ACTIVITY OF SOME ENZYMES IN THE SPOROCYSTS AND CERCARIAE OF Dicrocoelium lanceatum AND Eurytrema pancreiaticum

Z. ZDÁRSKÁ and V. Ya. PANIN

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and Zoological Institute, Academy of Sciences of the Kazakh SSR, Alma-Ata

Abstract. Differences in alkaline phosphatase, acid phosphatase and non-specific esterase activities have been observed in the sporocysts and cercariae of Dicrocoelium lanceatum Stiles et Hassal, 1896 and Eurytrema pancreiaticum (Janson, 1889). The walls of sporocysts of both species exhibit high activity of alkaline phosphatase. Non-specific esterase activity was demonstrated in the tegument of E. pancreiaticum sporocysts, whereas in D. lanceatum only acid phosphatase was observed in the pole with the birth pore. The collecting canals of the excretory system of E. pancreiaticum sporocysts have high activity of alkaline phosphatase and those of D. lanceatum demonstrate the activity of non-specific esterase. The tegument of E. pancreiaticum cercariae exhibits high activity of alkaline phosphatase, but this was not observed in D. lanceatum. The cystogenic gland cells of D. lanceatum cercariae contain acid phosphatase and non-specific esterase, whereas in those of E. pancreiaticum both these enzymes are lacking. Cholinesterase was demonstrated in the cerebral ganglion and main nerve trunks of cercariae of both species. The differences in the activity of the studied enzymes, both in sporocysts and cercariae, seem to be related to two factors, namely the particularities of the metabolic processes and the mode in which the cercariae are released from the body of the first intermediate host into the outer environment.

Both these trematode species, which cause great economic losses in domestic animals, have been studied by many authors. However, their life-cycles remained unclued for a long time. Initially it was supposed that only one intermediate host is involved and as late as in 1952 Krull and Mapes ascertained that D. lanceatum required the second intermediate host — the ant. The necessity of the second intermediate host (grasshopper) for the development of E. pancreiaticum was determined by Basch (1965). Under the conditions of Kazakhstan the life-cycle of this trematode was described in detail by Kaembaeva (1968). Although a large-scale control of these parasitoses was performed on the basis of these findings, their distribution is still wide.

The aim of the present paper is to elucidate the relationship between these larvae and the first intermediate host and the differences between larval stages of these two species of Dicrocoeliidae, which would contribute to a more effective control of these parasitoses.

MATERIAL AND METHODS

The material was obtained from snails collected in pasturelands in the vicinity of Alma-Ata in cooperation with the Zoological Institute of the Academy of Sciences of the Kazakh SSR. The larval stages of both trematode species parasitized the land snails Bradybaena laniti. After removal of the shell, the infected snails were fixed in Baker's solution at 4°C for 2 h, washed in 5% sucrose (4°C) for 2 h and then transferred either to Holt's syrup (4°C) or, using a shortened method, through cold acetone and benzene to paraffin. The material from Holt's syrup was cut with freezing microtome. The activity of individual enzymes was determined simultaneously on serial paraffin sections, frosted sections and control sections from mouse kidney (alkaline and acid phosphatases, non-specific esterase) and from mouse tongue (cholinesterase).
The alkaline phosphatase (APh) was demonstrated with \( x \)-naphthyl phosphate and Fast blue BB (Pearse 1968), acid phosphatase (AcPh) with \( x \)-naphthyl phosphate and hexazoparaarsamidin (HPR) (Pearse 1968). The specificity of this reaction was controlled by inhibition of 0.01 M NaF. The non-specific esterase (NE) was demonstrated by the method of Davis and Ornstein (1958) modified after Lojda and Papoušek (1967) with \( x \)-naphthyl acetate + HPR. For differentiation of esterases we used the inhibition tests with eserine (0.00001 M) and diethyl-p-nitrophenyl phosphate (Mintacol) at the concentration of 0.001 M. The cholinesterase (ChE) was demonstrated by a modified method of Karnovsky and Roots (1964) (Koch and Light). Inactivated sections (100 °C, 5 min) and sections incubated in media without substrate were used as controls.

RESULTS

ACTIVITY OF THE STUDIED ENZYMES IN E. PANCREATICUM LARVAE

a) Daughter sporocyst and endocyst

In the sporocyst wall the activity of APh was localized only in the walls of the excretory canals (Plate II, Fig. 2), whereas the whole endocyst exhibited high activity of this enzyme (Plate I, Figs. 1–3; Plate II, Figs. 1–4). None of the remaining enzymes under investigation has been demonstrated in the endocyst. The tegument of the sporocyst and large cells localized under muscular layer contained highly active NE (Plate III, Fig. 1). This NE was resistant to diethyl-p-nitrophenyl phosphate. AcPh and CHE were not identified in the sporocyst.

b) Cercariae

The cercariae (Plate I, Figs. 1, 3; Plate II, Figs. 1, 3, 4) exhibited APh activity in the tegument and collecting canals of the excretory system opening into the excretory bladder. CHE was detected in the cerebral ganglion and main nerve trunks.

ACTIVITY OF THE STUDIED ENZYMES IN D. LANCEATUM LARVAE

a) Daughter sporocysts

The daughter sporocysts exhibit the activity of APh along the whole length of their walls. At the pole with birth porc there are large cells with high activity of NE, which is inhibited by diethyl-p-nitrophenyl phosphate (Mintacol). AcPh is active only in the tegument on the pole with birth porc. CHE has not been observed in the sporocysts.

b) Cercariae

The cercariae exhibited high activity of NE (Plate III, Fig. 2; Plate IV, Figs. 1–2) and AcPh (Plate IV, Figs. 3–4) in cystogenic gland cells. NE was inhibited by diethyl-p-nitrophenyl phosphate. CHE was identified in cerebral ganglion and main nerve trunks.

DISCUSSION

The cercariae of D. lanceatum and E. pancreaticum substantially differ from one another in the enzyme activity in cystogenic gland cells. The cercariae of D. lanceatum exhibit high activity of AcPh and NE, whereas none of these enzymes is active in the cystogenic gland cells of E. pancreaticum cercariae. The penetration gland cells of both species did not contain any of the studied enzymes. The cercariae of D. lanceatum differ from those of E. pancreaticum also in the enzyme activity in the tegument of body and
tail. In *E. pancreaticum*, there is a high activity of APh, whereas in *D. lanceatum* this enzyme is completely lacking. The excretory system of *E. pancreaticum* cercariae exhibits the activity of APh, whereas in those of *D. lanceatum* this enzyme has not been identified. CHE is present in the cerebral ganglion and main nerve trunks of cercariae of both species.

The activity of enzymes in the sporocysts is also different in these two species. The tegument of *E. pancreaticum* sporocyst has active NE in both ends, while that of *D. lanceatum* sporocyst has active AcPh only in the pole with birth pore. In the subtegumental portion of sporocysts of both species (in *D. lanceatum* only on the pole with birth pore) there are large cells with high activity of NE, but these NE differ from one another. In *E. pancreaticum*, NE is resistant to diethyl-p-nitrophenyl phosphate, but in *D. lanceatum* NE is inhibited by this substance.

The excretory system of *E. pancreaticum* sporocysts exhibits APh activity, that of *D. lanceatum* sporocysts NE activity. A high activity of APh is present in the wall of both species, but it is localized differently. In *D. lanceatum* it is in the sporocyst wall, but in *E. pancreaticum* in the endocyst which is not developed in *D. lanceatum*.

The presence of enzymes in the tegument of daughter sporocysts of both trematode species shows its active role in the metabolic processes and in the transport of nutrients. The activity of NE is probably connected with the participation of lipids in the energetic processes of the sporocysts. The activity of APh shows the absorbing role of the wall of *D. lanceatum* sporocyst and *E. pancreaticum* endocyst. This is evidenced also by structural particularities of the tegument both in sporocysts and rediae, i.e. the presence of numerous microvilli which are thought to increase the surface (Ginetinskaya et al. 1966, Smyth 1966, Ginetinskaya 1968, Krupa et al. 1968, Negus 1968, Köie 1971 a, b, Erasmus 1972, Reader 1972).

The localization of APh, AcPh and NE in the sporocysts and cercariae of other species of trematodes is variable (Janoff and Ford 1965, James and Bowers 1967, Rifkin 1970, Ebrahimzadeh 1970, Köie 1971 a, b, c). APh is often detected on the surface, where an active transport of substances through membranes takes place. This was confirmed also in the sporocysts of some species of the family Schistosomatidae (Kinoti et al. 1971, Krupa and Bogitsch 1972, McManus and Brian 1973).

The cercariae of *E. pancreaticum* obtain the nutrients from the body cavity of the daughter sporocyst through the wall of the endocyst, whereas those of *D. lanceatum* obtain these substances only through the wall of the daughter sporocyst or through the tegument of the cercariae at the time when they are outside the sporocyst. This fact seems to condition the different localization of enzymes in sporocysts and cercariae of both examined trematode species.

On the other hand, these differences in the activity of the studied enzymes (APh, AcPh and NE) seem to be correlated with the different mode in which the cercariae leave their first intermediate host. The cercariae of *D. lanceatum* leave the sporocyst through the birth pore, assemble in the respiratory cavity and in form of slimeballs get into the outer environment where they are eaten by ants (second intermediate hosts). That means that the cercariae must actively pass through the tissues of the intermediate host. In *E. pancreaticum*, the cercariae do not leave the sporocyst, but the whole thick-walled sporocysts pass through the respiratory pore into outer environment where they are eaten by grasshoppers. In this case the entire sporocysts and not cercariae penetrate through the tissues of the snails and the sporocyst of *E. pancreaticum* thus plays the same role as the cercaria of *D. lanceatum*. This fact may elucidate the different localization of enzymes in the sporocysts and cercariae of these species. The similar high levels of NE activity in cystogenic gland cells of *D. lanceatum* cercaria and large cells in the wall of *E. pancreaticum* sporocyst

119
suggest that they probably discharge the same function. However, a further study is necessary to throw light upon this problem, especially with respect to the fate of both secretions while penetrating through the tissues of the first intermediate host and leaving it. In the trematode larvae studied by us (Žďárská 1968, 1969, 1970, 1971 a, b) which develop in freshwater snails, the secretion of cystogenic gland cells participated only in the formation of the tegument of cercariae and cyst wall of metacercariae and was not involved in the penetration through the intermediate host.

The necessity of change in leaving the first intermediate host, caused by the fact that the larvae of the suborder Dicrocoeliidae develop in land snails, led not only to the change of coenogenoses (Panin 1972, 1974) but also to greater changes of cellular structures and thus to changes of the enzymatic activity. The most important coenogenesis for these cercariae is the protection against the desiccation and of minor importance is the movement. This is reflected not only in histological, but also in histochemical changes, as is evident from our results.

АКТИВНОСТЬ НЕКОТОРЫХ ФЕРМЕНТОВ В СПОРОНИЦАХ И ПЕРКАРНЫХ DICROCOELIUM LANCEATUM И EURYTIEMA PANCREATICUM

3. Ждирска и В. Я. Панин

Резюме. Наблюдалась разница в активности щелочной фосфатазы, кислой фосфатазы и неспецифической эстеразы в спороницах и перкарных Dicrocoelium lanceatum Stiles et Hassal, 1896 и Eurytrema pancreaticum (Janson, 1880). В спороницах двух видов отмечена высокая активность щелочной фосфатазы. Активность неспецифической эстеразы обнаружена в тегументе спорониц E. pancreaticum, тогда как в спороницах D. lanceatum была найдена только кислая фосфатаза в области родиальной норы. Собирательные каналы экскреторной системы в спороницах E. pancreaticum показывают высокую активность щелочной фосфатазы, а у D. lanceatum активность неспецифической эстеразы. Тегумент перкарной E. pancreaticum показывает высокую активность щелочной фосфатазы, которая отсутствует у D. lanceatum. Пищевые железистые клетки перкарной D. lanceatum содержат кислую фосфатазу и неспецифическую эстеразу, но эти ферменты не были обнаружены в пищевых железистых клетках E. pancreaticum. Холениназа была обнаружена в молодом тангенсе и главных нервных стволах перкарней обоих видов. Различия в активности пищеваренных ферментов у спорониц и перкарных синопы, очевидно, с двумя факторами, а именно: с особенностями метаболических процессов и со способами выделения перкарней из организмов первого промежуточного хозяина во внешнюю среду.

REFERENCES


KINOTI I. S., BIRD R. I., BAKER M., Electronmicroscope and histochemical observations on the daughter sporocyst


Received 20 October 1976.
Activity of alkaline phosphatase in young sporocysts of *E. pancreatum* (α-naphthyl phosphatase + Fast blue BB). Fig. 1. Oblique section through a sporocyst with young cercariae (A) and sporocyst (B) with broken wall (a), from which emerges the endocyst filled with cercariae. High activity of APh is in the endocyst (arrows) and in the tegument of cercariae. (×75) Fig. 2. Longitudinal (A) and transverse (B) sections through the end part of sporocysts. Note high activity of APh in the endocyst (arrows). (×125) Fig. 3. Transverse sections through the middle (A, B) and end (C) parts of sporocysts with young cercariae with well visible high activity of APh in the tegument of young cercariae (a) and in the endocysts (arrows). ×100
Activity of alkaline phosphatase in sporocysts of *E. pancreaticum* with fully developed cercariae (α-naphthyl phosphate + Fast blue BB). Fig. 1. Section through the middle part of the sporocyst with fully developed cercariae, the tegument of which shows a high activity of APn. Activity of this enzyme is localized also in the endocyst (arrows). (×125) Fig. 2. Transverse section through the end part of a sporocyst with well visible activity of APn in the excretory canals (arrows) and in the endocyst (a). (×125) Fig. 3. Oblique section through the sporocyst in the site where the thick-walled end part passes to the thin-walled middle part. Note the activity of APn in the endocyst (arrow) and tegument of the cercariae. (×125) Fig. 4. Transverse sections through the sporocysts. The endocyst (arrows) and tegument of cercariae exhibit the activity of APn. (×125)
Fig. 1. Transverse section through the end part of the sporocyst of *E. pancreaticum*. Large cells with high activity of non-specific esterase are present in the sporocyst wall. This enzyme is also active in microvilli of the sporocyst tegument. (α-naphthyl acetate + HPR). (×325) Fig. 2. Section through several sporocysts of *D. lanceatum* and hepatopancreas of snail. Note high activity of non-specific esterase in the cystogenic gland cells and their ducts opening into oral sucker (arrow). The hepatopancreatic cells (a) display high activity of this enzyme. (α-naphthyl acetate + HPR) (×130)
Fig. 1. Transverse (A) and longitudinal (B) sections through a cercaria of *D. lanceatun* and horizontal section through the oral sucker (bottom right). High activity of non-specific esterase well visible in the cystogenic gland cells (a) and their ducts (arrows) opening into the oral sucker (b). (z-naphthyl acetate + HPR). (×425). Fig. 2. Detail of cystogenic gland cells in longitudinal section. The activity of non-specific esterase is localized in minute bodies. (z-naphthyl acetate + HPR). (×425). Fig. 3. Longitudinal section through two bodies of *D. lanceatun* cercariae showing high activity of acid phosphatase in the cystogenic gland cells (a). b — oral sucker. (z-naphthyl phosphate + HPR). (×260). Fig. 4. Section through *D. lanceatun* cercariae and hepatopancreas of snail. High activity of acid phosphatase is visible not only in the cystogenic gland cells of the cercariae (arrows) but also in the hepatopancreatic cells (c). (z-naphthyl phosphate + HPR). (×180).