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## The characteristics of redial generations in *Lymnaea truncatula* exposed to *Fasciola hepatica* miracidia after poisoning by sublethal doses of cupric chloride

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**Summary** — Adult *Lymnaea truncatula* were placed in a sublethal dose of copper chloride (0.1 mg/l) for 1 h, and then in normal water before being exposed to a single *Fasciola hepatica* miracidium on the 2nd or 9th day of the experiment. Infection rates were higher in untreated snails and in snails infected 9 d after treatment (73.5 and 71.4%) than in snails infected 2 d after copper contact (48.1%). Histological examination of surviving snail on the 30th day after miracidial exposure revealed that the total number of rediae was 25 and 31 per snail in snail groups infected 2 and 9 d respectively after copper contact, and 44 in untreated controls. There was a drop in the number of live, free rediae in infected snails after treatment contact. This decrease was more marked in the first redial generation than in the first cohort of the second generation. These results reveal that the infection rate in snails and the redial burden of *F hepatica* were reduced by the copper treatment of snails before miracidial exposure but placement of survivors in water for 8 d after treatment limited the consequences of chemical toxicity.

**copper / *Fasciola hepatica* / *Lymnaea truncatula* / redia**

**Résumé** — Les caractéristiques des générations rédiennes chez *Lymnaea truncatula* exposée aux miracidiums de *Fasciola hepatica* après un empoisonnement par des doses sublétales de chlorure cuivrique. Des *Lymnaea truncatula* adultes ont été placées dans une solution sublétale de chlorure cuivrique (0,1 mg/l) pendant 1 h, puis dans de l'eau normale avant d'être exposées chacune à un miracidium de *Fasciola hepatica* au 2<sup>e</sup> jour ou au 9<sup>e</sup> jour de l'expérience. Le taux d'infestation des mollusques était plus élevé chez les témoins non traités et les limnées infestées 9 j après le traitement (73,5 et 71,4%) que chez les mollusques infestés 2 j après le contact avec le cuivre (41,8%). L'examen histologique des limnées survivantes au 30<sup>e</sup> jour après l'exposition miracidienne a montré que le nombre total des rédies était respectivement de 25 et de 31 par mollusque chez les mollusques infestés 2 et 9 j après le contact avec le cuivre au lieu de 44 chez les témoins non traités. On a noté une chute dans le nombre des rédies indépendantes et en vie chez les limnées infestées après le traitement. Cette diminution était plus marquée dans la première génération rédienne que dans la première cohorte

de la seconde génération. Ces résultats montrent que le taux d'infestation des mollusques et la charge rédienne de *F. hepatica* ont été réduites par le traitement des mollusques par le cuivre avant l'exposition miracidienne mais le placement des survivants dans de l'eau pendant 8 j après le traitement a limité les conséquences de la toxicité chimique.

**cuivre / *Fasciola hepatica* / *Lymnaea truncatula* / rédie**

## INTRODUCTION

The efficiency of copper salts in the control of *Fasciola*-bearing snails has been well known for many years (Taylor, 1965; Boray, 1969). Copper sulphate and, more recently, cupric chloride were found to be highly lethal to the amphibious snail, *Lymnaea truncatula* and field trials were carried out using these chemicals over the snail habitats in swampy meadows (Pêcheur, 1974; Rondelaud, 1986).

Previous investigations were performed on the effects of cupric chloride in the *L. truncatula* habitats (Rondelaud, 1988). It was found that snail reproduction and movements of survivors are disturbed by the discharge of a 1 mg/l cupric chloride solution in the habitats. The fast emersion of *L. truncatula* led to colonization and egg deposits on the wet emerged areas. There was a reduction in the number of egg masses per snail and in the daily distance covered by these *L. truncatula* but these perturbations were maximum after the chemical discharge and gradually disappeared over time.

Few data are currently available on the bionomics of *Fasciola* infection in *L. truncatula* when miracidial penetration into the snail occurs after the end of snail poisoning. Our first results raised the following questions. Is the infection rate of *L. truncatula* decreased when miracidial exposure is performed with snails which survive poisoning by cupric chloride? What are the consequences on the redial generation development? To answer these questions, we exposed snails to a sublethal solution

of cupric chloride and we infected the surviving snails 1 or 8 d after their stay in normal water. The present work reports our observations on the infection rate and development of redial generations on the 30th day after miracidial exposure.

## MATERIALS AND METHODS

Adult *L. truncatula* measuring 4 mm in height were collected in ditches along the D20 road in the communes of Migné and Nuret-le-Ferron, department of Indre (France). The 750 collected snails were divided into 3 groups. The control group comprised 150 untreated snails, which were each exposed to a single *F. hepatica* miracidium for 4 h at 20°C. The other 600 snails were placed in a 0.1 mg/l solution of  $\text{CuCl}_2$  in breeding-container water (calcium ion content, 60 mg/l) for 1 h (Rondelaud, 1986). There were 50 snails/l of solution. The snails were then placed under normal conditions (oxygenated water at 20°C, lettuce *ad libitum*) and the survivors were counted on the 2nd day. Half of the 298 survivors were then exposed to *F. hepatica* miracidia (1 parasite per snail) and the other half 1 week later (day 9 of the experiment).

The snails were then raised for 30 d (at 20°C) in closed-circuit aquaria (10 snails/l of water). On the 30th day following miracidial exposure, the shell height of survivors was measured. The snails were killed by immersion in Bouin's fixative followed by immediate breaking of the shell. Serial sections (5 µm thick) were stained with Harris' hematoxylin and modified Gabe's trichrome. The use of these stains permitted a good recognition of immature parasites in the snail body.

Snail infection rates were calculated using the ratio of the numbers of infected snails to the number of survivors on d 30.

The criteria used to recognize the redial generations have previously been defined by Ron-

delaud and Barthe (1987). Rediae were classified depending on the form and size of their pharynx into the following categories: first generation; first cohort of the second generation; subsequent generations. We also considered redial independence or dependence (when the rediae are free in the snail body, or when they are located in the sporocyst or in the body of another redia), the physiological state (whether living or degenerating), and maturity as determined by the presence of cercariae and procercariae. Redial maturity was expressed as a percentage in relation to the total number of live, independent forms.

Mean values and standard deviations were determined in each group for shell heights. They were also calculated from the individual redial burden values while taking into account the redial generation. The mean values were subjected to Anova.

## RESULTS

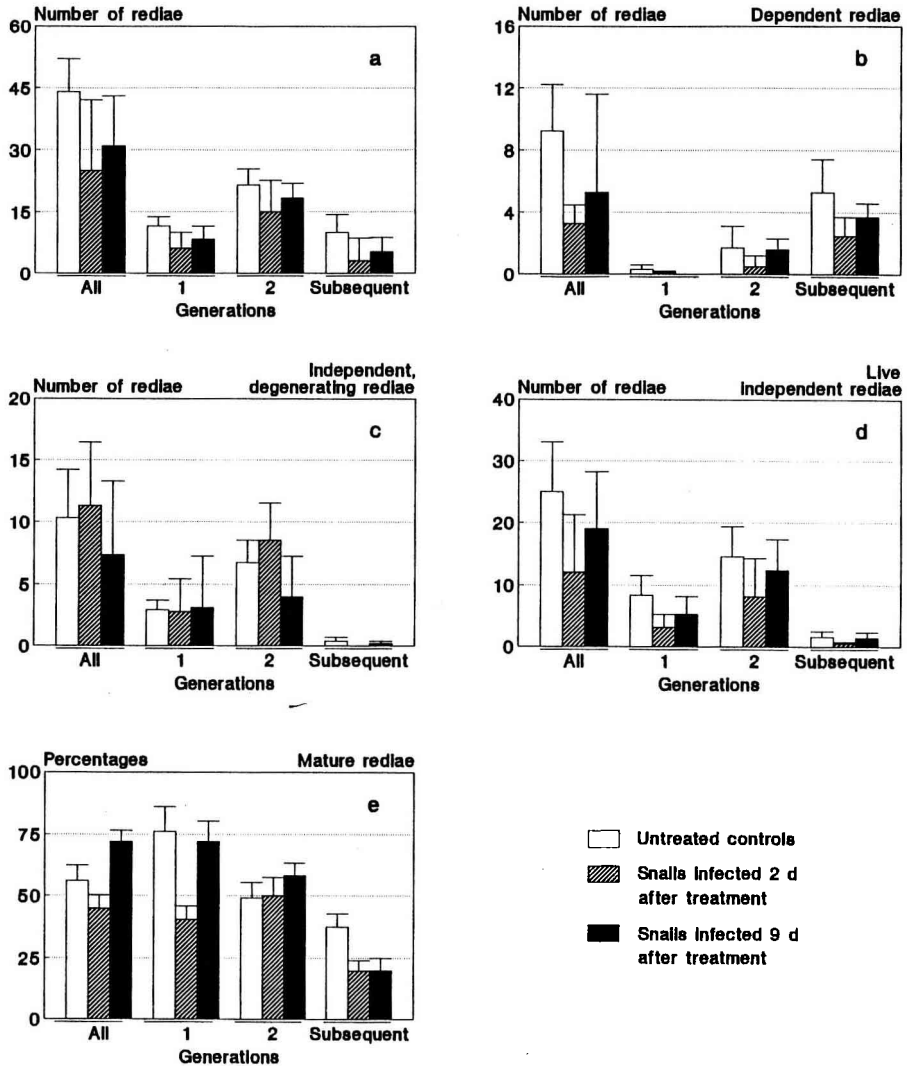
The numbers of surviving snails on the 30 th postexposure day were 87/150 in untreated controls, 27/199 in snails infected 2 d after treatment, and 21/199 in snails infected 9 d after copper contact. The infection rates were higher in untreated snails and in snails infected 9 d after treatment (73.5 and 71.4%) than in snails infected 2 d after copper contact (48.1%).

Shell height was  $7.8 \pm 1.1$  mm in the controls and was lower in the treated groups ( $5.7 \pm 2.3$  mm in snails infected 2 d after treatment, and  $6.1 \pm 2.7$  mm in snails infected 9 d after treatment). Significant differences in the mean heights between controls and treated snails were noted ( $P < 0.01$  and  $P < 0.05$ ) but the difference between the values of both treated groups was not significant.

Figure 1a shows the distribution of redia numbers in the 3 groups and demonstrates that the mean values obtained for all the generations were lower in treated groups than in controls (a mean of 25 and 31 rediae instead of 44). Differences in the mean numbers of rediae between controls and

the other groups were significant ( $P < 0.01$ ) but the difference between treated groups was not significant. When compared with control values, the number of the first generation rediae in the group infected 2 d after treatment was 47% lower ( $P < 0.01$ ) whereas the first cohort of the second generation was 30.3% lower ( $P < 0.05$ ). In group infected 9 d after treatment, these percentages were 27% ( $P < 0.05$ ) and 15% (not significant) respectively. In subsequent generations, the number of rediae was 69% lower in snails infected 2 d after copper contact ( $P < 0.01$ ) and 47% lower in the other treated snails ( $P < 0.05$ ).

Quantitative variations were noted in the distribution of rediae in relation to their physiological state. First, the dependent rediae of all the generations (fig 1b) in snails infected 2 d after treatment decreased in numbers from 64% ( $P < 0.01$ ) and those of subsequent generations were 66% lower ( $P < 0.01$ ) when compared with control values. In snails infected 9 d after copper contact, the differences in the mean numbers of dependent rediae between controls and treated groups were not significant for all the generations like for each generation considered separately. Secondly, independent, degenerating rediae of all the generations in the group infected 2 d after treatment were similar to those of controls (a mean of 10.3 vs 11.3 rediae), and slightly lower (non-significant difference) in the group infected 9 d after copper contact (7.3 rediae). There were no significant differences in the mean numbers of independent, degenerating rediae between controls and treated groups for each generation considered separately. Thirdly, live independent rediae of all the generations (fig 1d) were most affected with a number 52% lower in the group infected 2 d after treatment when compared with controls ( $P < 0.001$ ), and 24% in the other treated group ( $P < 0.001$ ). The first generation was more affected by this drop ( $P < 0.01$  and  $P < 0.05$ , respectively) than the



**Fig 1.** Distribution of *F hepatica* rediae in controls and treated groups in relation with their generation and their physiological state. Bars correspond to standard deviations.

first cohort of the second generation ( $P < 0.05$  and  $P < 0.05$ , respectively). The differences in the mean numbers of live, independent rediae between controls and treated groups were not significant for the subsequent generations.

The percentage of mature rediae for all the generations (fig 1e) was lower ( $P < 0.01$ ) in the group infected 2 d after treatment (45%) than in controls (56%), and clearly higher ( $P < 0.01$ ) in the other treated group (72%). For the first generation per-

centages, the only significant difference noted in the mean percentage of mature rediae was between controls and snails infected 2 d after treatment ( $P < 0.01$ ). Those of the second generation were similar in controls and treated groups. In subsequent generations, the percentages in both treated groups were considerably lower than in controls ( $P < 0.01$ ).

## DISCUSSION

Little is known on the effect of chemicals on the development of trematode parthenitae in freshwater pulmonate snails. In snails infected by *Schistosoma* sp, praziquantel stops the emission of cercariae for several days and histological examination of these treated snails revealed a total destruction of many mature cercariae (Andrews, 1978; Combes, 1982; Touassem and Combes, 1986; Yi and Combes, 1987). The results reported in this study demonstrate a reduction in the infection rate and the number of rediae in *L. truncatula* exposed to miracidia after copper contact. Chemical poisoning of the snail at a sublethal dose can therefore be added to the factors known already, such as snail body volume or habitat desiccation, which have an influence on the development of *F. hepatica* redial generations (Kendall 1949; Kendall and Ollerenshaw, 1963; Smith 1984; Rondelaud, 1994).

From our results, it should be noted that the copper action was limited over time since snails infected following an 8-d period in normal breeding water had an infection rate and a redial burden clearly higher than those found in infected snails following a single day in normal conditions. It is logical to suggest that the effects of this sublethal copper dose progressively disappeared as the stay of surviving snails in normal breeding water increased. The disappearance of

these effects may be interpreted in the light of observations by Moukrim *et al* (1988). According to these authors, the exposure of the snail *L. peregra* to a sublethal concentration of trichlorfon for 7 d and placing the surviving snails in normal breeding water for 28 d was followed by the development of epithelial necrosis in several viscera during 21 d at least with a further reconstitution and resumption of normal function. As these studies were carried out on *L. peregra*, which is a more resistant snail species than *L. truncatula*, it is difficult to extrapolate the results obtained with trichlorfon to the effects caused by copper chloride. However, epithelial necrosis has already been reported in the viscera of molluscs exposed to copper (Sparks, 1985) and it could be assumed that the disappearance of copper effects would be the consequence of these lesions' development in the *L. truncatula* organs because this visceral pathology is not specific of a single aggressive factor in this snail (Moukrim and Rondelaud, 1992). Other studies are necessary to verify this hypothesis by histological examination of *L. truncatula* exposed to sublethal doses of cupric chloride.

Our results demonstrate a drop in the number of live, independent rediae in infected snails after exposure to cupric chloride and the correlative increase in independent and degenerating parasites. These quantitative variations were more marked in the first redial generation than in the first cohort of the second generation. From these results, it is logical to think that the copper effects become manifest in the days that follow penetration of an *F. hepatica* miracidium into the intermediate host. If the developmental diagram of redial generations proposed by Rondelaud and Barthe (1982) is considered, it can be suggested that the copper would affect the initial sporocyst and the first redia of the first generation that arose from it.

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