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Toxic Effects of Chromium on *Schistosoma haematobium* miracidia

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Various heavy metals, among which numerous copper compounds (Cheng and Sullivan 1974, 1977; Wolmarans and Van Aardt 1986; Wolmarans, Van Aardt and Coetzee 1986), have recently been evaluated as molluscicides for freshwater snails, which act as intermediate hosts of trematode parasites of medical or veterinary importance. Very little information, however, is available on heavy metals that may be suitable to eliminate the parasites as such. Hunter, Kemp, Smalley, Wilkins and Dixon (1956) tested 127 compounds for their potential to eliminate the cercariae of schistosome parasites. Suitable compounds, however, should also, amongst others, inhibit the penetration ability of parasites as well as stunt the development of those who do penetrate their hosts.

In the light of these requirements, the present study evaluated the effect of chromium on the miracidia of Schistosoma haematobium, which causes urinary bilharzia. Attention was mainly focussed on (1) the chromium concentration which resulted in 100% mortality (2) the effect of chromium on the external and internal morphology of the miracidia, and (3) the ability of the miracidia to form sporocytes in vitro and in vivo and to penetrate their intermediate host snail, Bulinus africanus.

MATERIALS AND METHODS

Freshly hatched miracidia were used in all the experiments. Schistosome eggs were isolated from homogenized livers of infected Saccostomys campestris mice by means of a helminth filter and transferred to freshwater to hatch. Chromium chloride solutions were made up at concentrations of 4.5, 3.6, 2.7, 1.8 and 0.9 mmol, 4.5 umol and 4.5 nmol in 0.45 um Millipore filtered tap water. All experiments were carried out in exposure volumes of 10mm /miracidium at room

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temperature.

In order to determine the chromium dose which resulted in 100% mortality within 1 hr of exposure, 7 groups of 100 miracidia each, were transferred separately to all the chromium solutions.

During this period, the miracidia were monitored microscopically to determine which chromium concentrations resulted in 100% mortality. Miracidia which survived were rinsed and transferred to chromium-free water for 2 hr after which they were re-examined for further mortalities. This period was important for the subsequent experiments because miracidia surviving the chromium exposure were left for a further 2 hr to penetrate the snails.

The effect of chromium on the external morphology was investigated in 5 groups of 100 miracidia each. These groups were separately exposed for 5, 15 and 60 minutes respectively to 4.5 mmol chromium and for 60 minutes to 4.5 umol and 4.5 nmol chromium. After these periods the miracidia were rinsed 3 times in chromium-free water and fixed in a 1:1 mixture of 2% glutaraldehyde and 2% formaldehyde. The active swimming control miracidia were kept in freshwater for 1 hr and then fixed. The fixed material was prepared for electron microscopy according to the instructions of Ekly-Natey, Wuest, Swiderski, Striebel and Huggel (1985). The presence of chromium on the miracidium surface was investigated with the aid of an X-ray microanalyzer coupled to an electron microscope (Cambridge model SEM 250).

The pathological effect of chromium on the internal morphology of the miracidia was studied by means of a transmission electron microscope (Philips model CM10). The exposure and fixing procedures closely resembled those used for the morphological investigations except that one group of 100 miracidia was killed instantly by heat, left for 1 hr and then fixed. These miracidia acted as controls for the miracidia which died during exposure to chromium and which were then left for a period up to 1 hr. The fixed miracidia were then bedded into 1.5% agar blocks of 2 x 3 mm and prepared for microscopy (Wolmarans, van Aardt and Coetzee 1986).

The ability of the exposed miracidia to transform in vitro to sporocysts was investigated in 7 groups of 100 miracidia each. These groups were separately exposed to the different chromium solutions for 1 hr, rinsed in chromium-free water and transferred to sporocyst culture medium (DiConza and Basch 1974) for 48 hr. The number of sporocysts formed during this period was hereafter determined by means of a light microscope.

In vivo transformation was studied in 3 groups of 25 miracidia each, separately exposed to 4.5 mmol chromium for 5 minutes and to 4.5 umol and 4.5 nmol chromium for 1 hr. The miracidia were then rinsed in chromium-free water after which 15 snails were each challenged for 2 hr with 5 miracidia exposed to a specific concentration. The snails were then kept for 35 days at 25 C after which they were periodically screaned for cercaria shedding.

The ability of chromium exposed miracidia to penetrate snails was investigated as mentioned for the in vivo transformation, except that 250 miracidia were used in each of 3 groups. Each of these groups were subdivided into 5 subgroups of 50 each. The snails were then challenged for 2 hr with 1 group of 50 miracidia. For the control experiment, 5 additional snails were challenged with 50 miracidia each that had not been exposed to chromium. After the 2 hr penetration period, the snails were removed and the miracidia which failed to penetrate were counted.

RESULTS AND DISCUSSION

From Table 1 it can be seen that only the 4.5, 3.6, 2.7 and 1.8 mmol chromium solutions resulted in 100% mortality within a period of 1 hr. None of the other concentrations caused any mortalities within this period. It should be noted that no mortality occurred among miracidia which survived exposure in the remaining concentrations and were kept in chromium-free water afterwards.

The effect of chromium on the external morphology of the miracidia is illustrated in Fig. 1 to 6. From these figures it can be seen that only the miracidia that had been exposed to 4.5 mmol chromium (Fig. 4-6), changed their shape substantially when compared to the control. This can probably be ascribed to the contraction of the underlying longitudinal muscle tissues. Longer exposure periods to 4.5 mmol chromium did not lead to noticably more extensive morphological changes. The presence of chromium aggregates on the outer surface of all the exposed miracidia were striking. Although well differentiated epidermal plates were not observed on miracidia exposed to 4.5 mmol chromium, no loss of epidermal plates or cilia was observed on any of the miracidia.

The effect of chromium on the internal morphology of the miracidia is illustrated in Fig 7 to 10. It can be seen from Fig 7 (miracidia exposed to 4.5 mmol chromium for 60 minutes) that a noticable degeneration of tissue occurred. The possibility that this phenomenon may be attributable to the natural degeneration of tissue

before the dead miracidia were fixed plays a minor role since miracidia that were killed instantly with boiling water (Fig. 12) and fixed after 60 minutes, showed markedly less degeneration. In contrast to this, it is clear that miracidia exposed for 5 and 15 minutes to 4.5 mmol chromium (Fig 8 and 9) and for 60 minutes to 4.5 umol and 4.5 nmol chromium (Fig 10 and 11) did not show signs of damage when compared to the control miracidia (Fig. 12).

From Table 1 it can be seen that 100% sporocyst transformation occurred in control miracidia and miracidia exposed to 4.5 nmol chromium. In the miracidia exposed to 4.5 umol and 0.9 mmol chromium, 93% and 91% transformation occurred respectively.

The results obtained from in vivo sporocyst transformation (Table 2), indicate that no further development occurred in miracidia exposed for 5 minutes to 4.5 mmol chromium. This finding can on the one hand be ascribed to the direct effect of chromium and on the other hand to the possibility that the eradication of these weakened miracidia by the immune system of the snails was more successful. Contrary to this, all 5 snails challenged with control miracidia and miracidia exposed to 4.5 nmol chromium for 60 minutes shed cercariae, while 4 out of 5 of the snails challenged with miracidia exposed to 4.5 umol chromium produced cercariae. It is further clear that only 50% of the miracidia exposed for 5 minutes to 4.5 mmol chromium managed to penetrate. Of the miracidia exposed for 60 minutes to 4.5 umol and 4.5 nmol chromium, 62% and 56% respectively penetrated snails while 76% of the control miracidia, penetrated. However, the trends observed in penetration can not be ascribed solely to the effect of chromium, since not all miracidia that hatch are capable of penetration, regardless of external influences. Meuleman (1980) found inter alia that only 34% of S. mansoni miracidia were capable of penetration under normal conditions. However, it remains difficult to distinguish without doubt whether those miracidia that did make contact with the snails penetrated, or only swam into the mantle cavity of the snail, but had not yet penetrated.

From the present study it seems as if chromium has the potential, due to its toxic effect on miracidia, to break the transmission cycle of S. haematobium. According to Edungbola (1980), the development of compounds with molluscicidal, miracidicidal and cercaricidal properties will contribute substantially to the control of schistosomiasis. Therefore it is evident that the effect of chromium on the cercarial stage of the parasite should also be considered.

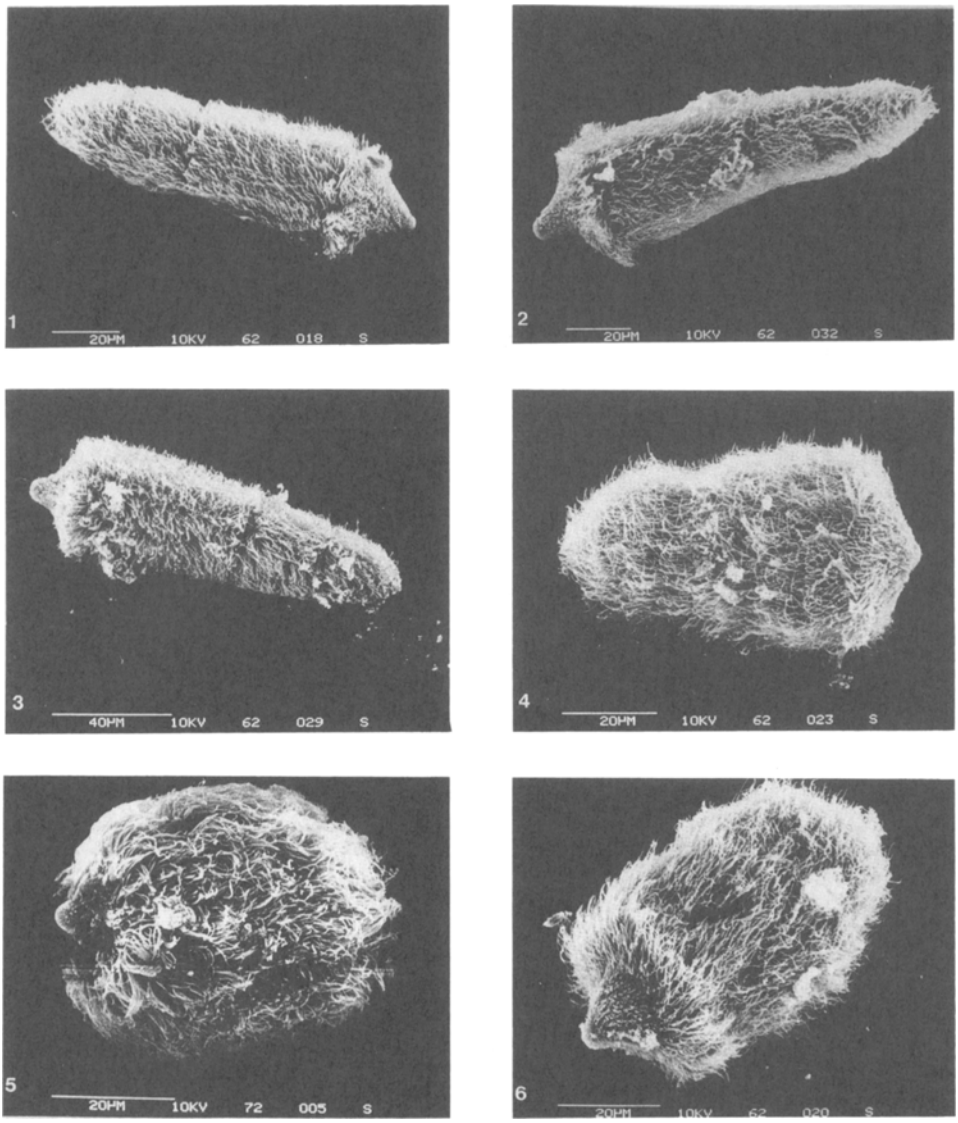

Figure 1 Electron micrograph of the external morphology of a control miracidium.

Figure 2-3 Electron micrographs of the external morphology of miracidia exposed to 4,5 nmol and 4,5 µmol chromium for 1 hr respectively.

Figure 4-6 Electron micrographs of the external morphology of miracidia exposed to 4,5 mmol chromium for 5, 15 and 60 minutes.

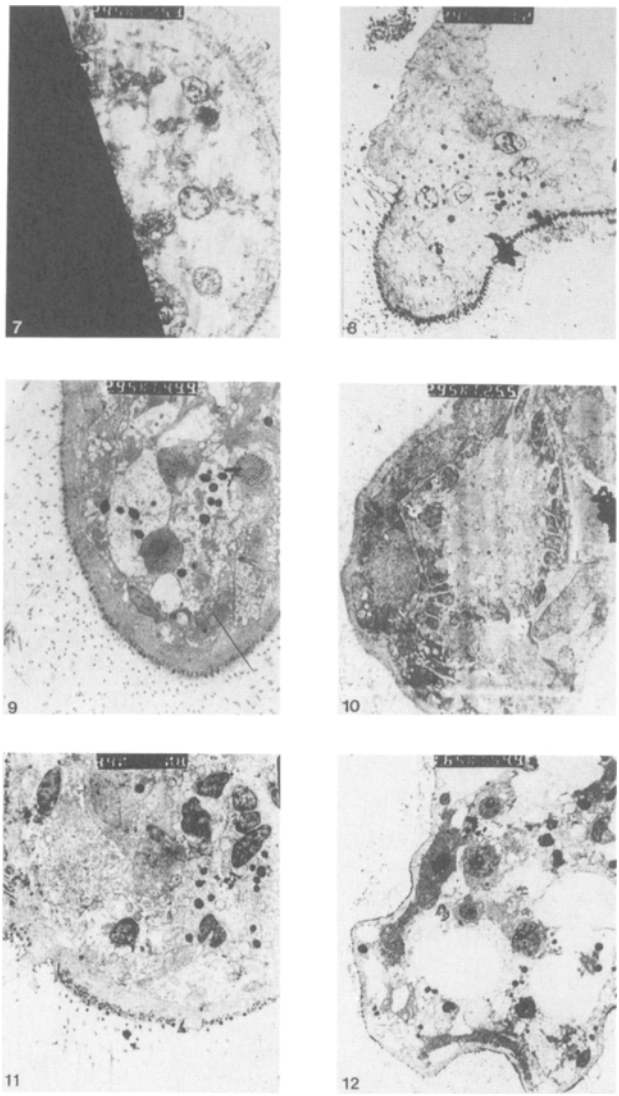

Figure 7-9 Electron micrographs of the internal morphology of miracidia exposed to 4,5 nmol chromium for 60, 5 and 15 minutes respectively.

Figure 10-11 Electron micrographs of the interal morphology of miracidia exposed to 4,5 µmol and 4,5 nmol chromium for 1 hr respectively.

Figure 12 Electron micrographs of the interal morphology of a miracidium killed instantly with boiling water and fixed 60 minutes later.

Table 1. The percentage of (i) mortality of miracidia within 1 hr of exposure (ii) miracidia that transformed to sporocysts and (iii) that penetrate after exposure to chromium, as well as the number of snails out of 5 challenged with miracidia which shed cercariae

Chromium Concentration	Exposure period	% Mortality within 1hr	% In vitro sporo= cyst formation	% Miracidia that penetrated	Number of snails that shed cercaria
4.5 mmol	60 min	100	-	50	0
	5 min			50	0
3.6 mmol	60 min	100	-		
2.7 mmol	60 min	100	-		
1.8 mmol	60 min	100	-		
0.9 mmol	60 min	0	91		
4.5 umol	60 min	0	93	62	4
4.5 nmol	60 min	0	100	56	5
Control	60 min	0	100	76	5

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