Experimental evidence that copper is taken up by the freshwater snail *Bulinus tropicus* through a process of adsorption

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In this investigation the mechanism of copper uptake by freshwater snails was examined. The experiments investigated the following: the uptake of copper by the snail, the release of copper from the snail, the decrease of copper in the exposure medium, and the relationship between copper uptake and snail metabolism. The results of the investigation provide evidence that copper is taken up by a process of adsorption rather than by active absorption.

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Die meganisme van koperopname deur varswaterslakke is in hierdie ondersoek nagegaan. Eksperimente wat uitgevoer is, het onder andere die opname van koper deur die slak, die vrystelling van koper uit die slak, die afname van koper in die blootstellingsmedium en die verband tussen koper-opname en slakmetabolisme ingesluit. Uit die resultate van die ondersoek is bewyse verkry dat koper eerder deur 'n proses van adsorpsie as aktiewe absorpsie deur die slakke opgeneem word.

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The need to investigate how copper is taken up by the snail arose because copper exerts a toxic effect on freshwater snails (Cheng & Sullivan 1974; Van Aardt & Coertze 1981). Indirect proof that copper is taken up through a process of adsorption by the land snail *Agriolimax reticulatus* was presented by Ryder & Bowen (1977). In contrast, Zylstra (1972) showed that colloidal ferritin is taken up actively by *Lymnaea stagnalis*, and this uptake of ferritin can therefore be regarded as an absorption process. Cheng & Sullivan (1974) also concluded that copper is absorbed rather than adsorbed by the rectal ridge and head-foot epithelial tissue of *Biomphalaria glabrata*. However, it is important to note that none of the authors mentioned, did experiments to investigate specifically the means of copper uptake by snails. To investigate the means of uptake four experiments were performed. These were designed to:

(i) Investigate the increase of copper in the snail over a period of 6 h;
(ii) monitor the decrease of copper in the exposure medium during exposure;
(iii) investigate copper release by the snails;
(iv) investigate copper uptake at increasing exposure temperatures.

Materials and Methods

Fully grown *Bulinus tropicus* specimens were collected in a dam on the grounds of the Potchefstroom Agricultural College (grid reference 26° 44′ S/27° 04′ E). The snails were maintained in aquaria as described by De Kock & Van Eeden (1980) at a temperature of 25°C ± 1°C and a water conductivity of 500 µS. The snails were provided with fresh Tetramin (TetraWerke, West Germany) daily. Before the start of each experiment the experimental animals were starved for 12 h.

To examine the increase of copper in the snails, 70 snails were divided into seven groups of ten each. One group served as the control while the remaining six groups were exposed to 1 mg kg^{-1} $CuSO_4$ at a pH of 8,4 for 1, 2, 3, 4, 5 and 6 h. After exposure, the snails were rinsed quickly, weighed and individually digested in 0,2 cm^3 55% analytically pure HNO_3 for 6 h. One cubic centimetre distilled water was then added to each digested snail, and the copper concentrations in the digested solutions were determined with a Varian atomic absorption spectrophotometer (Model no AA 775).

The decrease of copper in the exposure medium during exposure was examined as follows. Thirty snails were divided into three groups of ten each, after which each group was separately exposed to 30 cm^3 1 mg kg^{-1} $CuSO_4$ at a pH of 8,4 for 6 h. During the exposure period 1-cm^3 samples of each

exposure medium were taken hourly for 6 h from all three exposure mediums. This copper concentration was determined immediately after sampling, and each removed sample was then replaced with 1 cm^3 water with a copper concentration corresponding to that of the measured sample.

The release of copper from exposed snails was investigated as follows. Thirty snails were divided into three groups of ten each and separately exposed to 1 mg kg^{-1} $CuSO_4$ at pH 8,4 for 6 h. The exposure volume was 3 cm^3 per snail. After exposure, the snails were rinsed in distilled water and transferred as three separate groups of ten each to 30 cm^3 copper-free aquarium water (Van Aardt & Coertze 1981). Samples of 1 cm^3 of the copper-free medium were taken hourly for the first 6 h, and then at 18 and 24 h after transfer. The copper content of the snails was analysed.

Snails have different metabolic rates at different temperatures. The snails were exposed to copper at different temperatures and the copper uptake was investigated as follows. One hundred snails were divided into five groups of 20 each. Each group was acclimatized for 24 h at a temperature of 5, 10, 15, 20 or 25°C in aquarium water. The snails were starved only for the last 12 h of the acclimatization period. The groups were then exposed to 1 mg kg^{-1} $CuSO_4$ at pH 8,4 for 6 h at temperatures corresponding to the acclimatization temperatures. After exposure, the concentration of copper in each snail was determined with an atomic absorption spectrophotometer.

The metabolic activity of the snails at these temperatures was also investigated by determining the locomotor activity of the snails (Jones 1975). Twenty-five snails were divided into five groups of five each, and every snail in each group was marked on the apex with a different colour of a quick-drying paint. The distance which each snail moved in water at a specific temperature, could thus be determined. The bottom surfaces of the dishes in which each group was placed were divided into squares of 4 cm^2 to facilitate the measurement of the distance that each snail moved. The five groups of snails were transferred into separate dishes of water with temperatures of 5, 10, 15, 20 or 25°C, and were allowed to move for 30 min. The total distance each snail covered, was measured, and the average distance at each temperature was determined.

Results and Discussion

The results in Table 1 show clearly that there was a significant increase in copper concentration in the total snails after 1 h of exposure to copper. It is also clear that the concentration of copper did not increase further with longer periods of exposure (Table 1). These differences, however, were not statistically significant. A possible explanation for the failure to accumulate copper over a period of 6 h could be the withdrawal of the snails into their shells. Such withdrawal would result in less external epithelium being exposed to the copper, so that an apparent equilibrium is established.

From the results in Figure 1 it is clear that there was a rapid decrease in the concentration of copper in the exposure medium during the first hour of exposure, which closely paralleled the increase in the copper concentration in the snails after 1 h of exposure. Furthermore, it is clear from Figure 1 that very little copper was released by the exposed snails into the aquarium water, which excluded the possible establishment of an equilibrium between copper uptake and copper release. The copper release which did occur, could possibly have been due to the release of copper-containing mucus (Cheng & Sullivan 1974). Cheng & Sullivan (1974) also found that copper is present in the thick mucous layer which is secreted by the freshwater snail, *Biomphalaria glabrata*, in reaction to contact with poisonous heavy metals.

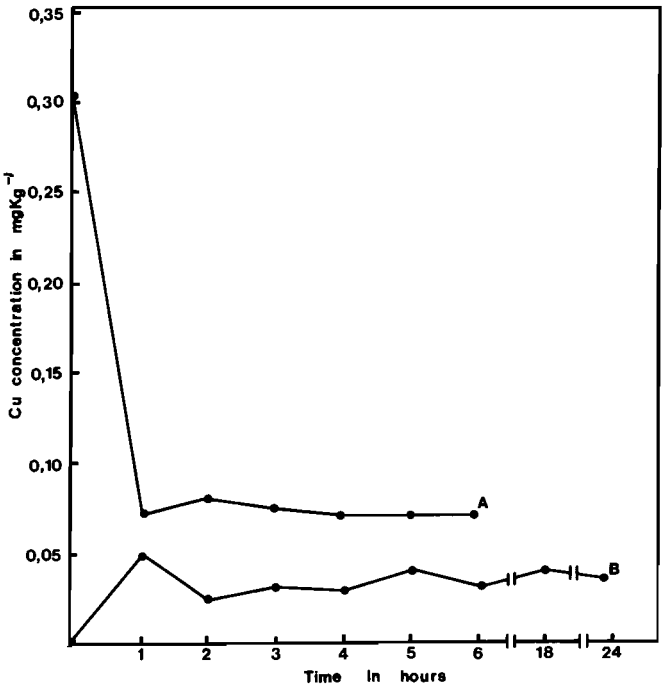

Figure 1 (A) The decrease in the copper concentration of the exposure medium. (B) The increase of copper from exposed snails to clean aquarium water at a pH of 8,4.

Table 1 The mean copper concentration in mg kg^{-1} in total snails which were exposed for periods of 0, 1, 2, 3, 4, 5 and 6 h to 1 mg kg^{-1} $CuSo_4$ at pH 8,4

Period of exposure in hours	Mean snail mass in mg	Copper concentration per total snail	Copper concentration per 100 mg total snail	Standard deviation
0	81,60	$5,47 \times 10^{-3}$	$6,85 \times 10^{-3}$	$2,37 \times 10^{-3}$
1	89,05	$8,66 \times 10^{-3}$	$8,85 \times 10^{-3}$	$2,29 \times 10^{-3}$
2	85,76	$8,64 \times 10^{-3}$	$10,59 \times 10^{-3}$	$3,24 \times 10^{-3}$
3	84,88	$9,00 \times 10^{-3}$	$10,36 \times 10^{-3}$	$2,53 \times 10^{-3}$
4	77,27	$8,72 \times 10^{-3}$	$11,50 \times 10^{-3}$	$2,77 \times 10^{-3}$
5	89,15	$10,33 \times 10^{-3}$	$11,95 \times 10^{-3}$	$2,92 \times 10^{-3}$
6	88,74	$9,33 \times 10^{-3}$	$11,53 \times 10^{-3}$	$4,43 \times 10^{-3}$

From these results, it can be assumed with reasonable certainty that copper is taken up through a process of adsorption. This is particularly supported by the fact that copper is rapidly taken up by the snail, this uptake does not increase any further after exposure for an hour, and almost no copper is released by the snail. This conclusion is further supported by the results in Figure 2, which show that the temperature of exposure had no effect on the uptake of copper. However, there is a direct relationship between temperature and snail movement (Jones 1975) which reflects the metabolism of the snail. Thus it is clear that an accelerated metabolism does not lead to an increase in copper uptake (Figure 2). This implies that active absorption of copper does not take place, but that adsorption, either physical or chemical, probably occurs.

The experiments performed in this investigation should be

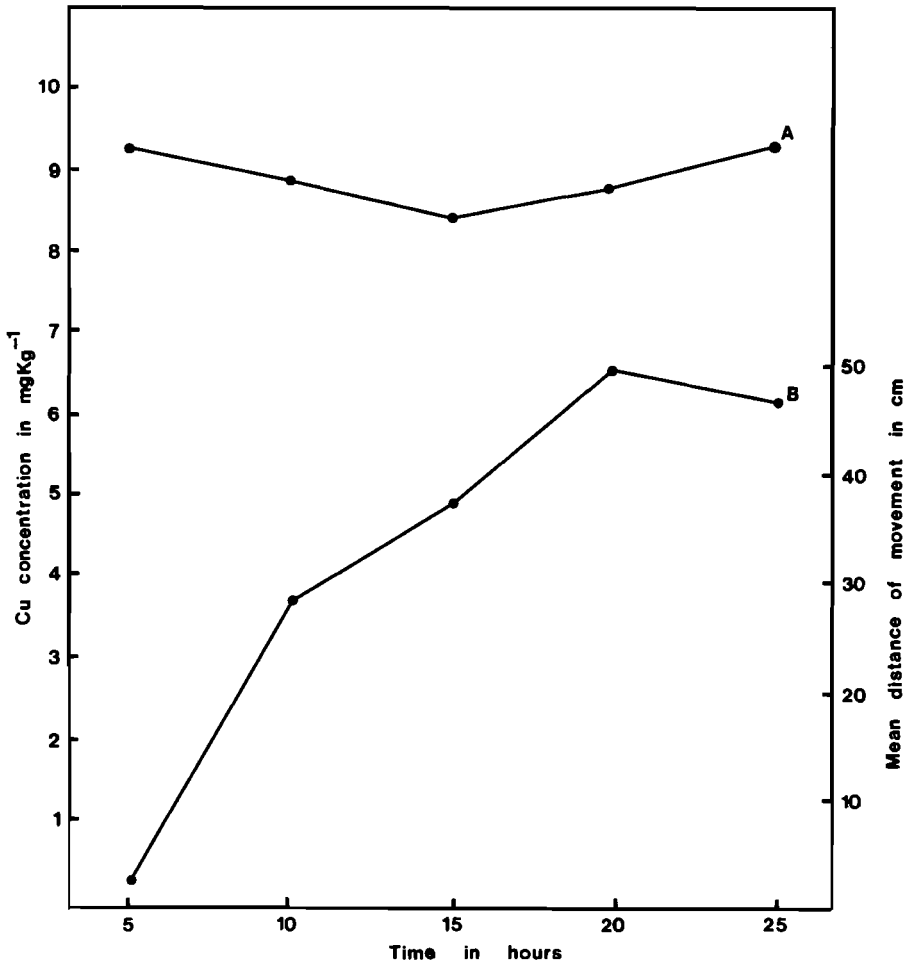

Figure 2 (A) The relationship between the amounts of copper which are taken up by the snails at different temperatures. (B) The distances moved by the snails at these temperatures.

useful for studying the mechanisms in snails of copper uptake, as well as that of other heavy metals such as chromium, lead and cadmium.

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