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Research Note

Effects of copper sulphate on *in vitro* encystment of the cercariae of *Echinostoma caproni*

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Abstract

The effects of various concentrations of copper sulphate were studied on *in vitro* encystment of *Echinostoma caproni* in a Locke's–artificial spring water (ASW) (1:1) medium. Cercariae were killed in 10,000 mg l^{-1} $CuSO_4$ in Locke's–ASW (1:1) within 24 h and extruded cystogenous material to produce an abnormal cyst wall. The 'emergency response' of encystment to high concentrations of copper reported for *Parorchis acanthus* cercariae did not occur in *E. caproni.* Concentrations of 1000 mg l^{-1} and 100 mg l^{-1} $CuSO_4$ in Locke's–ASW (1:1) also killed the cercariae without encystment by 48 h. A concentration of 10 mg l^{-1} $CuSO_4$ in Locke's–ASW (1:1) allowed for normal *in vitro* encystment within 48 h and these cysts were capable of excystation in a trypsin–bile salts medium.

Fried & LaTerra (2002) described a Locke's–artificial spring water (ASW) (1:1) medium that allows for the successful *in vitro* encystment of the cercariae of *Echinostoma caproni*. They reported 67% encystment of cercariae in this medium within 48 h at 23°C. The cysts formed *in vitro* could be excysted chemically in the trypsin and bile salts medium of Fried & Roth (1974) and were infective to mice (Fried & LaTerra, 2002). The production of cysts *in vitro* of echinostomes allows for experiments usually done only on cercarial species that encyst outside a host on surfaces such as the shells of crabs and molluscs and on the inside of laboratory glassware. One such species that encysts on surfaces is *Parorchis acanthus*, which has been used to study the *in vitro* effects of toxicants on survival, infectivity and encystment of cercariae, e.g. Bennett *et al.* (2003). Bennett *et al.* (2003) examined the effects of copper on encystment of *P. acanthus* cercariae, and noted that in high concentrations of copper, the percentage of cysts formed was greater than that of the sea water controls. They suggested this phenomenon represents an 'emergency response' that cercariae can use in the presence of polluted waters. The 'emergency response' idea reported by Bennett *et al.* (2003) prompted us to test the effects of various concentrations of copper on the *in vitro* encystment of *E. caproni* cercariae.

Cercariae were obtained from experimentally infected *Biomphalaria glabrata* as described in Idris & Fried (1996) and were tested at 23°C in 6-cm Petri dishes containing 15 ml of the encystment medium; about 30 cercariae per dish were tested as described in Fried & LaTerra (2002). Each dish contained various concentrations of copper in the form of cupric sulphate pentahydrate ($CuSO_4{\cdot}5H_20$, Sigma). The diluent used for the $CuSO_4$ was the Locke's–ASW (1:1) *in vitro* encystment medium of Fried & LaTerra (2002) and the concentrations of $CuSO_4$ used were 10,000 mg l^{-1}, 1000 mg l^{-1}, 100 mg l^{-1} and 10 mg l^{-1}. Observations were made 24 and 48 h after the cultures were set up. Each experiment at concentrations of 10,000 mg l^{-1}, 1000 mg l^{-1} and 100 mg l^{-1} was repeated twice, while the experiment in 10 mg l^{-1} $CuSO_4$ was repeated three times. Controls always consisted of the Locke's–ASW (1:1) without $CuSO_4$.

All cercariae were dead in 10,000 mg l^{-1} and 1000 mg l^{-1} $CuSO_4$ within 24 h. In 10,000 mg l^{-1} $CuSO_4$, more than 50% of the dead cercariae showed the presence

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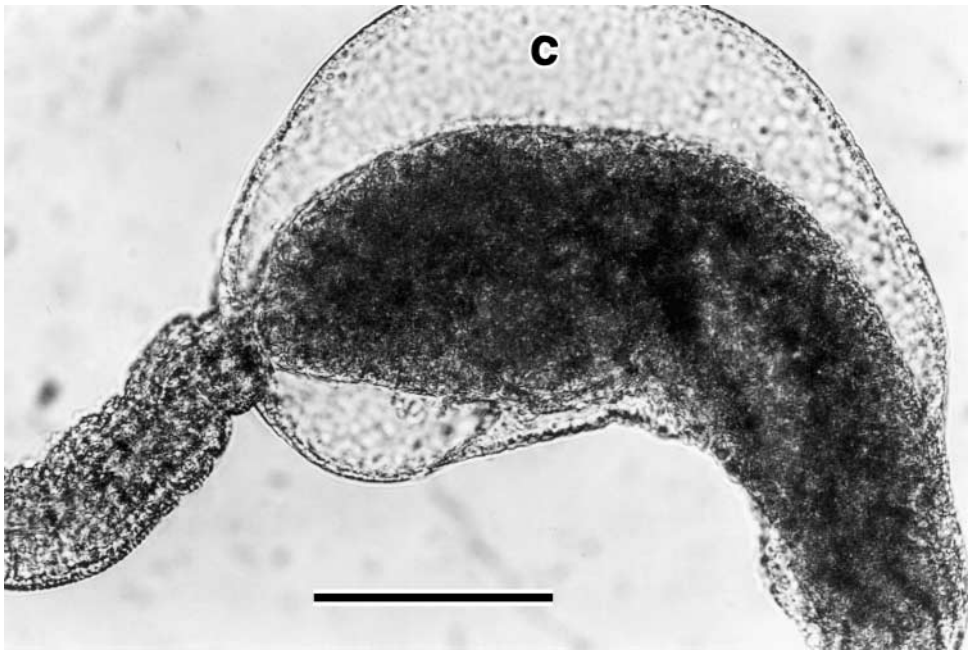

Fig. 1. Formation of an abnormal cyst (C) around the dead cercarial body of *Echinostoma caproni* in 10,000 mg l^{-1} $CuSO_4$ in Locke's-ASW (1:1). Scale bar = 100 μm.

of an abnormally formed outer cyst as shown in fig. 1. The tail was usually flexed toward the body; the body was granular and no internal structure was apparent. Cercariae in 1000 mg l^{-1} $CuSO_4$ were also dead within 24 h; their tails were intact and typically flexed toward the body. However, these organisms did not show the presence of an abnormally formed outer cyst. The effects of encystment in 100 mg l^{-1} $CuSO_4$ showed that all cercariae were dead by 48 h and no cysts were formed. Only cercariae in 10 mg l^{-1} $CuSO_4$ had encysted within 48 h.

Of the 99 cercariae tested in 10 mg l^{-1} $CuSO_4$, 30 (30%) had encysted. This compared to 55 of 80 (or 69%) that encysted in the controls. Chemical excystation studies on 16 of the cysts formed *in vitro* in the 10 mg l^{-1} $CuSO_4$ solutions showed that 9 of 16 (56%) excysted within 2 h compared to 12 of 14 (86%) that had excysted from the controls.

In conclusion, the 'emergency response' producing normal-appearing cysts in high concentrations of copper as reported for *P. acanthus* by Bennett *et al.* (2003) did not occur in *E. caproni.* The high concentration of $CuSO_4$ (10,000 mg l^{-1}) in the Locke's–ASW (1:1) medium killed within 24 h the *E. caproni* cercariae and induced the extrusion of cystogenous material from many of the organisms resulting in the occurrence of abnormal cyst walls. This study also showed that *E. caproni* cercariae can encyst *in vitro* in the Fried & LaTerra (2002) medium containing 10 mg l^{-1} $CuSO_4$ and that cysts formed therein were normal and capable of excysting in the trypsin and bile salts medium of Fried & Roth (1974).

It may be worthwhile to speculate why the *E. caproni* cercaria does not demonstrate an 'emergency response'. Cercariae of this species ordinarily encyst within the kidney–pericardial region of gastropod hosts, whereas cercariae of *P. acanthus* encyst on surfaces. Perhaps the *E. caproni* cercariae, protected by the internal environment of its host, has not evolved the need for an 'emergency response' as seen in the cercaria of *P. acanthus*, which encysts on surfaces.

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