during starvation even in mammals. At the end of each starvation period total carbohydrate recorded a maximal abatement followed by total protein

Table 1. Levels of inorganic and organic constituents in the blood of the garden slug *L. alte* upon starvation. Numbers in parentheses indicate number of estimations.

Parameter	Normal	Starved					
		I Week	% Change	II Week	% Change	III Week	% Change
Sodium mM/l	108·67 ± 9·69 (15)	114·36[a] ± 9·25 (15)	5·24	122·81[a] ± 8·12 (15)	13·01	132·25[a] ± 6·70 (15)	21·71
Potassium mM/l	2·61 ± 0·46 (15)	2·64[NS] ± 0·44 (15)	+ 1·15	2·68[NS] ± 0·40 (15)	+ 2·68	2·71[NS] ± 0 33 (15)	+ 3·83
Calcium mM/l	3·71 ± 0·40 (15)	3·76[NS] ± 0·37 (15)	+ 1·35	3·83[NS] ± 0·31 (15)	+ 3·23	3·91[a] ± 0·22 (15)	+ 5·93
Magnesium mM/l	11·04 ± 0·90 (15)	11·24[NS] ± 0·90 (15)	+ 1·81	11·81[a] ± 0·86 (15)	+ 6·97	12·52[a] ± 0·67 (15)	+ 13·41
Chloride mM/l	92·56 ± 7·13 (15)	96·14[NS] ± 6·96 (15)	+ 3·87	101·34[a] ± 6·72 (15)	+ 9·48	108·27[a] ± 6·59 (15)	+ 16·97
TAPS mg/100 ml	188·3 ± 36·0 (12)	146·4[a] ± 23·2 (12)	−22·25	126·8[a] ± 18·6 (12)	−32·66	89·6[a] ± 2·3 (12)	− 52·43
Glucose mg/100 ml	5·83 ± 0·09 (12)	3·14[a] ± 0·04 (12)	−46·14	1·08[a] ± 0·02 (12)	−81·47	Trace	−100·00
Total Protein mg/100 ml	2845 ±405 (12)	2560[d] ±345 (12)	−10·02	2224[a] ±263 (12)	−21·83	1836[a] ±160 (12)	− 35·46
TNPS mg/100 ml	7·39 ± 0·70 (12)	7·81[NS] ± 0·84 (12)	+ 5·68	8·27[a] ± 0·96 (12)	+11·91	9·32[a] ± 1·04 (12)	+ 26·11
Total Lipid mg/100 ml	743·0 ± 96·6 (12)	712·0[NS] ± 91·5 (12)	− 4·17	662·0[b] ± 84·7 (12)	−10·30	579·6[a] ± 65·2 (12)	− 22·07

P Value : $a < 0{\cdot}001$; $b < 0{\cdot}01$; $c < 0{\cdot}02$; $d < 0{\cdot}05$. NS = Not Significant.

and total lipid. As against the diminution observed in these organic reserves TNPS showed a stepwise increase which could be due to protein degradation. This is justifiable in view of the depletion noticed in protein content (table 1).

It is certain that even though the garden slug utilizes all the three major food stuffs at differential rates, the carbohydrates appear to be the primary food source indicating the carbohydrate oriented metabolism during starvation as shown in pulmonate, *P. corneus* (Emerson 1967) and the terrestrial pulmonate, *C. semirugata* (Ramamurthi and Subramanyam 1976).

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