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SHORT COMMUNICATIONS

DEMONSTRATION OF CALCIUM SALTS IN EXCRETORY CORPUSCLES OF LARVAL STAGES OF TREMATODES

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Abstract. Calcium salts were demonstrated by Kóssa's method in excretory corpuscles of five species of cercariae and six species of metacercariae. The interference of calcium salts in excretory corpuscles in Gomori's method for the detection of alkaline phosphatase is discussed.

In contrast to the authors studying the activity of alkaline phosphatase by means of Gomori's method we have not managed to detect this enzyme in excretory corpuscles of trematode larvae using the methods with diazonium salts. The activity of alkaline phosphatase was demonstrated only in the walls of excretory canals. Since it is known that calcium salts give false positive results in Gomori's method for the detection of alkaline phosphatase (Pearse 1968), we have attempted to demonstrate calcium salts in the excretory corpuscles of some cercariae and metacercariae by means of Kóssa's method.

MATERIALS AND METHODS

Cercariae of *Notocotylus ephemera*, cercariae and metacercariae of *Echinostoma revolutum*, *Hypoderaeum conoideum*, *Echinoparyphium aconiatum*, *E. recurvatum* and metacercariae of *Opisthioglyphe ranae* and *Cotylurus cornutus* (*Tetracotyle*) were fixed by Baker's fluid for 24 h at 20 °C and embedded into paraffin. Calcium salts were demonstrated in sections by means of Kóssa's method (Pearse 1968).

RESULTS

Excretory corpuscles present in the main excretory canals of all of the mentioned cercariae were found to be positive for calcium salts. Intensively stained excretory corpuscles are visible in Plate I, Figs. 1 and 2, showing longitudinal and transverse sections through *Echinoparyphium aconiatum* cercaria and in Plate I, Fig. 3, showing longitudinal and transverse sections through *Echinostoma revolutum* cercaria. Calcium salts present in excretory corpuscles accumulated in the excretory bladder of metacercaria are shown in Plate II, Fig. 4 (metacercaria of *E. revolutum*) and in Plate II, Fig. 2 (metacercaria of *Opisthioglyphe ranae*).

DISCUSSION

Calcium has been demonstrated in excretory corpuscles of trematode larvae only occasionally, namely in the metacercaria of *Acanthoparyphium spinulosum* (supravitally by alizarin red S) directly in the excretory system and in freed concretions by

quinalizarin and microanalysis (Martin and Bils 1964). Mitchell and Crang (1976) demonstrated calcium and magnesium in metacercariae of *Posthodiplostomum minimum* using X-ray microanalysis. The rather simple Kóssa's method was not used for the detection of calcium in trematode larvae. It was used for the first time for the detection of calcium salts in excretory corpuscles of *Echinostoma revolutum* (Žďárská and Našincová 1985). The inconsistencies in the data of alkaline phosphatase activity led us to a verification of the presence of calcium salts in excretory corpuscles in histological sections. Some authors, who used Gomori's method for the detection of alkaline phosphatase, found the activity of this enzyme also in excretory corpuscles (Cheng 1964, Fried et al. 1984). Since it is known that different substances in tissue, among them also preformed deposits of calcium and phosphates, interfere with the positive results of Gomori's method for the detection of alkaline phosphatase, it may happen that these false positive reactions are considered a real activity of alkaline phosphatase, if careful two controls (inactivation and incubation without substrate) are not performed. This concerns the excretory corpuscles of trematodes, in which calcium has been demonstrated. In our experiments, the method with diazonium salts (α-naphthyl phosphate + Fast blue BB — see Pearse 1978) was used for the detection of alkaline phosphatase. The activity of this enzyme, however, was demonstrated only in the walls of the excretory system of some cercariae (Žďárská and Panin 1977, Žďárská and Soboleva 1980, 1984a, b, Žďárská et al. 1980, 1984), but not in excretory corpuscles (Žďárská and Našincová 1985). Questionable is the statement of Le Flore and Bass (1983) who used also diazonium salts and described alkaline phosphatase activity in excretory corpuscles of *Himasthla rhigedana* metacercaria, though it is evident from their Fig. 2 that the excretory corpuscles are negative in this method. We have verified the presence of calcium in excretory corpuscles of several cercariae and metacercariae to eliminate the false results obtained by Gomori's method. Calcium was found to be present in excretory corpuscles of all larvae studied by us. We used Kóssa's method for the detection of calcium salts on the basis of our previous experience with calcareous corpuscles of cestode larvae (Žďárská 1975, Valkounová 1982). We considered the fact that the layering in excretory corpuscles of adult trematodes demostrated by ultrastructural studies (Martin and Bils 1964, Erasmus 1972 and Gibsin 1973) is very similar to the layering in calcareous corpuscles in cestodes (Von Brand et al. 1960). Our observations confirmed that Kóssa's method is suitable not only for the detection of calcium salts in calcareous corpuscles of cestode larvae, but also in excretory corpuscles of trematode larvae.

ОБНАРУЖЕНИЕ ИЗВЕСТКОВЫХ СОЛЕЙ В ЭКСКРЕТОРНЫХ ТЕЛЬЦАХ ЛИЧИНОК ТРЕМАТОД

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Резюме. С помощью метода Косса обнаружили известковые соли в экскреторных тельцах пяти видов церкарий и шести видов метацеркарий. Обсуждается интерференция известковых солей в экскреторных тельцах в методу по Гомори для выявления щелочной фосфатазы.

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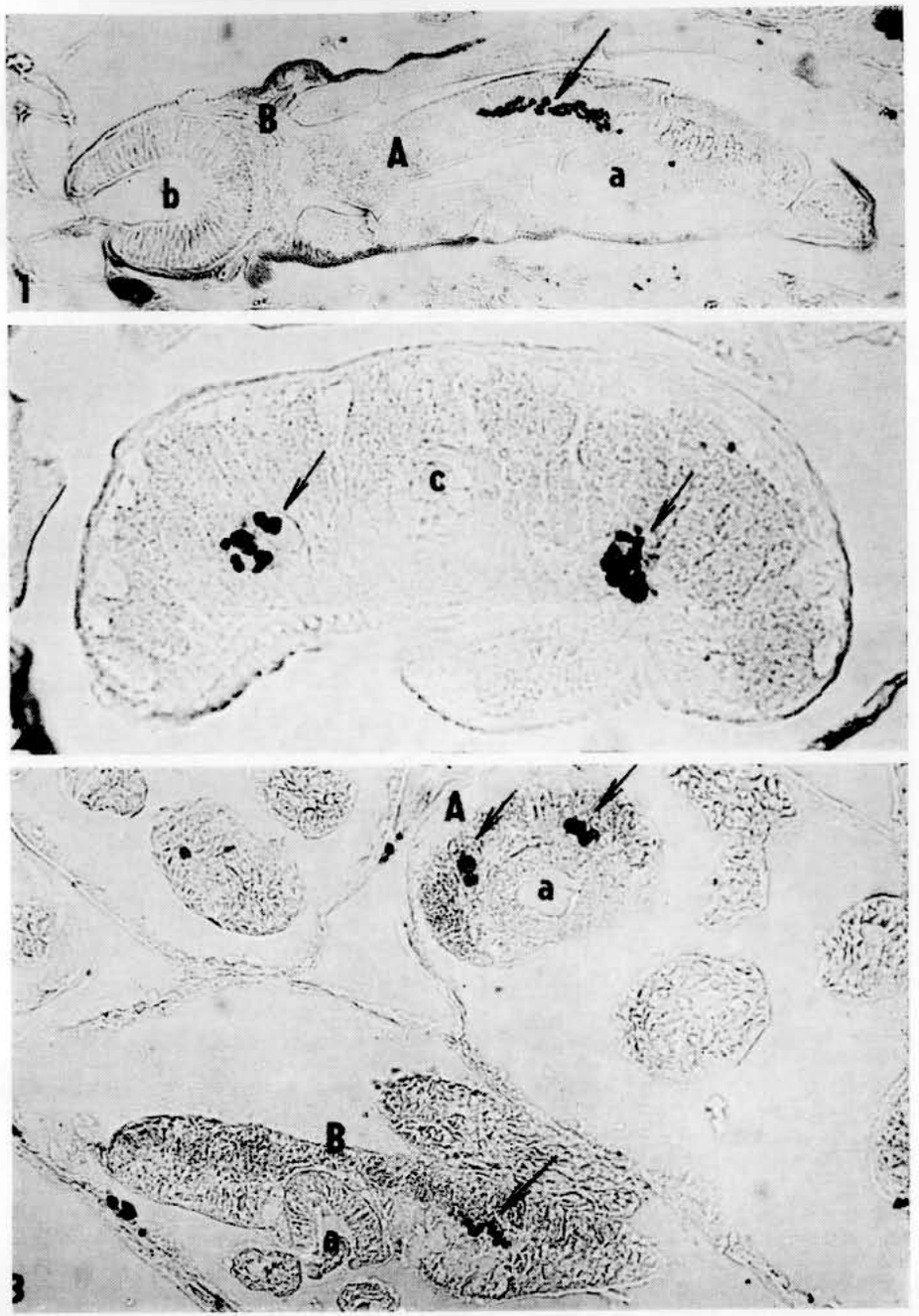

Fig. 1. Longitudinal section through the cercaria (A) and redia (B) of *E. aconiatum*. Excretory corpuscles of cercaria (arrow) in the collecting canal in front of the ventral sucker (a) are intensively stained by Kóssa's method. b — pharynx of redia (×200). Fig. 2. The same in transverse section through *E. aconiatum* cercaria. Arrow — excretory corpuscles, c — oesophagus (×600). Fig. 3. Transverse (A) and longitudinal (B) section through *E. revolutum* cercaria. Arrow — excretory corpuscles, a — ventral sucker. Kóssa (×350).

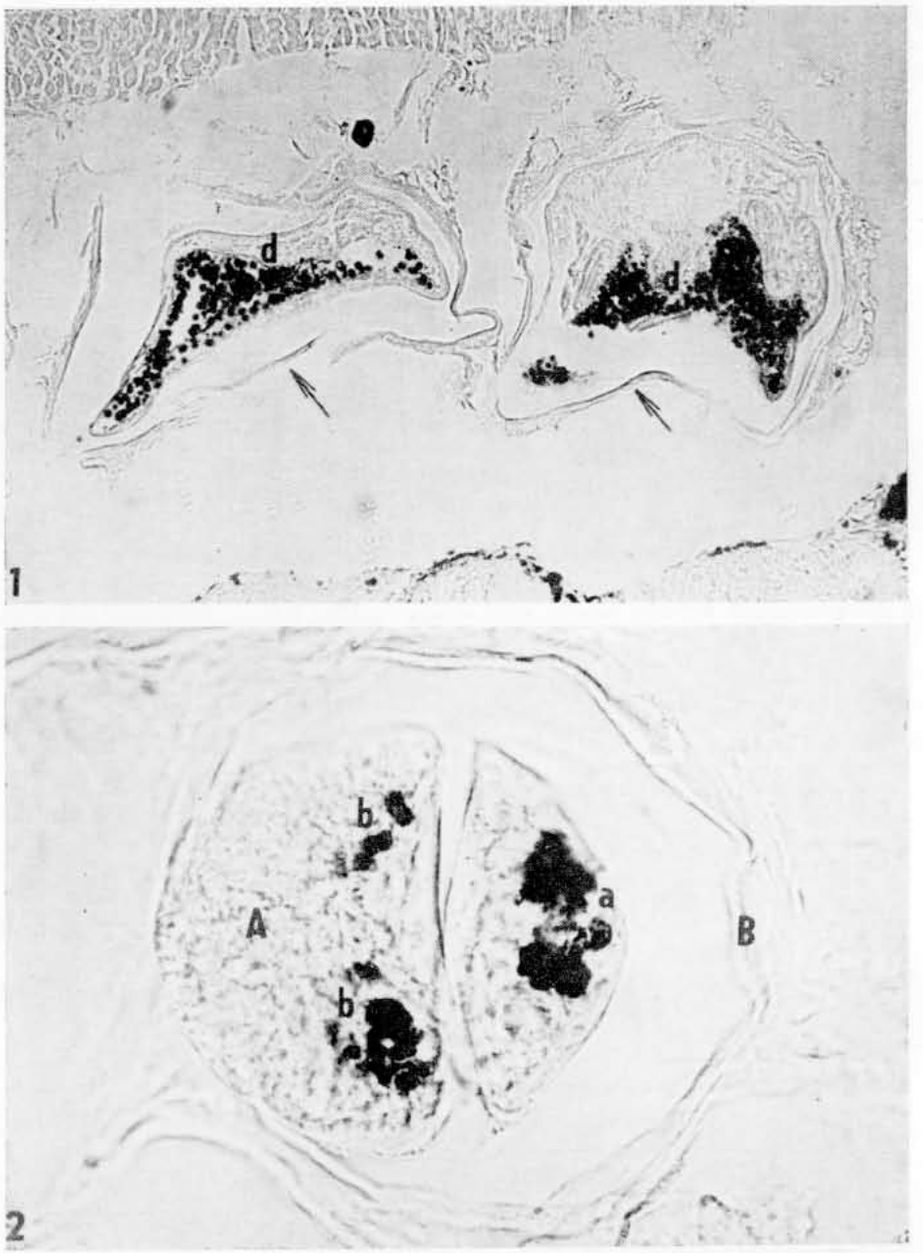

Fig. 1. Section through *O. ranae* metacercaria with excretory bladder (d) filled with excretory corpuscles intensively stained by Kóssa's method for the detection of calcium salts. Arrow — cyst wall of metacercaria (×400). Fig. 2. Section through body (A) and cyst wall (B) of *E. revolutum* metacercaria. Excretory corpuscles in excretory bladder (a) and collecting excretory canals (b) stained by Kóssa's method (×700).