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Uptake and Distribution of Copper Sulphate and Its Effect on the Respiration Rate of the Hemocyanin–producing Freshwater Snail *Lymnaea natalensis*

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Copper sulphate was one of the earliest compounds suggested as a molluscicide and although several new compounds have since been developed, copper sulphate is still widely used against freshwater snail intermediate hosts of trematode parasites causing bilharzia. A large variety of freshwater snail species also act as intermediate hosts for other trematode parasites which are important for medical and veterinary reasons. However, the toxic effect that copper sulphate may have on these species has not yet been investigated adequately. This incomplete picture of the action of copper sulphate on freshwater snails is further complicated by the fact that some of these snail species have hemocyanin (a protein containing copper) as respiration pigment. Because of the existence of a copper metabolic pathway, these species may handle external copper differently from those species with hemoglobin as respiration pigment.

Various toxic effects of copper on hemoglobin-containing snails have already been demonstrated. These include a loss of certain essential electrolytes in Bulinus tropicus (Van Aardt and Coertze 1981) during exposure to copper, a disturbance of the osmoregularity mechanism of Biomphalaria glabrata (Cheng and Sullivan 1977) and a reduction in the respiration rate of B. glabrata (Cheng and Sullivan 1973).

Wolmarans, Van Aardt and Coetzee (1986) demonstrated the development of certain histological changes in the surface epithelium of B. tropicus after this snail species was exposed to copper sulphate for 6 h. Wolmarans and Van Aardt (1985) showed that in B. tropicus the uptake of copper from the water takes place by means of adsorption onto the snail surfaces that are exposed to the water. Although these results are in agreement with those of Ryder and Bowen (1977), Zylstra (1972) demonstrated the active uptake of ferritin by Lymnaea stagnalis which indicates that

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there are different ways in which the various ions are taken up or that the uptake can differ in different snail species.

In the present study, the uptake of external copper in the form of copper sulphate, as well as the effect of this ion on respiration rate, was investigated in Lymnaea natalensis, the intermediate host of Fasciola gigantica. This snail possesses hemocyanin as respiratory pigment.

MATERIALS AND METHODS

Fully grown specimens of L. natalensis collected in the field, were used for the experiments. After collection, the snails were kept in aquaria according to the conditions as described by De Kock and Van Eeden (1980) and supplied daily with fresh Tetramin (Tetra Werke, West Germany).

A pilot study was carried out to determine whether the snails do take up external copper. Sixty snails were divided into 2 groups. One group acted as control. The other group was exposed for 6 h to 1 mg/kg^{-1} copper sulphate dissolved in 0.45-um millipore-filtered aquarium water at a temperature of 25 °C and a pH of 7.2. The exposure volume was 5 ml per snail and during the experiment air was bubbled through the exposure medium continually. These exposure conditions were maintained during all the experiments. The control snails were kept under the same conditions except that they were not exposed to copper.

After the exposure period, the snails were transferred three times for 5 min at a time to 0.45-um millipore-filtered aquarium water with a conductance of 450 µs and a pH of 7.2 so that they could move around in order to get rid of excess exposure medium on the snail and in the mantle cavity. The snails were then dried with paper towels, weighed and digested after which they were prepared for copper analysis (Wolmarans and Van Aardt 1986) with a Varian atomic absorption spectrophotometer (model 775).

In order to determine the distribution of copper in the snails, 120 snails were divided into four groups. One group was kept as a control, while the remaining three groups were exposed to copper for 2, 4 and 6 h, respectively. After this exposure period, the snails were rinsed as described previously. Hemolymph was then collected from each group of snails according to the method of Lever and Bekius (1965) and pooled in polyethylene microcuvettes and stored at 4 °C for the determination of copper. The snails were killed with dry heat (Van Aardt and Coertze 1981) and the soft

tissues separated from the shells. The head-foot region and the digestive gland were separated from the rest of the soft tissue by dissection and the excess fluid drained from the tissues with Whatman no 1 filter paper. The tissues were then weighed and digested according to the description of Wolmarans and Van Aardt (1986). The head-foot region was selected in order to investigate whether the copper found in B. tropicus (Wolmarans and Van Aardt 1985) adsorbed onto this epithelial surface, while the digestive gland was selected because this gland was found in several snail species to accumulate and store heavy metals (Schipp and Hevert 1978).

Sample shells of the control snails and the snails exposed for 6 h to copper, were rinsed well with distilled water and dried for 6 h in an oven at 70 ^{o}C. They were then weighed and digested just as the soft tissues, 0.8 ml distilled water was added to each digested sample and the copper concentration determined.

The uptake of copper from the water was investigated by determining the copper concentration in the water during exposure. Three groups of snails with the same total mass were separately exposed to copper for 6 h. At 10-min intervals 1 ml exposure medium was collected from each group and analyzed for copper.

In order to determine the loss of copper from the snails, both the snails and the copper-free water into which the snails were placed after exposure, were analyzed for copper. Twenty-five snails were kept as controls. Sixty snails were exposed to copper for 2 h after which the hemolymph of 30 snails were collected as already described 6 and 12 h after exposure. These snails were killed by heat, and the digestive gland and head-foot region of each snail were removed, weighed and digested. The copper concentration in these tissues was then determined. During these periods the snails were kept in 5 ml/snail well-aerated copper-free aquarium water.

In order to investigate the loss of copper from the snails to the copper-free aquarium water, three groups of 25 snails each were exposed to copper for 2, 4 and 6 h respectively. The snails were rinsed and placed in 5 ml aquarium water per snail for 30 h. During this period 12 water samples of 1 ml each were collected at different times from all three snail groups and analyzed for copper.

For the determination of the effect of copper on the respiration rate of snails, one group of 30 snails was taken and placed in a closed off respiration chamber

(Wolmarans 1987) that was filled with oxygen-saturated water. After the oxygen uptake of these snails was determined with the aid of a oxygen electrode (Radiometer), the snails were removed from the respiration chamber and they were left for 1 h in aquarium water to move around. The snails were then placed back into a respiration chamber filled with oxygen-saturated 1 mg/kg^{-1} copper sulphate solution. The rate of oxygen uptake was thereafter determined again.

RESULTS AND DISCUSSION

From Table 1 it can be seen that the copper concentration in total snails after an exposure period of 6 h differed significantly from the concentration in the control snails on the Student t test ($p < 0.05$). It is also clear from Table 1 that an increase in copper concentration occurred in the head-foot tissue, digestive gland and hemolymph after exposure to copper for 2 h. The copper concentration differed significantly ($p<0.05$) from the control values in all these cases but in the case of the head-foot and the hemolymph did not increase significantly after longer periods of exposure.

On the other hand, the copper concentrations in the digestive gland showed an increase with increasing exposure periods.

The concentration after 4 h of exposure also differed significantly from the concentration found after 2 h. Likewise, it was also found that the concentration measured after 6 h of exposure differed significantly from the concentration found at 4 h.

No difference was found in the copper concentration of the shells of snails exposed to copper when compared with those of the controls. This finding is in agreement with those of Wolmarans and Van Aardt (1986) on *B. tropicus*.

The copper concentration in the exposure medium decreased from an initial 0.34 mg/kg^{-1} to a mean of 0.16 mg/kg^{-1} after 1 h exposure (Fig. 1). Although this uptake of copper from the exposure medium was not as large as that found with *B. tropicus* (Wolmarans and Van Aardt 1985), it must be kept in mind that the exposure volume in the case of *B. tropicus* was only 3 ml per snail. It is, however, striking that in *L. natalensis*, as in *B. tropicus*, no further decrease in the copper concentration occurred in the exposure medium after 1 h. In contrast to this, there was a considerable increase of copper in the exposure medium after 6 h of exposure in the case of *L. natalensis*.

Table 1. The copper concentration in the whole snail, digestive gland, head-foot, shell, and hemolymph samples after exposure to 1 mgkg^{-1} $CuSO_4$, for different periods of time.

Sample	Exposure time in h	Number of samples	Mean sample mass (mg)	Copper concentration (mg/kg^{-1}) wet wet weight directly after exposure
Whole snail	0	30	159.4	$5.6 \times 10^{-3} \pm 0.6 \times 10^{-3}$/mg sample
	6	30	187.4	$7.5 \times 10^{-3} \pm 2.0 \times 10^{-3}$ " "
Shell	0	30	61.7	$1.71 \times 10^{-2} \pm 0.70 \times 10^{-2}$ " "
	6	30	74.6	$1.25 \times 10^{-2} \pm 0.70 \times 10^{-2}$ " "
Head-foot	0	30	39.8	$5.2 \times 10^{-3} \pm 0.33 \times 10^{-3}$ " "
	2	30	19.8	$7.0 \quad 10^{-3} \pm 0.23 \times 10^{-3}$ " "
	4	30	23.3	$7.2 \times 10^{-33} \pm 2.8 \times 10^{-3}$ " "
	6	30	21.2	$6.4 \times 10^{-3} \pm 2.5 \times 10^{-3}$ " "
Digestive gland	0	30	40.6	$9.3 \times 10^{-3} \pm 7.0 \times 10^{-3}$ " "
	2	30	23.1	$16.0 \times 10^{-3} \pm 6.0 \times 10^{-3}$ " "
	4	30	17.8	$17.2 \times 10^{-3} \pm 11.0 \times 10^{-3}$ " "
	6	30	21.2	$18.6 \times 10^{-3} \pm 12.5 \times 10^{-3}$ " "
Hemolymph	0	30	*	3.48×10^{-1}
	2	30	*	4.41×10^{-1}
	4	30	*	4.71×10^{-1}
	6	30	*	4.66×10^{-1}

* The copper concentration in the hemolymph was determined in unweight pooled samples.

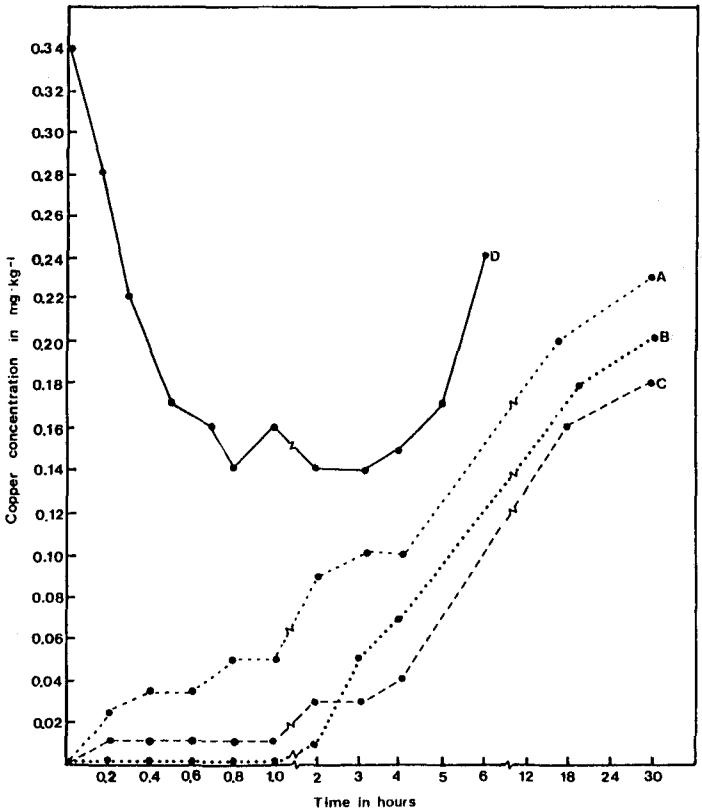

Fig. 1 The decrease in the copper concentration of the exposure medium (D) as well as the increase in the copper concentration of initially copper free mediums due to the excretion of copper from snails which were exposed to copper for 6 hours (A), 4 hours (B) and 2 hours (C).

Table 2 The copper concentration in the hemolymph, head-foot and digestive gland samples of three snail groups at zero, 6 and 12 h after exposure to 1 $mgkg^{-1}$ $CuSO_4$ for a period of 2 h

Sample	Time in h	Mean sample mass (mg)	Copper concentration in mh/kg^{-1}
Digestive gland	0	40.6	$9.3X10^{-3} \pm 7.0x10^{-3}$
	6	22.2	$10.2x10^{-3} \pm 6.6x10^{-3}$
	12	22.8	$11.4x10^{-3} \pm 4.5x10^{-3}$
Head-foot	0	39.8	$5.2x10^{-3} \pm 0.33x10^{-3}$
	6	26.1	$12.0x10^{-3} \pm 7.0x10^{-3}$
	12	22.0	$15.5x10^{-3} \pm 8.3x10^{-3}$
Hemolymph	0	*	$3.48x10^{-1}$
	6	*	$4.66x10^{-1}$
	12	*	$5.28x10^{-1}$

* The copper concentration in the hemolymph was determined in unweighed pooled samples.

This phenomenon also occurred in the snail groups exposed to copper for 2, 4 and 6 h and which were transferred to clean aquarium water afterwards. In this case it was clear that the loss of copper from the snails exposed to copper for 6 h was greater than from the snails exposed for 2 and 4 h (Fig. 1). It is clear from Table 2 that, in spite of the fact that the snails were only exposed to copper for 2 h and that they were kept in copper-free aquarium water for 6 and 12 h, respectively, a large increase in the copper concentration occurred in the head-foot region of these snails. In contrast, the copper concentration of both the digestive gland and the hemolymph showed a much smaller increase after the 2 h exposure period. From these results on the uptake of copper by snails, it can be deducted that copper is probably taken up by adsorption at the head-foot region and metabolic interaction in the digestive gland. This reasoning is supported in the case of the head-foot region by the finding that a rapid decrease in copper occurred in the exposure medium and that the copper concentration of the head-foot region did not increase with increasing exposure time. A similar finding was made by Wolmarans and Van Aardt (1985) with B. tropicus.

It would appear as if copper reached the digestive gland via the blood pathways and then accumulated there for as long as exposure was maintained. When the snails were transferred to a copper-free medium, a loss of copper occurred from the hemolymph as well as the digestive gland. This loss was reflected in an increase in copper in the copper-free medium. This released copper probably became available for uptake by the snails again. This may possibly explain the increased copper concentration in the head-foot region and to a much smaller degree in the hemolymph and the digestive gland after the snails exposed to copper were transferred to a copper-free medium.

No difference in the rate of oxygen uptake occurred in the snails before or during exposure to copper. Although the copper ion in this case was in a stereochemical sense at a pH of 7.2 biologically exposed, it did not result in a decrease in respiration rate of the snails. This was in contrast to the findings of Cheng and Sullivan (1973) but could possibly be attributed to the fact that L. natalensis possesses a copper metabolic pathway.

It seems further that the copper taken up in the hemolymph does not influence the copper-containing respiration protein of the snails. This leads to the question how this compound can promote lethality in snails. The authors are of the opinion that lethality results from a negative effect of copper on the exposed

epithelium, which in turn leads to a disturbance in the osmoregulatory processes of the snails. This explanation is also presumed to operate in the case of B. tropicus (Wolmarans, Van Aardt and Coetzee 1986) and B. glabrata and Cheng and Sullivan 1977).

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