Bull. Environ. Contam. Toxicol. (1985) 34:396–402

Bulletin of Environmental Contamination and Toxicology

Effect of Copper Sulphate on Respiration, Electron Transport, and Redox Potential in the Digestive Gland of the Snail Host, *Lymnaea luteola*

G. Ramesh Babu and P. Venkateswara Rao*

Department of Zoology, S. V. University, Tirupati-517 502 (A.P.), India

Bioaccumulation of heavy metals such as copper has shown various effects on behaviour (Kleere Koper et al 1973), enzyme activity (Iordachesw et al 1978). The toxic action of heavy metals is generally attributed to their inhibitory effect on enzyme system, except at high concentrations which act on the surface tissues as protein precipitates.

Copper sulphate is a cheap and commonly used molluscicide. Yet actual toxic mechanism involved is unknown. Therefore the effect of copper sulphate on respiration of the common freshwater gastropod snail Lymnaea luteola, has been assessed as has been done with other molluscs (Scott & Major, 1972; Brown & Newell, 1972; MacInnes & Thurberg, 1973). Its effect on terminal oxidase of electron transport chain and on the general NAD linked dehydrogenase activity as reflected by the redox state and the contribution of peroxidase under this treatment has been evaluated.

MATERIAL AND METHODS

Collection, maintenance and separation of uninfected snails (free from larval trematode infections) were done as reported elsewhere (Manohar et al. 1972). Fully fed, actively moving snails of 300-450 mg weight range were selected for experimentation. 6 hr exposure to 20 ml of 2 ppm copper sulphate solution prepared in dechlorinated tap water was recorded lethal (LD_{50}). 25 snails were exposed every time in 500 ml of 2 ppm copper sulphate, solution keeping the ratio of 1 animal to 20 ml of solution constant. Digestive gland was isolated in ice cold snail ringer (Carriker, 1946). This particular tissue is preferred in view of its metabolic importance and also of its remote position which does not have direct contact with the ambient medium.

* For correspondence and Reprints

Oxygen consumption was measured of whole snail and the digestive gland with the help of the conventional Warburg apparatus (Umbreit et al. 1959). Method of Hall et al. (1971) was adopted for determination of cytochrome oxidase activity whereas peroxidase was studied as given by Bergmeyer (1965). Protein the sample was estimated by folin-phenol method of Lowry et al. (1951). Oxidized or reduced forms of nicotinamide adenine dinucleotide were estimated by dichlorophenol indophenol (DCPIP) reduction method as described by McCormick & Wright (1971). Student's 't' test was used for statistical analysis.

RESULTS AND DISCUSSION

No change was observed in whole animal respiration. Significant drop was seen in endogenous respiration of digestive gland of the treated snail in 1st 10 min, whereas in 2nd 10 min no difference was maintained (Table 1).

Significant decrease in cytochrome oxidase activity as well as in NADH to NAD ratio with concomitant rise in peroxidase activity were observed (Table 2).

The rate of O_2 consumption is influenced by many factors such as activity, temperature, body size, nutritional status, stage in the life cycle, season, time of the day, previous respiratory experience and genetic background. Therefore it is surprising that the whole animal respiration is not altered on treatment, although the animal appears very much disturbed. This struggle itself could have raised the O_2 consumption. But in the present study, O_2 consumption has been recorded in the absence of copper ions in the reaction vessels unlike in the study of Sullivan & Cheng (1975) with Biomphalaria glabrata. Therefore the question of recovery can not be ruled out altogether. The immediate response of mussels to inorganic ions was to secrete copious amounts of mucus (Scott & Major, 1972). The same is true of the snail in the present study. Just as is the case with many other invertebrates here in Lymnaea luteola also, the main problem on treatment, could have been not one of absorption, but one of removal through body surface, gut or urine.

The tremendous lowering of endogenous respiration of digestive gland of the treated snail definitely shows that the copper has reached this organ although it is remotely placed at the closed end of the spiral shell. Copper might be accumulated in digestive gland as in

gills of Mytilus edulis as reported by Brown & Newell (1972). These accumulated copper ions might have reached toxic levels in the course of 6 hr exposure causing a severe drop in its respiration. Reduction in endogenous respiration due to heavy metal ions is not uncommon (MacInnes & Thurberg, 1973). Disruption of membrane permeability (Yager & Harry, 1966) or O_2 uptake and osmoregulatory function of epithelium (Sullivan & Cheng, 1975) was reported on heavy metal ion toxicity. Lethal effects of copper have been tentatively ascribed to inhibition of respiratory enzymes and/or enzymes related to excretion of metals (Hubschmann, 1967). This may be the case even with Lymnaea luteola for the observed low O_2 consumption in the digestive gland during treatment.

The effects of copper sulphate treatment on cytochrome oxidase and peroxidases are quite opposite, cytochrome oxidase being inhibited and peroxidase being activated. This is quite meaningful in the light of the role of peroxidase in catalysing the oxidation of various xenobiotic compounds. Since peroxidase can catalyse by means of hydrogen peroxide, the oxidation of cytochrome c, it is highly likely that, such a reaction is being catalysed by the increased peroxidase activity. To that extent mitochondrial oxidation of cytochrome c is inhibited. Probably this is the reason why the whole animal O_2 consumption does not change on treatment. This surmise is further substantiated by the reported presence of respiratory enzyme activity in cytosomes and their inhibition by the well known inhibitors (Zs Nagy, 1977). Thus with the demonstration of respiratory enzyme activity in cytosomes, extramitochondrial oxidations seem to be feasible in the treated snail particularly by means of peroxidation.

In the present study NAD levels increased and NADH content dropped significantly, thereby lowering substantially NADH to NAD ratio. This lowered ratio in digestive gland of the treated snail apparently means that the oxidative capacity of the snail is very high on treatment in spite of the significant decrease in cytochrome oxidase activity. This increased oxidative potential must be attributed to the significant increase in the peroxidase activity recorded in the present study. The presence of haemoproteins with pseudoperoxidase activity (Schindelmeiser et al. 1979) and carotenoproteins with an important role as terminal electron acceptors of redox chain (Zs Nagy, 1977) could possibly come into play on treatment with copper sulphate. Thus it is inferred that copper ions accumulate in digestive gland and upset ultimately the redox

Table 1. Effect of copper ions on respiration. Values expressed as um of O_2 consumed/gm wet wt/hr (mean±SD). Gas phase is air and temperature 37°C.

Particulars	Untreated	Treated * (2 ppm $CuSo_4$x6 hr)		% Difference		't'	
Whole Animal respiration	12.72	9.92		-22		NS	
	I	II	I	II	I	II	I	II
Endogenous respiration of digestive gland	56.36 ±25.46 (29)	14.91 ±10.75 (28)	24.27 ±17.60 (27)	12.76 ±8.51 (27)	-57	-14	S	NS

Number in the parentheses indicates the number of individual observations made. 'S' indicates that the difference is significant at 5% level whereas NS stands for not significant. I for Ist 10 min and II for 2nd 10 min.

* 50% of the snails that survived the treatment after 6 hr have been used.

Table 2. Effect of copper ions on respiratory enzymes and co-factors of digestive gland of the snail Lymnaea luteola.

Enzyme/Co-factor	Untreated	Treated	% Difference	't'
Cytochrome oxidase (mean±SD, um of cytochrome c oxidized/mg protein/hr)	2.23 ±0.73 (12)	0.57 ±0.09 (12)	-75	S
Peroxidase (mean±SD, um of H_2O_2 reduced/mg protein/hr)	5.31 ±2.28 (15)	8.24 ±2.90 (15)	+55	S
NAD (mean±SD, um of NAD/gm wet wt)	0.703 ±0.253 (12)	1.777 ±0.605 (12)	+143	S
NADH (mean±SD, um of NAD/gm wet wt)	3.775 ±0.641 (12)	2.652 ±0.575 (12)	-30	S
NADH to NAD ratio	5.37	1.49	-72	-

Number in the parenthesis indicates the number of individual observations made. 'S' indicates that the difference is significant at 5% level whereas NS stands for not significant.

potential by reducing the activity of cytochrome oxidase and NADH to NAD ratio.

Acknowledgements. The authors thank Council of Scientific and Industrial Research, Government of India for the financial grant to Dr. P. Venkateswara Rao for his research project and Dr. G. Ramesh Babu thanks UGC for the subsequent research fellowship.

REFERENCES

Bergmeyer HU (1965) Methods of Enzymatic Analysis. Academic Press, New York and London

Brown BE, Newell RC (1972) The effect of copper and zinc on the metabolism of the mussel Mytilus edulis. Mar Biol (Berl) 16(2): 108-118

Carriker MR (1946) Observations on the functioning of the alimentary system of the snail Lymnaea stagnalis appressa (Say). Biol Bull 91: 88-111

Hall FR, Hollingworth RM, Shankland, DL (1971) Cyanide tolerence in millipedes: The biochemical basis. Comp. Biochem. Physiol. 38B: 723-737

Hubschmann JH (1967) Effect of copper on the cray fish O. rusticus. II. Mode of toxic action. Crustacea 12: 141-151

Iordachesw D, Dumitru IP, Niculescu S (1978) Activation by copper ions of Mytilidases - Acid proteolytic enzymes obtained from Mytilus gralloprovincialis. Comp. Biochem. Physiol 61B: 119-122

Kleere Koper H, Waxman JB, Matis J (1973) Interaction of temperature and copper ions as orienting stimuli in the locomotor behaviour of the goldfish (Carassius auratus) J Fish Res Bd Canada 30: 725-728

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193: 265-275

Mac Innes JR, Thurberg FP (1973) Effects of metals on the behaviour and oxygen consumption of the mud snail. Mar Poll Bull 4: 185-186

Manohar L, Venkateswara Rao P, Swami KS (1972) Variations in aminotransferase activity and total free aminoacid level in the body fluid of the snail Lymnaea luteola during different larval trematode infections. J Invert Pathol 19: 36-41

McCormick DB, Wright LD (1971) Methods in Enzymology. 28 Part B Academic Press, New York. London, pp 24-28

Schindelmeiser I, Kuhlman D. Nolte A (1978) Localization and characterization of haemoproteins in the central nervous tissue of some gastropods. Comp Biochem Physiol 64B: 149-154

Scott D, Major C (1972) The effect of copper (II) on survival, respiration and heart rate in the common blue mussel Mytilus edulis. Biol Bull 143: 679-688

Sullivan JT, Cheng TC (1975) Heavy metal toxicity to Biomphalaria glabrata (Mollusca Pulmonate) Ann New York Acad Sci 266: 437-444
Umbreit WW, Burris R, Stauffer J (1959) Manometric Techniques. Burgess, Minneapolis
Yager CM, Harry HW (1966) Uptake of heavy metal ions by Taphius glabratus a snail host of Schistosoma mansoni. Exp Parasitol 19: 174-182
Zs Nagy I (1977) Cytosomes (yellow pigment grannules) of Molluscs as cell organelles of Anoxic Energy production. Intl Rev Cytol 49: 331-377
Received April 23, 1984; accepted August 10, 1984