Influence of copper sulphate on the water and electrolyte balance of the freshwater snail *Bulinus (Bulinus) tropicus*

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The water and electrolyte balance of *Bulinus (Bulinus) tropicus*, a freshwater pulmonate, was determined when subjected to different sublethal concentrations of copper sulphate. It was found that the lethal dose (LD_{50}), which is the dose lethal to 50% of the snails, is 1,0 ppm in water of pH 8,5. Increasing dosages disturb the ionic and water balance. Haemolymph concentrations of Na^+, Ca^{2+} and Cl^- decrease markedly. The rates of ion influx and efflux for Ca^{2+}, Na^+, Cl^- and ^3H_2O, measured with their corresponding radioisotopes, were drastically changed. The results indicate that the physiological effects of copper are not limited to particular organs or tissues but probably occur throughout the snail body. The possible effect of copper sulphate on the integumental exchange mechanisms for Ca^{2+}, HCO_3^-, Na^+, K^+ and Cl^- are discussed.

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Die water- en elektrolietbalans van *Bulinus (Bulinus) tropicus*, 'n varswater pulmonaat, is bepaal tydens blootstelling aan subletale konsentrasies kopersulfaat in die water. Dit is gevind dat die letale dosiswaarde (LD_{50}) waarby 50% van die slakke by 'n pH van 8,5 vrek, 1,0 dpm is. Toenemende dosisse versteur die water- en ioonbalans. Die hemolimfwaardes van Na^+, Ca^{2+} en Cl^- neem opvallend af. Die iooninfluks- en uitflukskoerse vir Ca^{2+}, Na^+, Cl^- en ^3H_2O, soos gemeet met hul ooreenstemmende radioisotope, word dramaties verander. Die resultate toon aan dat die fisiologiese beïnvloeding van koper nie slegs beperk is tot bepaalde organe of weefsels nie maar dat die hele slak daaronder ly. Die moontlike effek van kopersulfaat op die uitruilmeganismes van die slakhuid ten opsigte van Ca^{2+}, HCO_3^-, Na^+, K^+ en Cl^- word bespreek.

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It is well known that solutions of copper sulphate act as molluscicides to freshwater snails. Most of the recent work dealing with the development and application of molluscicides in controlling schistosomiasis has been documented by Cheng (1974). It appears that our knowledge of the biological effects of copper compounds on snails concerns mainly the determination of lethal dose values and the usefulness in practice of copper-containing chemicals under field conditions. Little is kown about the physiological changes which take place inside the snail when lethal or sublethal doses are applied. In this regard Cheng & Sullivan (1974) did pioneering work by demonstrating that the oxygen consumption and heart rate of *Biomphalaria glabrata* decrease measurably when snails are exposed to copper-containing compounds. They also found that exposure of *B. glabrata* to 0,06 ppm copper sulphate for a few hours results in a substantial decrease in the osmotic pressure of the haemolymph. This causes an osmotically induced inflow of water into the tissues, resulting in the snail's death.

The present study was designed to investigate water and electrolyte balance of *Bulinus (Bulinus) tropicus* exposed for short times to copper sulphate solutions of various sublethal concentrations. To accomplish this, most of the experiments were devised to measure the fluxes of water and of several major electrolytes and to establish their concentrations in the snail's haemolymph and tissues. The lethal doses of copper sulphate were determined as LD_{50}.

Materials and Methods

Specimens of *B. (B.) tropicus* were reared in the laboratory according to the methods of Van der Schalie & Berry (1973) and De Kock & van Eeden (1980). The egg masses were obtained from snails collected in a dam near Potchefstroom (grid ref. 2627 Ca). After having been fed for 6 – 8 weeks on Tetramin (Tetramin Werke, West Germany), the snails were considered large enough (190 ± 67 mg) for experimentation. To determine the LD_{50} values for each copper sulphate concentration, 100 specimens, comprising ten replicates (ten snails per replicate) were used. One hundred millilitres of space per snail were available during 12 h exposure in $CuSO_4$ solutions and 24 h observation period in water. The temperature was kept at 26 °C ± 1 °C.

Borehole water (water analysis in ppm: total alkalinity

179; Cu < 0,02; Fe 28; Ca 19; Mg 81; Na 21; K 4,1; Cl 24; SO_4 97; PO_4 0,7), diluted with distilled water from 650 μS to 450 μS, and having a pH of 8,5, was used as aquarium water. After exposure to the copper solutions, the snails were quickly washed with three changes of aquarium water. The water was changed at 3-hourly intervals, during which time snails were also observed under a stereomicroscope to determine whether they were alive or dead. Dead snails were identified by the colour of the head-foot changing from dark to light grey, by the lack of response of the tentacles and foot muscles to mechanical stimulation and by floating motionless on the surface of the water.

From a graph of percentage mortality against logarithm of the dose (Figure 1), doses of 0,2, 1,0 and 4,0 ppm were chosen to test the sublethal effects of copper sulphate on the water and electrolyte balance of the snail.

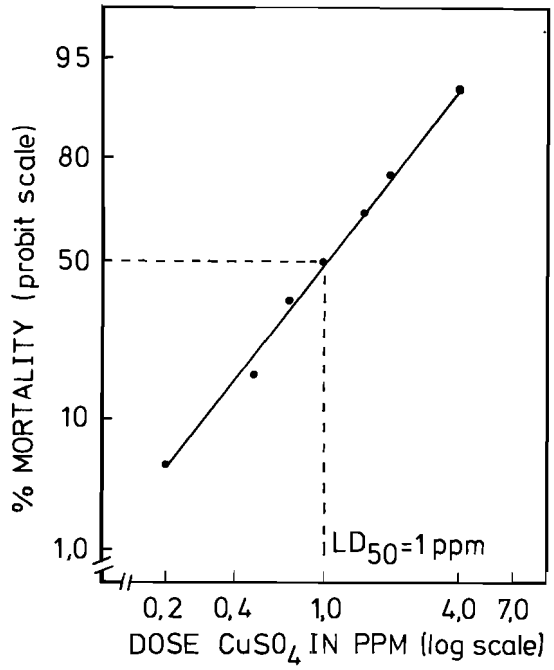

Figure 1 Dose-mortality curve of laboratory snails exposed for 12 h to various concentrations of copper sulphate. Data from this figure was used to establish the sublethal copper sulphate doses used in the experiments.

Aquarium water was used for controls and the experimental snails were exposed to copper solutions for 6 rather than 24 h. They were observed for 24 h in aquarium water, and it was found that all snails survived these doses.

Measurements made directly after six hours exposure to $CuSO_4$

Haemolymph samples (20 – 30 μl per snail) were collected according to the technique of Van Aardt & Frey (1979). The osmotic pressure of the haemolymph was measured by the freezing-point method of Ramsay & Brown (1955) while the pH of the haemolymph was determined using a micro-electrode unit (Model E5021, Radiometer, Denmark). The total CO_2 concentration (CO_2, HCO_3^-, CO_3^{2-}) in 30 μl haemolymph samples was determined according to Holaday & Veresky (1956). To prepare the snail tissue for the sodium and potassium determinations the following procedure was followed. Snails were killed by exposure to dry heat in a test tube immersed for 45 s in boiling water. The wet body mass was removed from the shell and placed on a glass coverslip, weighed and then dried in an oven at 105 °C. After determination of the dried body mass the total tissue water was calculated by subtraction. Sodium and potassium concentrations in the dried body mass can now be determined (EEL 227 integrating flamephotometer, England) by dissolving it in a known volume (= total tissue water per snail) of a 5:1 nitric/perchloric acid mixture (Parry & Potts 1965). Sodium and potassium in the haemolymph was also determined by flamephotometry.

Chlorides in the tissues were determined using the Aminco-Cotlove titrator (Model 4-4413, U.S.A.). For this measurement the wet body mass was initially freeze-dried (Moss 1964), pulverized and then dried in an oven at 105 °C. Four millilitres distilled water was then added and shaken for 8 h for chloride extraction. After centrifugation, 400 μl concentrated acetic acid and 25 μl concentrated nitric acid were added to the supernatant before the analysis. To express the values in mmol l^{-1}, the water content by freeze drying and the freeze-dried body mass for each snail must be known (Potts & Parry 1964). For chlorides in haemolymph, known volumes of haemolymph were dissolved directly in distilled water before acetic and nitric acid were added.

The calcium concentration was determined by flame-atomic absorption spectrometry (Varian Techtron, Model 1100, U.S.A.). Haemolymph samples and also the wet body masses were dissolved in 0,5 ml concentrated HNO_3. The samples were dried at 80 °C and dissolved in 0,1 N HCl. Into each sample, 0,5 ml strontium nitrate (5%) was pipetted and diluted to 10 ml with 4% potassium chloride. The analyses were carried out at a wavelength of 422,7 nm and a split width of 0,2 nm.

Measurements made during the six hour exposure to $CuSO_4$

The rates of influx and efflux of Ca^{2+}, Na^+, Cl^- and ^3H_2O into and out of the snails were determined with the aid of radio-isotopes (Radiochemical Centre, Amersham, England). For each copper concentration tested, including the controls, 30 snails, (divided into three groups) were used to determine the influx and efflux rates.

To be able to determine the water (as ^3H_2O), calcium (as $^{45}CaCl_2$) and chlorine (as $Na^{36}Cl$) efflux rates, groups of snails were loaded for 4, 48 and 12 h respectively with the corresponding radio-isotope in aquarium water. The loading time in tritiated water (1μCi ^3H_2O) was chosen according to van Aardt (1968). To determine the ^{22}Na loading time, 20 snails were subjected to 10 μCi $^{22}NaCl$ in 30 ml aquarium water. At hourly intervals the snails were individually removed, washed in radioactive free water and the ^{22}Na determined with a scintillation spectrometer (Model 3314, Packard, U.S.A.). It was found that after 12 h ^{22}Na reaches equilibrium between the snail tissues and the surrounding water. In the same manner the loading time for ^{45}Ca (loaded with 0,1μCi ml^{-1} H_2O) and ^{36}Cl (loaded with 0,47μCi ml^{-1} H_2O) was determined. For these measurements, however, the snails from each group

were killed at appropriate time intervals and predigested (0,2 ml 60% perchloric acid, 0,4 ml 30% hydrogen peroxide) before being counted in a liquid scintillation mixture (6 g PPO and 7 ml 2-methoxy ethanol in 1 l toluene.)

The efflux rate was individually determined for each radio-isotope on different snail groups. Each group of ten snails was placed in 30 ml aquarium water. The increased radioactivity of the water over a 6-h period was monitored with a liquid scintillation spectrometer (Model 3390, Packard Instruments, U.S.A.). Instagel (Packard Instruments) was used as liquid scintillation mixture. The efflux rate for $^{22}NaCl$ was, after a 12-h loading period, measured more directly: after being washed in three changes of aquarium water, individual snails were immediately analysed in a well-type scintillation spectrometer (Model 3314, Packard Instruments). Six hours after replacing them in water free of radioactivity the snails (individually marked) were again analysed for ^{22}Na activity. The snails were then killed by dry-heating and the sodium concentration in the haemolymph and tissues measured by flame photometry.

The influx rates of chlorine, sodium and calcium were calculated from the decrease in radioactivity of the external medium, in which the snails were placed over a period of 6 h (Shaw 1961; Little 1965; Potts, Foster, Rudy & Parry-Howells 1967; Greenaway 1970, 1971a).

In order to express the influx and efflux rates in micromoles per litre, ionic concentrations in the snail and in the experimental water at the start and the end of each experiment had to be known. To accomplish this on *B. (B.) tropicus* the procedures and calculations outlined by Rudy (1966) and Potts *et al.* (1967) were followed.

To determine the water influx rate snails were each exposed to tritiated water for 20 min (Van Aardt 1968). Subsequently all the water was removed from the animals by freeze-drying (Moss 1964; Van Aardt & van Eeden 1972). The specific radioactivity of the water thus collected was measured with a liquid scintillation spectrometer (Model 3390, Packard Instruments). In addition the activity of the tritiated water in the experimental container was also measured after a sample had been freeze-dried. The water influx constant was calculated according to Rudy (1967). The potential difference (in mV) between the haemolymph in the haemocoel and the water surrounding the snail was measured according to the micro-electrode technique of Woodbury (1966) as applied by Wright (1975) and Dietz & Branton (1975) to intact aquatic animals. For *B. (B.) tropicus* a small hole (± 1 mm in diameter) was drilled through the shell between the kidney and the pericardial cavity without damaging the underlying body epithelium. After penetration of the epithelial layer with the glass micro-electrode the other electrode was placed in the water. The measured electric potential difference was then fed into an operational amplifier and registered on an oscilloscope (Model R5031, Techtronix, U.S.A.).

In all experiments the water temperature was kept at 26 °C ± 1 °C. Air was bubbled through the experimental water with the aid of polyethylene capillaries. Apart from the laboratory reared snails, LD_{50} values, ion fluxes and electrolyte measurements were also done on field snails having approximately the same body mass as the laboratory snails. These field snails were collected over a period of two years from the same habitat as that of the laboratory animals.

Results

After exposure to the different copper sulphate solutions the snails quickly became immobile. At lower concentrations (0,2 to 0,7 ppm) most snails did not retract into their shells but showed uncoordinated movements of the head and foot muscles. After 12 h of exposure some snails were visibly swollen. It was noted, however, that the recovery of the snails, after having been transferred to a copper-free solution, was remarkably quick; within 60 s some snails started moving about. Snails exposed to higher copper concentrations (1,0 to 4,0 ppm) retracted immediately, releasing the air bubble from the mantle cavity and sinking to the bottom of the container. A copious mucous secretion was observed on the ventral surface of the foot.

The results (Figure 1) indicate that for copper the lethal dose or LD_{50} (i.e. where 50% of the individuals die) is 1,0 ppm. For snails from the field the LD_{50} value was 0,7 ppm in aquarium water, but this increases to 1,5 ppm when tested in water from their natural habitat. The values found for *B. (B.) tropicus* were much higher than those for *Australorbis glabratus* where an LD_{50} of 0,1 ppm (under field conditions, no measurements made on pH of water) was found (Ritchie *et al.* 1965). From this it can be presumed that *A. glabratus* is more susceptible to the copper ion. It is known, however, that copper sulphate becomes progressively insoluble at pH values higher than 7,5 (Meyling 1966; Paulini 1974) resulting in lower toxicity to snails. It was found, however, that at a pH of 8,5 the LD_{50} value for *Lymnaea natalensis* was less than 0,2 ppm (unpublished data). The more 'open' structure of the shell in this species possibly allows the exposure of a larger body surface to the copper solutions, thus reducing the amount of copper needed to be lethal. The retraction of individual specimens of *B. (B.) tropicus* into their shells in response to higher $CuSO_4$ doses had a similar effect, i.e. the reduction of exposed body surface to direct contact with the copper solution. The reduction in body area exposed, however, does not seem to affect the intake of the copper ion. The LD_{50} results show that there is a constant relationship between mortality and dose, even at higher concentrations (Figure 1).

The results depicted in Table 1 show that increasing the dosage of $CuSO_4$ disturbs the ionic and water balance mechanisms of the snail. At a concentration of 4,0 ppm, $CuSO_4$ reduces the sodium and chloride concentrations in the snail to 43,9 and 40,5% of the control values. The total potassium in the body as well as the potassium in the haemolymph, however, remained unchanged compared to the controls. Calcium, on the other hand, increased to 260,5% in the haemolymph, but total calcium decreased to 64,2% for the whole animal compared to the controls. Contrary to expectations, a sharp decrease in the osmotic pressure of the haemolymph was not found; it only dropped to 15% below that of the controls. Similar results were found for the field snails kept in aquarium water. The total CO_2 values of the control snails are virtually the same as those of snails exposed to 1 ppm copper.

The results of the flux experiments (Table 2) show that with increasing dosages the amount of calcium, sodium

Table 1 The influence of copper sulphate on the electrolyte composition of *B. (B.) tropicus* after 6 hours sublethal exposure to $CuSO_4$. Total Na^+, Ca^{2+} etc. values are given for the snail without the shell. Each measurement represents the average and standard deviation (±) of 30 snails at 26 °C with a water pH of 8,50. Values are expressed in millimoles per litre

	Copper sulphate (ppm)			
	Controls	0,2	1,0	4,0
Haemolymph:				
m Osm kg^{-1} H_2O	138 ± 7,9	132 ± 3,9	129 ± 3,4	117 ± 3,4
total CO_2	13,6 ± 2,3	–	13,3 ± 0,9	–
pH	7,60 ± 0,03	7,4 ± 0,04	7,28 ± 0,04	7,15 ± 0,04
Calcium:				
total (less shell)	26,1 ± 7,0	20,5 ± 5,4	18,5 ± 4,9	16,7 ± 3,5
haemolymph	5,7 ± 1,6	5,5 ± 1,5	12,6 ± 3,5	14,8 ± 4,2
Sodium:				
total (less shell)	33,7 ± 2,5	20,2 ± 3,3	18,9 ± 3,0	14,8 ± 4,2
haemolymph	36,9 ± 3,2	31,2 ± 2,9	27,8 ± 2,7	16,6 ± 2,6
Potassium:				
total (less shell)	21,0 ± 3,3	21,7 ± 2,8	22,0 ± 3,0	21,6 ± 3,7
haemolymph	3,0 ± 0,2	2,5 ± 0,6	2,0 ± 2,0	2,6 ± 2,5
Chlorides:				
total (less shell)	28,1 ± 2,8	23,1 ± 1,8	13,3 ± 2,3	14,4 ± 1,6
haemolymph	36,7 ± 1,1	24,2 ± 1,4	21,6 ± 2,1	13,8 ± 0,7
Water:				
pH	8,50	8,40	8,50	8,47
Epithelial potential (mV)*	−19,9 ± 1,4	18,3 ± 0,6	−14,9 ± 1,1	−9,4 ± 1,6

*Negative inside snail

Table 2 Influence of copper sulphate on the ion fluxes for *B. (B.) tropicus*. The values for sodium, calcium and chlorine are expressed in micromoles per litre per hour and for water the influx and efflux rates are expressed as rate constants, K_i and K_e per hour. Each measurement represents the average and standard deviation (±) of 30 snails at 26 °C with a water pH of 8,50

	Copper sulphate (ppm)			
	Controls	0,2	1,0	4,0
Na-influx	33,8 ± 2,4	26,9 ± 0,3	2,6 ± 0,3	1,8 ± 0,2
Na-efflux	23,1 ± 7,1	28,8 ± 9,1	32,4 ± 9,8	43,5 ± 13,4
Ca-influx	67,8 ± 6,3	57,0 ± 4,9	32,7 ± 2,7	12,6 ± 3,1
Ca-efflux	33,4 ± 1,2	36,6 ± 6,5	56,1 ± 4,8	75,0 ± 14,8
Cl-influx	22,6 ± 1,6	24,2 ± 5,3	2,2 ± 1,2	0,88 ± 0,19
Cl-efflux	39,3 ± 1,1	44,9 ± 1,2	56,3 ± 1,4	235,1 ± 9,7
^3H_2O-influx	6,75 ± 1,4	4,26 ± 0,89	1,5 ± 0,60	0,84 ± 0,16
^3H_2O-efflux	0,20 ± 0,01	0,19 ± 0,01	0,15 ± 0,01	0,15 ± 0,02
Net osmotic influx ($\mu l h^{-1}$)	0,78	0,55	0,39	0,36

(Figure 2) and chlorine leaving the snail (measured as efflux rates) increases remarkably. Contrary to this the sodium and chlorine influx rates virtually come to a standstill. The influx of tritiated water is about 85% less in a 4 ppm copper sulphate solution compared to the controls. The net osmotic inflow which is not coupled to active transport processes (Potts *et al.* 1967) fell to about half the value found for the controls. The rapid increase

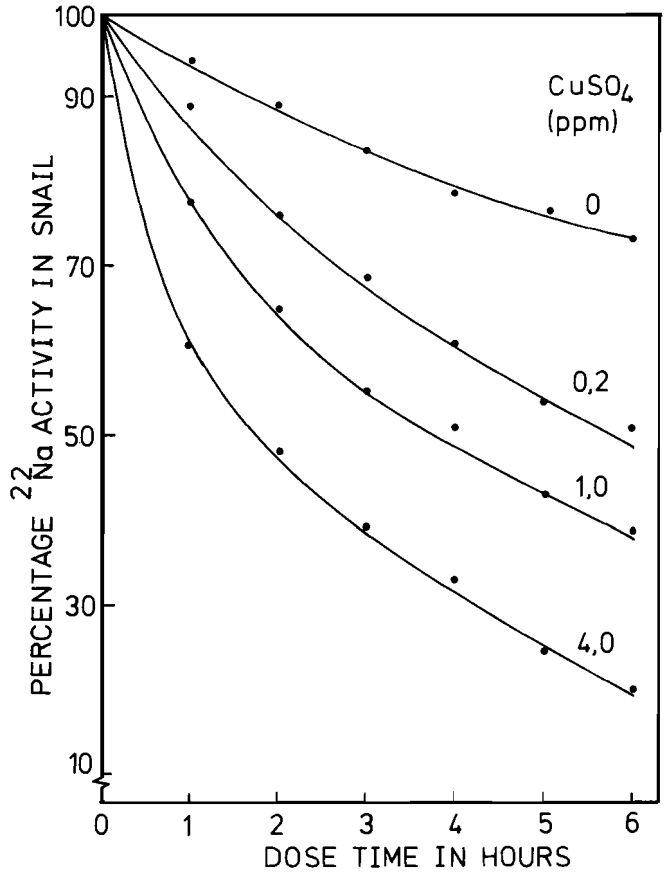

Figure 2 Percentage decrease of sodium-22 activity in laboratory snails exposed for 6 h to various concentrations of copper sulphate.

of calcium-45 in the water from snails loaded with calcium-45 for 48 h and the concommitant decrease of the calcium concentration in the snail (Table 1) supports the notion that this element is not just adsorbed on the body wall or lodged only in the mucous secretion during the loading period but that it penetrates through the integument of the snail. This is true also for the radioisotopes used for sodium and chlorine in the snails loaded for a period of 12 h. These isotopes are transported into the snail's haemolymph or tissues.

The rapid decline of sodium (Figure 2), chlorine and calcium isotopes in the water for the controls undoubtedly points to a very effective intake mechanism through the body wall or some other specific site. Again, as regards flux measurements of the above-mentioned ions the field snails tested show exactly the same results as those obtained for the laboratory reared snails.

Discussion

During exposures of six hours, the three sublethal concentrations of $CuSO_4$ have a deleterious effect on the water and ionic balance of *B. (B.) tropicus*. Contrary to the literature, copper sulphate is very effective on this species of snail when dissolved in the highly alkaline water found in the Potchefstroom area. Spronk, Brinkman, van Hoek & Knook (1971) studying other physiological effects of copper on *Lymnaea stagnalis*, mentioned a considerable decrease of sodium and chlorine in the haemolymph. The work of Cheng & Sullivan (1977) proved that sublethal concentrations of copper sulphate drastically altered the osmoregulation of *Biomphalaria glabrata*. They attribute the cause of death to a high osmotic inflow of water and argue against a massive leakage of electrolytes from the snail. Other physiological effects caused by copper included the change in rate of heart beat and the rate of oxygen consumption observed in *B. glabrata* (Cheng & Sullivan 1974). Using the latter physiological parameters as a quick bio-assay method they found considerable reductions in both the rate and amount of oxygen consumed. According to Hanumante, Nagabhushanam & Vaidya (1979), copper sulphate increases the nuclear diameter of certain neuro-secretory cells in *Indoplanorbis exustus*, probably causing an increase in the rate of transport and release of neurosecretory material.

From these observations it is evident that the patho-physiological effects of copper are not limited to particular organs, tissues or cells but occur throughout the body of the snail. Furthermore, the patho-physiological effects are undoubtedly the direct cause of the copper itself as it has been shown that this anion is absorbed by the snail (Yager & Harry 1964; Spronk *et al.* 1971; Brown & Newell 1972; Cheng & Sullivan 1974). In accounting for our own results on *B. (B.) tropicus* it seems plausible to postulate that the basic cause of the gross disturbances of the water and ionic balance is the change in permeability of the cell membranes, particularly of the epithelial cells. If the differential permeability of the cell membrane for Cl^-, Ca^{2+} and Na^+ were abolished by the copper ion, this would explain the high efflux rates found. To substantiate this, Walshe (1966), showed that the ATP-ase activity necessary for active membrane transport is inhibited by the copper ion. This in turn could explain why the heart rate and oxygen consumption decrease when *B., glabrata* is exposed to copper solutions (Cheng & Sullivan 1977). Why the water efflux, contrary to the Na^+, Cl^-, Ca^{2+} efflux, decreases is not clear. It seems logical to explain it in terms of the larger ionic radius of the water molecules compared to those of sodium and chlorine, or the smaller difference in water potential between that inside and outside the animal. The passive movement of water may, however, also be coupled to an active transport process (Diamond & Bossert 1967) explaining the low water influx and efflux rate values of copper-treated snails. The sites for these passive movements of water coupled with active water transport via epithelial cells would most likely be situated in the kidney (Wendelaar-Bonga & Boer 1969) and to a lesser extent in the integumental epithelium.

The decrease in the Cl^- and Na^+ concentrations in the haemolymph of copper treated *B. (B.) tropicus* was similar to that found by De With, Witteveen & van der Woude (1980) using ethoxzolamide on *Lymnaea stagnalis*. They demonstrated that this compound inhibits the high carbonic anhydrase activity of the epithelial cells of the body wall, blocking of this enzyme having a dramatic effect on the integumental Na^+ to H^+ and Cl^- to HCO_3^- exchange mechanisms. In the haemolymph Na^+ and Cl^- levels decrease with a corresponding increase in the H^+ and HCO_3^- levels to maintain electroneutrality inside the snail (De With *et al.* 1980). We could, however, not demonstrate a concommitant increase in the HCO_3^- haemolymph concentration (measured as total CO_2) when snails were exposed to

1 ppm copper sulphate. In addition we found the negative potential inside *B. (B.) tropicus* to become progressively less when snails were exposed to increased copper concentrations.

It is not clear why the potassium concentration in the haemolymph and tissues stays constant when exposed to copper sulphate. De With (1977), however, found a marked ability to regulate potassium concentration in the haemolymph by *Lymnaea stagnalis* when specimens were kept for 10 days in potassium-free water. To explain this he suggested a buffer system for K^+ in the haemolymph controlled by a process of sustained release of K^+ from the snails' tissues. This view, however, does not explain the constancy of potassium content in the tissues when snails are treated with copper. It is possible, though, that many of the cell membranes in the muscular body wall of the snail are not yet affected after a short period of 6 h exposure to the copper. Determinations of the influx and efflux values for potassium could provide an answer to this phenomenon.

The decrease of calcium in the snails' tissue and the corresponding increase of this electrolyte in the haemolymph during copper exposure can be seen as a massive leakage of calcium from the tissues. This is caused by the increasing release of calcium from the calcium cells (Greenaway 1971b; Sminia, De With, Bos, van Nieuwmegen, Witter & Wondergem 1977; De With & Sminia 1980). How the copper affects the release from the calcium cells is not clear. The high accumulation of calcium in the haemolymph may be temporary as high efflux values were found for this electrolyte. It is possible that copper, like other inhibitors (De With *et al.* 1980), impairs the calcium transport system at the mantle's edge to the tissues, resulting in an overall decrease of Ca^{2+} initially in the tissues of the snail.

The dramatic decrease of sodium and chloride concentrations in the haemolymph of *B. (B.) tropicus* may be used as a bio-assay method in the laboratory and in preliminary tests for copper-releasing compounds as a molluscicide. Haemolymph sampling is relatively easy to perform on *B. (B.) tropicus*. The determination of sodium and chlorides is fast, reliable and relatively simple. It can be used in conjunction with other proven bio-assay methods such as measuring the oxygen consumption and heartbeat rate. For most of the South African freshwater snails, however, including *B. (B.) tropicus*, the heart beat cannot be visually observed because of overlying pigmentation and a rather thick shell. We have also found considerable variation in the oxygen consumption rate between individuals of *B. (B.) tropicus* under normal conditions, which limits the use of this parameter (Van Aardt & Frey 1979). The very small differences found in the ionic and water balance between field and laboratory snails justifies the use of field snails for testing molluscicides provided that the snails are not infected with parasites causing physiological alterations.

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