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Effect of Copper Sulfate and Sodium Pentachlorophenate on Adenine and Adenosine Phosphates in *Lymnaea luteola* (Mollusca : Gastropoda)

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Use of copper in the form of $CuSO_4$ as a molluscicide had its origin with CHANDLER (1920). Although this compound is still widely used, especially for the control of fresh water gastropods, major intermediate hosts of larval trematodes, little is known about the exact cidal mechanism(s) of the copper ion. Similarly sodium pentachlorophenate (NaPCP) has been used as a fungicide and as a molluscicide and is described as a strong uncoupling agent of oxidative phosphorylation (BEVENUE & BACKMAN 1967). In work on snails, WEINBACH (1956) postulated that uncoupling of the oxidative phosphorylation provides a biochemical mechanism of pentachlorophenol's molluscicidal action. OLIVER & HASKINS (1960) showed that sublethal concentrations of NaPCP, could reduce the fecundity and egg viability of Biomphalaria glabrata. MASSOUND & WEBBE (1969) and HIRA & WEBBE (1972) have investigated the effect of sublethal concentrations of trifenmorpha and triphenyl lead acetate, respectively, on B. glabrata and on the development of Schistosoma mansoni within the snail. There are, therefore, a number of interesting and potentially useful effects in the application of low dosages of molluscicides as opposed to the usual short-term higher dosages, so that these low concentrations may not kill the non-target animals such as fish. When the snails are exposed to lower concentrations of $CuSO_4$ and NaPCP, they are able to survive for a short period only. The basic phenomena of the toxicity of these molluscicides in these animals are unknown.

To elucidate the physiological and biochemical effects of $CuSO_4$ and NaPCP on Lymnaea luteola, a locally abundant species acting as host for larval trematodes, and by so doing to provide a more rational approach to the synthesis of new copper and phenolic compounds and to evaluate their toxicity, for the control of this gastropod, a series of studies has been initiated.

TOMIZAWA & FUKAMI (1957) reported that muscle from rotenone-poisoned locusts showed a mild deficiency in its ability to synthesize adenosine triphosphate (ATP). Bodily processes are designed to utilize ATP, whose synthesis is, therefore, of utmost importance. ATP together with ADP and AMP forms adenylate pool. The energy charge, which depends on the relative concentrations of these adenylate compounds, exercises an integrated control, on the overall metabolism of the animal.

Therefore, it seemed to be of great importance to measure adenosine phosphate concentrations and calculate the energy charge in snails, to know the metabolic disturbances which might occur when the snails are exposed separately to two selected molluscides at low concentrations, and to compare their effectiveness.

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MATERIALS AND METHODS

Animals. Fresh water snails (L. luteola) weighing 400-500 mg (including shell) were used. They were collected in January-March from the rice fields nearby Tirupati, Andhra Pradesh, India, and were acclimated to laboratory conditions in dechlorinated tap water for 7 days. Snails were fed ad libitium with Amaranthus viridis leaves. Healthy animals were used.

Test substances. $CuSO_4$ was dissolved in dechlorinated tap water (2 ppm). Pentachlorophenol (technical grade, BDH) was dissolved in a minimum solution of 2N NaOH and diluted with dechlorinated tap water to get 2 ppm.

Treatment. Two groups of 25 snails were placed separately in a litre of test solution at 2 ppm and a control group of 25 animals were placed in a litre of dechlorinated tap water. After the snails were exposed to 15 h and 10 h in $CuSO_4$ and NaPCP, respectively, they were sacrificed. Duration of exposure was selected on the basis of concentration-duration products determined by us as recommended in W.H.O. monograph series No. 50 (1965).

Preparation of tissue extract. Animals were deshelled, bodies blotted and quickly (20 s) chilled in liquid air. After 30 min they were transferred into cold 6% perchloric acid (PCA) and their weights determined. Extracts were prepared in cold 6% PCA (0-5°C) from groups of at least 8 snails weighing 2-3 g in all, for the estimation of adenine and adenosine phosphates by employing ion-exchange chromatography (COHN 1957, GLICK 1954). The ion-exchange column (10x0.7 cm) was packed with Dowex 1-x8 resin (200-400 mesh, chloride form). Known volume of the extract was taken on to the column with a flow rate of 1 mL/min. The following eluents were applied:

For adenine+adenosine	: 0.01 M NH_4Cl
For AMP	: 0.005 N HCl
For ADP	: 0.01 N HCl + 0.04 N NaCl
For ATP	: 0.01 N HCl + 0.2 N NaCl

50 mL of the first eluent and 160 mL of each of the other eluents were used. 20 mL fractions were collected at a flow rate of 2 mL/min. Adenine contents of the fractions were measured spectrophotometrically at 260 nm and the concentrations were calculated from the standard graph prepared separately for adenine, AMP, ADP and ATP. The column was regenerated with 1 N HCl, then washed with distilled water till it became completely chloride free.

RESULTS

Survival of the animals during the treatment with molluscicides.

a) $CuSO_4$: Snails started dying from 12th h of exposure and 50% mortality was reached by 15th h. The animals used in this experiment are, therefore, exposed to $CuSO_4$ for 15 h.
b) NaPCP: Animals started dying from 3rd h of treatment and reached 50% mortality by 10th h. So the snails had 10 h exposure to NaPCP.

Normal animals in the tap water are designated as "untreated". Animals were considered dead when the mantle was not retracted into the shell upon mechanical stimulation; they were discarded.

Adenine and adenine nucleotide levels in untreated and treated animals.

The results are summarized in the Table 1. The untreated values are the averages of 8 measurements.

TABLE 1. Concentrations of Adenine and Adenosine Phosphatases (u moles/g wet weight) in Untreated and Treated Lymnaea luteola

	Untreated	Treated (2 ppm)		
		$CuSO_4$-15 h		NaPCP-10 h
AMP	1.3±0.1	0.8±0.1 S	S*	1.1±0.1
ADP	0.7±0.2	0.7±0.1 NS	NS*	0.6±0.2
ATP	0.5±0.1	0.4±0.1 NS	NS*	0.3±0.1 S
Total adenosine phosphates.	2.5	1.9		2.0
Adenine+Adenosine as adenine.	1.6±0.1	1.2±0.2 S	S*	2.2±0.3 S
ATP	0.7	0.7		0.5
ATP/AMP	0.4	0.5		0.3
ATP as per cent of normal level.	100	92.2		72.7
$\frac{ATP + 1/2\ ADP}{ATP+ADP+AMP}$	0.3	0.4		0.3

S, NS - The values are significant (S) or not significant (NS) at 0.05 level over untreated.

S*,NS*- The difference in between the two treatments is significant (S*) or not significant (NS*) at 0.05 level.

Treated values are from 6 observations. Snails exposed to $CuSO_4$ for 15 h could maintain 92% of the normal ATP level, while it was 72% for NaPCP treated snails. In the $CuSO_4$ treatment the ATP/ADP ratio remained unchanged while the ATP/AMP ratio increased. In NaPCP treatment both the parameters decreased. The adenine+adenosine fractions decreased on $CuSO_4$ treatment and increased on NaPCP treatment.

DISCUSSION

Nucleoside phosphate concentrations reported here for Lymnaea luteola are quite low compared to the values for other molluscs (ARAI & SATIO 1961, WYLIE & SMITH 1964, RAGHUPATHIRAMI REDDY & SWAMI 1967, Zs.-NAGY & ERMINI 1972, Zs.-NAGY 1973, BEIS & NEWS-HOLME 1975, WIJSMAN 1976). This is quite surprising when viewed in relation to its high rate of tissue respiration (1.18 mL/gm wet weight of foot slices/h according to VENKATESWARA RAO & ONNURAPPA 1978). This apparent discrepancy, can only be explained in terms of the relationship

between oxidations and phosphorylations.

Inhibitory effects of copper compounds on respiration (CHENG & SULLIVAN 1973) and the uncoupling action of PCP on oxidative phosphorylation (BEVENUE & BECKMAN 1967, WEINBACH 1956) are expected to be reflected in the adenosine phosphate levels. The lack of any statistically significant difference in ATP levels of $CuSO_4$-treated and untreated snails as well as $CuSO_4$-treated and NaPCP-treated snails, suggests that there is something special in the oxidative phosphorylative capacity of L. luteola. This is further corroborated by the lack of any statistically significant difference in the ADP levels of the treated and untreated snails. The significant drop of ATP levels in NaPCP-treated snails may be due to the hypermetabolic state induced by PCP (HOLMBERG et al. 1972). There appears to be not much of a change in ATP/ADP ratios as well, on treatment. This apparent lack of any effect of $CuSO_4$ and NaPCP on the ATP and ADP levels of L. luteola again points towards the uncoupled state of the mitochondria of this snail. $CuSO_4$ might inhibit respiration as reported by CHENG & SULLIVAN (1973), but cannot have any effect on ATP and ADP levels as oxidations involving molecular oxygen are already uncoupled with phosphorylation. NaPCP might not uncouple oxidations, involving molecular oxygen, from phosphorylation any further and therefore cannot have any marked effect on the snails' ATP and ADP levels.

But there is the significant loss of AMP, further reflected in reduction in total pool size of the adenosine phosphates and adenine in the treated snails. This lowered total adenosine phosphate concentration in both $CuSO_4$-treated as well as NaPCP-treated snails may be responsible for the observed mortality. Therefore, if at all there is any molluscicidal action on the part of these two compounds, the biochemical mechanism involved may have something to do with the metabolism of adenine/adenosine compounds, rather than with oxidations or phosphorylation. This is further corroborated by the lack of any real change in the "energy charge" of the treated snails.

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