THE ACTIVITY OF ALKALINE AND ACID PHOSPHATASE AND NON-SPECIFIC ESTERASE IN EXPERIMENTAL BOVINE CYSTICERCOSIS AT DIFFERENT STAGES OF DEVELOPMENT*

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Dedicated to Academician B. Ryšavý on the occasion of his 60th birthday

Abstract. The activity of alkaline and acid phosphatase and non-specific esterase was detected both in the parasite and in the tissue reaction on days 21, 23, 42, 168 and 261 after experimental infection. A very high activity of all enzymes was found in 21- and 23-day-old C. bovis in the tegument of whole bladder. In 42, 168 and 261 days old cysticerci the activity of alkaline and acid phosphatase was limited only to a part of bladder surrounding the opening of the spiral canal, whereas the activity of non-specific esterase was present in the whole bladder. The activity of non-specific esterase was localized in subtegmental cells of the bladder wall and in small bodies in the bladder and scolex. These bodies increased in number with the age of the cysticercus. In the tissue reaction, a high activity of alkaline phosphatase was detected only in the period of about 20 days after infection in the layer of activated fibroblasts. The activity of acid phosphatase was demonstrated in the tissue reaction in all time periods and was localized in the histiocytes, macrophages, necrotic exudate, necrotic foci and pigment cells at the periphery of tissue reaction. These cells exhibited also the activity of non-specific esterase.

Previous histochemical studies on bovine cysticercosis dealt with the cysticercus and tissue reaction as late as on days 83 and 108 after infection (Ždárská 1973, 1975, 1976, Ždárská and Machnicka 1978 a, b, 1979) and literary data on the activity of some enzymes in the larva and at the early phase of pathologic reaction have been lacking. The presence of enzymes in this period and the dynamics of enzymatic activity during the infection are important facts necessary for a better knowledge of the function of individual parts of the cestode larva during the development and for the understanding of the parasite-host interaction. We have therefore studied the activity of alkaline and acid phosphatase and non-specific esterase in Cysticercus bovis and host tissue reaction on days 21, 23, 42, 168 and 261 after experimental infection.

MATERIAL AND METHODS

Calves at the age of 4 months were experimentally infected with Taenia saginata (Goeze, 1782) eggs. The methods of experimental infection are described in the paper by Blažek et al. (1980). For histochemical studies, the cysticerci were recovered from heart and skeletal muscles immediately after the animal had been killed. They were fixed either together with the surrounding tissue or released from solid foci and cysts. No macroscopic signs of degeneration were observed.

The activity of acid and alkaline phosphatase and non-specific esterase was studied in C. bovis and in the pathologic reaction on days 21, 23, 42, 168 and 261 after infection. Histochemical methods described in the paper by Ždárská and Machnicka (1978a) were used for the detection of individual enzymes.

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RESULTS

A. Cysticercus bovis

The bladder wall of 21- and 23-day-old C. bovis (Plate I, Figs. 1, 2, Plate II, Figs. 1, 2, Plate III, Figs. 1, 2, Plate IV, Fig. 1) exhibits a high activity of alkaline and acid phosphatase and non-specific esterase only in the whole surface tegument. The remaining layers of bladder wall are negative. There is no difference in the activity and localization of the above enzymes between the cysticerci developing in the skeletal muscles and those developing in the heart.

In 42-day-old cysticerci localized both in skeletal muscles (Plate IV, Fig. 1) and in heart the activity of alkaline and acid phosphatase was demonstrated not only in the tegument but also in the subtegmental cells in the part of bladder surrounding the opening of the spiral canal. The remaining portion of the bladder does not exhibit any activity of these two enzymes. The activity of non-specific esterase (Plate IV, Fig. 2) is localized in subtegmental cells and in occasional small bodies in the wall of the whole bladder. In the scolex portion, the activity of alkaline phosphatase is present in the nerve trunks and that of non-specific esterase in small bodies identical with bodies in the bladder wall.

In C. bovis at the age of 168 (Plate V, Figs. 1, 2) and 261 days (Plate VI, Fig. 2), the localization of alkaline and acid phosphatase activity is the same as in 42-day-old cysticercus, but the non-specific esterase activity is different. In the bladder wall and scolex portion, the bodies with high non-specific esterase activity are more numerous and concentrate particularly in the part of bladder lacking alkaline phosphatase, i.e., in the part distant from the spiral canal, particularly around the wide and ribbed canals and in the scolex portion around the walls of both collecting canals, in sucker and parenchyma around suckers and rostellum.

B. Tissue reaction

On days 21 and 23 after infection, the cysticercus in a solid focus is surrounded by erythrocytes or leucocytes or by numerous eosinophils. The exudate is necrotic in some foci and is surrounded by large light macrophages. At the periphery, the nodule is formed from a vascularized granular fibroblastic tissue with lymphoid cells.

In the tissue reaction, the high activity of alkaline phosphatase localized in activated fibroblasts prevails both in skeletal (Plate II, Fig. 1) and in heart muscles (Plate II, Fig. 1). A high activity of this enzyme was demonstrated also in the wall of capillaries visible particularly in the non-affected muscles. The acid phosphatase activity localized in macrophages is highest at the border of the necrotic exudate around the cysticercus and adjacent further layer of tissue reaction (Plate II, Fig. 2, Plate III, Fig. 2). The non-specific esterase activity was not present (Plate I, Fig. 2).

On day 42 after infection, the cyst wall at the sites adjacent to the part of bladder around the spiral canal opening is markedly thickened by a granular fibroblastic tissue. In the remaining parts it is weakly or strongly infiltrated by lymphocytes and eosinophils. The eosinophils are sometimes accumulated in the cyst cavity close to the scolex.

The activity of alkaline phosphatase is localized only in the wall of capillaries and in occasional elongated cells of fibroblast type at the periphery of tissue reaction in heart. Acid phosphatase activity is present in histiocytes, macrophages, small necrotic foci and pigment cells on the border of tissue reaction.
On days 168 and 261 after infection, the cyst wall with live cysticercus is thickened by histiocytes only in the region of the scolex part of cysticercus and the greater remaining part of the wall is formed from connective tissue cells at resting stage.

In principle the enzymatic activity is the same as in the tissue reaction on day 42 after infection. The alkaline phosphatase is present only in capillaries (Plate V, Fig. 1), acid phosphatase in histiocytes and acid phosphatase with non-specific esterase in pigment cells.

**DISCUSSION**

The results obtained provide new data on the histochemistry of developing *C. bovis*. In the youngest cysticerci (21 and 23 days old) of the whole complex of experimental material, the whole bladder surface exhibits the activity of alkaline and acid phosphatase and non-specific esterase. The bladder at this stage of development is not yet differentiated in the portion with an intensive transport of substances and that without this intensive exchange. The whole bladder surface of a 21- and 23-day-old cysticercus has an intensive nutritional function, as its development, i.e., the growth and morphological differentiation, is most intensive in the first weeks after infection (Blažek et al. 1980). The functional differentiation of the bladder wall occurs gradually with the growth. Already in a 42-day-old cysticercus, the bladder wall can be clearly differentiated into two portions on the basis of alkaline and acid phosphatase activity — the portion with enzymatic activity, i.e., a portion of bladder close to developing scolex and that without any activity of these enzymes. These sites of alkaline and acid phosphatase activity remain preserved even in the oldest (261 days old) cysticerci of the material under study.

The activity of non-specific esterase changes considerably during the development of *C. bovis*. In the youngest cysticerci (21 days old), the non-specific esterase activity is localized only in the tegument. At the age of 42 days, the activity of non-specific esterase cannot be demonstrated any more in the tegument, but it is present in subtegumental cells, particularly in the bladder portion without alkaline and acid phosphatase activity, i.e., in the portion distant from the differentiating scolex. A high activity of non-specific esterase is localized also in small bodies present both in bladder wall and in scolex portion. In the scolex portion, these bodies are particularly in suckers and in the parenchyma around them. In the remaining part of scolex, i.e., at places with calcareous bodies, they are less numerous. They exhibit also a low activity of acid phosphatase and can be first demonstrated in a 42-day-old *C. bovis*. They become more numerous with the age of cysticercus (168 and 261 days) and are then concentrated in the bladder especially around wide and ribbed excretory canals and in the scolex in suckers and surrounding parenchyma. The function of these bodies is not clear, but they are supposed to be metabolic products accumulating in the tissue of ageing cysticercus.

Another new fact detected in the studied material is the high activity of alkaline phosphatase in the tissue reaction around 21- and 23-day-old cysticercus. It is localized in activated fibroblasts which suppress the muscle tissue both in skeletal muscles and in heart. The high activity of alkaline phosphatase in this portion of nodular proliferation indicates an intensive formation of fibrils which has already been demonstrated at this stage of infection at an ultrastructural level (Blažek and Schramlová 1979). The tissue reaction around 42-day-old cysticercus did no more exhibit alkaline phosphatase activity in skeletal muscles and in the heart it was limited only to a narrow stripe of fibroblasts at the periphery of tissue reaction marked only in sectors. Acid phosphatase activity is highest in macrophage around the young larva, but it is detectable also in
АКТИВНОСТЬ ЩЕЛОЧНОЙ И КИСЛОЙ ФОСФАТАЗЫ И НЕСПЕЦИФИЧЕСКОЙ ЭСТЕРАЗЫ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЦИСТИЦЕРКОЗЕ КРУПНОГО РОГАТОГО СКОТА В РАЗНЫХ СТАДИЯХ РАЗВИТИЯ

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Резюме. Изучена активность щелочной и кислой фосфатазы и неспецифической эстеразы как в нарастае, так и в тканевой реакции на 21-й, 23-й, 42-й, 168-й и 261-й день после экспериментального заражения. У C. bovis в возрасте 21 и 23 дня обнаружена высокая активность приведенных ферментов во всем тегументе пузыря. У цистицерка в возрасте 42, 168 и 261 день активность щелочной и кислой фосфатазы обнаружена только в части пузыря, окружающей отверстие спирального канала, тогда как активность неспецифической эстеразы проявилась во всем пузыре. Неспецифическая эстераза была обнаружена в субтегументальных клетках пузыря и мелких тельцах в пузыре и сколексе. Количество этих тележков повышалось с возрастом цистицерка. В тканевой реакции обнаружена активность щелочной фосфатазы только приблизительно на 20-й день после заражения в слое активированных фиброblastов. Активность кислой фосфатазы выявлена в тканевой реакции во всех исследованных периодах, в гистиоцитах, макрофагах, некротическом экссудате, некротических очагах и пигментных клетках на периферии тканевой реакции. Эти клетки проявляли также активность неспецифической эстеразы.

Plague belongs to classic diseases characterized by the natural foci phenomenon. Today, there are about 50 countries in whose territories natural foci of plague have been found or their occurrence is anticipated. One of the countries which is paying long-term constant attention to plague research, is the Soviet Union. Extensive network of anti-plague stations, qualified personnel and perfect complex of preventive measures ward off epidemics of this disease despite the existence of natural foci of plague in the USSR territory. Although much work has been done in studying the consistent patterns of the plague incidence and the characteristics of its natural foci, and hundreds of publications have been devoted to these problems a number of questions still remains unsolved and opinions of specialists in the evaluation of some phenomena are differing.

The subtitle of M. P. Kozlov’s book is “Natural foci, epizootology, epidemic manifestation”. It contains a survey of concrete data and contemporary views, primarily from this field. Apart from the introduction and conclusion the book is divided into 8 chapters:

I. General biological patterns facilitating the bacteria to become parasites of certain animal species; II. Global origin of the causative agent and of natural foci of plague; III. Interrelationships between the causative agent and the plague carriers; IV. Interrelationships between the plague microbe and its vectors, the fleas; V. The role of main carriers in the formation of natural foci of plague and in the maintenance of epizootic process; VI. General consistent patterns in the epizootic process of plague; VII. Epidemic manifestation of plague in natural foci in the USSR territory and adjoining countries; VIII. Problems of plague control.

The book presents a good survey on the status quo in the research of plague in the USSR territory, contains many original views of the author, does not avoid disputable problems. However, not all papers cited in the text are included in the list of literature. The publication will be surely of interest to specialists in the research of natural focality of diseases, to parasitologists, medical zoologists and epidemiologists as well.

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Fig. 1. Acid phosphatase activity in bladder tegument of 21-day-old *C. bovis*. $\alpha$ — naphthyl phosphate $+$ HPR. (140×). Fig. 2. Non-specific esterase activity in bladder tegument of 21-day-old *C. bovis*. The enzyme is not active in tissue reaction in skeletal muscles — compare with activity of other enzymes in Plate II, Figs. 1, 2. $\alpha$ — naphthyl acetate $+$ HPR (32×).
**Fig. 1.** Localization of alkaline phosphatase activity in *C. bovis* and in tissue reaction in skeletal muscles on day 21 p.i. α — naphthyl phosphate + Fast blue BB (32×). **Fig. 2.** Localization of acid phosphatase in the same material as in Fig. 1. α — naphthyl phosphate + HPR (32×).
Fig. 1. Localization of alkaline phosphatase activity in C. bovis and in tissue reaction in heart on day 21 p.i. α — naphthyl phosphate + Fast blue BB (33×). Fig. 2. Localization of acid phosphatase in the same material as in Fig. 1. α — naphthyl phosphate + HPR (33×).
Fig. 1. Localization of alkaline phosphatase in *C. bovis* and in tissue reaction in skeletal muscles on day 42 p.i. α — naphthyl phosphate + Fast blue BB (29×). **Fig. 2.** Non-specific esterase activity in the same material as in Fig. 1. In the cysticercus, it is localized in subtegmental cells of bladder and in small bodies in scolex, particularly around suckers. α — naphthyl acetate + HPR (39×).
Fig. 1. Localization of alkaline phosphatase activity in *C. bovis* and in tissue reaction in skeletal muscles (only in capillaries) on day 168 p.i. \( \alpha \) — naphthyl phosphate + Fast blue BB (32×).

Fig. 2. Non-specific esterase activity in the same material as in Fig. 1. It is lacking in the bladder portion around spiral canal opening at the site of alkaline phosphatase activity (see Fig. 1). \( \alpha \) — naphthyl acetate + HPR (32×).
Fig. 1. Non-specific esterase activity in the cysticercus and in tissue reaction in heart on day 21 p.i. (compare with activity of other enzymes in Plate III, Figs. 1,2). α — naphthyl acetate + HPR (33×). Fig. 2. Non-specific esterase activity in C. bovis and in skeletal muscles on day 261 p.i. High activity is localized in bladder wall and scolex portion with suckers. α — naphthyl acetate + HPR (18×).