TISSUE REACTION OF THE SKELETAL MUSCLES OF CATTLE BOTH TO A SPONTANEOUS AND EXPERIMENTAL INFECTION WITH CYSTICERCUS BOVIS

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Abstract. The reaction of the tissues to an infection with Cysticercus bovis was studied on material from cattle with a spontaneous and an experimental infection. The authors examined a total of 232 cysts from the skeletal muscles. A tissue reaction to C. bovis was inflammatory in nature and originated always at the site at which the invaginated scolex opened onto the surface. Typical of these changes were the origin of a pseudoeplithelial rim and a zone of granulation tissue maturing on the periphery. At a later phase marking the start of necrotic changes in the cysticercus, the inflammation accompanied by exudation started to develop anew. Calcified structures stirred up a giant cell, cleaning up reaction, and cicatization followed the resorption of the parasite and the exudate. We identified two types of necroses in a tissue reaction to C. bovis: a focal necrosis of the exudate and the inflammatory rim with a subsequent dystrophic calcification, and a focal necrosis and calcification of collagenous fibres and their groups. Necrotic-like foci typical of a reaction to C. bovis were seen in the inflammatory rim. Using specific staining procedures, we succeeded in distinguishing these foci which did not succumb completely to a dystrophic calcification, from concomitantly present necrotic foci.

Studies on tissue reactions appeared to us to be of utmost importance in a complex investigation of bovine cysticercosis. The results might be useful in the diagnosis, prevention and control of cysticercosis in cattle, and of taeniarynchosis in man. The results obtained in the present study might contribute to a better understanding of the pathogenicity of the agent of cysticercosis, nowadays regarded as the most serious zoonosis from the standpoint of economy, health and veterinary medicine.

MATERIALS AND METHODS

Tissue reactions to a spontaneous cysticercosis in cattle were studied on material from 114 bovine animals with spontaneous cysticercosis killed at abattoirs in Prague and Studená (district Jindřichův Hradec). A weak infection (1–3 cysts discovered in sections during a routine inspection of carcasses at the abattoir) was found in 70 of these animals; a heavy infection was present in 44 animals. Tissue reactions of the skeletal muscles to a spontaneous cysticercosis were evaluated in 140 cysts from 70 cases with a weak infection, and in 36 cysts from 44 cases of heavy infections. The tissue reaction of the skeletal muscles to an experimentally induced cysticercosis was studied on material from 3 calves infected with doses from 125,000—180,000 eggs. The material was obtained by courtesy of Associate Professor Dr. B. Machnicka, Parasitological Institute, Polish Academy of Sciences, Warsaw. We examined with histological methods a total of 56 cysts from muscles of the plexus of the thoracic extremity, shoulder muscles, muscles of the forearm, lumbar muscles, outer pelvic muscles and dorsal muscles of the femur, and 4 cysts from the tongue. Ten of the 56 cysts from skeletal muscles were collected on day 83 p.i., 46 cysts on day 102 p.i. The experiment has earlier been described by Štěrba (1974).

Slices of tissues were fixed for histological examination with 10 % neutral formol or 4 % Backer's formol, and treated with standard paraffin techniques. Partial or complete histological series were made of tissue samples from an experimental infection. Calcified structures were treated with a method suggested by Slais (1960, 1970) and a modification of this method (Štěrba and Slais 1972,
Tissues were stained with haematoxylin eosin, van Gieson's method, van Gieson's elastic method, trichrome (Masson and Goldner's method complemented with Gomori's impregnation method); Kossa's method for the detection of calcium, the PAS method for the detection of mast cells.

Of the histochemical methods for the detection of polysaccharides we used PAS with acetylation, desacetylation and digestion of the control sections with diastase; Hale's method in Müller's modification employing the effect of colloidal iron on control slides, and Hale's method combined with PAS; metachromasia was detected with thionine and toluidine blue; for the detection of proteins we used Millon's test, Adams'test and a reaction to arginine.

RESULTS

TISSUE REACTION IN THE SKELETAL MUSCLES OF ANIMALS WITH A SPONTANEOUS INFECTION

Apart from two findings (in a massive infection) cysts from the skeletal muscles of bovine animals with a spontaneous infection contained completely developed specimens of *C. bovis* (their invaginated portion was inside the spacious bladder).

Fully developed larvae were present mostly in dilated lymphatic capillaries of the muscles. The reaction of the surrounding tissue was indistinct (Plate I, Fig. 1). The parasites were surrounded by finely granular coagulations of protein. An atrophy of the individual muscle fibres, rarely accompanied by a lymphoid infiltration, was caused by pressure of the growing parasite. In the case of disappearance of complete bundles of muscle fibres, the connective tissue layers of the perimysium internum merged into wider strips of connective tissue. The course of the lymphatic vessel was not changed. This accumulation of connective tissue around the parasite (or a perivascular fibrosis) mimicked its fibrous encapsulation. At this phase, faint signs of an inflammatory reaction, i.e., a multiplication of plasma cells and an activation of histiocytes, could be noted in the perimysium.

Signs of a tissue reaction were always more marked at the site facing the opening of the invaginated canal on the surface of the bladder (Plate I, Fig. 2). The nuclei of endothelial cells were swollen, the connective tissue was imbied and of a yellowish colour in staining with van Gieson, and its layered organisation persisted on the periphery only. The affected sector of the lymphatic capillary was surrounded by activated histiocytes, exudative cells having the character of neutrophil and eosinophil leukocytes, and loose connective tissue of the original granulation tissue (Plate II, Fig. 1).

A greater development of inflammatory changes evidenced itself in a concentration of the serous exudate containing an increased number of eosinophil leukocytes in the affected wall of the lymphatic capillary which harboured the parasite. In the further development of inflammatory changes, the growing parasite distended part of the lymphatic capillary which started to separate and take on a cyst-like appearance.

Eosinophil exudate entered the spiral canal, distended it and gradually succumbed to necrosis. A fibrinoid substance among the histiocytes in the inflammatory rim increased in volume. The histiocytes became organized in palisades, giant cells started to appear (Plate II, Fig. 2). The periphery of the inflammatory rim was composed of a vascular granulation tissue and contained foci of macrophages. The number of lymphoid cells increased in the rim and formed folliculoid structures, i.e., spurious lymph nodes (Plate II, Fig. 2). Atrophied muscle fibres could be demonstrated in the maturing zone of the granulation tissue.

It was typical of *C. bovis* that inflammatory changes originated and developed at a time at which the parasite was fully vital. Mostly, they displayed a marked predilection to the site facing the opening of the spiral canal onto the bladder surface. If, by movements of the parasite, the opening of the canal came to face another part of the
wall, other inflammatory changes originated individually in these parts of the wall. Inflammatory changes in the vicinity of one of the pointed poles of the bladder were evidently associated with the original polar location of the scolex.

Typical of the histological picture of a muscle cysticercosis were conspicuous foci necrotic in appearance in the lymphoidly infiltrated granulation tissue surrounding the acutely inflamed site. We observed in them a coarser basophilic granularity and a coarse reticulum with fragmented fibres. The foci stained well with Hale’s method and were highly metachromatic. Most of them were calcified. Foci lined with fibrotic granulation tissue, frequently containing multinucleate giant cells, were seen in their immediate vicinity (Plate III, Fig. 1). In our opinion, a necrobiosis of the epithelial rim was set off directly under the influence of the parasite, and should be held responsible for the origin of these foci. Similar focal changes could be seen at several sites of the inner surface of the inflammatory rim. These focal changes have regularly been observed in an infection with *C. bovis*, but have never been found in an infection with other larval cestodes or parasites. A direct metaplasia of the bone was observed in foci formed on the very periphery of the inflammatory encapsulation within the course of further development (Plate III, Fig. 2).

In the next phase of development of the tissue reaction, inflammatory changes occurred in the whole periphery of the cyst which still contained a fully vital cysticercus. The process of maturation of the outer zone of the granulation tissue was greatly advanced. The inner zone of the vascular granulation which was infiltrated heavily with eosinophil leukocytes, formed a remarkable fold at the site facing the parasite. Eosinophil leukocytes were concentrated also in the space surrounding the parasite. The first signs of death of the parasite appeared at the time at which the whole periphery was affected by inflammatory changes (Plate IV, Fig. 1). Wrinkled, and later collapsed, cysticerci were surrounded by a large quantity of cellular exudate composed of eosinophil leukocytes. The necrosis which affected both the parasite and the exudate, spread sometimes to the resorptive, inflammatory, rim or the fibrous capsule of the cyst. The resorption of parasitic remnants and the exudate lead to the origin of scars heavily infiltrated with lymphoid cells and a folliculoid aggregation of lymphoid cellular elements enclosing unresorbed and dystrophically calcified remnants of both the parasite and the necrotic exudate (Plate IV, Fig. 2; Plate V, Fig. 1).

**TISSUE REACTION OF THE SKELETAL MUSCLES OF ANIMALS WITH AN EXPERIMENTAL INFECTION**

In 8 cysts examined histologically on day 83 p.i., the enclosure of the parenchymal part of the parasites in the bladder was not yet complete. The life parasite resembling an elongate ovoid structure occupied the spacious cavity of the distended lymphatic capillary. Mostly, he was surrounded by a small quantity of a noncellular, homogeneous, exudate; the exudate of 4 cysts contained several eosinophil leukocytes. The finding of neutrophil leukocytes was most sporadic. Infiltrating cells were always present close to the opening of the spiral canal onto the bladder surface, or near the poles of the ovoid bladder.

The tissue reacted with an inflammation to the presence of the parasite. It was heaviest in the vicinity of the parenchymal portion of the cysticercus. Contrary to all findings in animals with a spontaneous infection, it was more marked in the wall of the vessel. The area on which the bladder abutted directly the endothelium was rather small. The inflammatory reaction shaped as an unevenly wide folded rim was extended to the poles of the ovoidly elongate bladder, sometimes exceeding the posterior half of the cyst.
At these sites, the folds of the rim were most noticeable. The inner layer of the inflammatory rim closest to the parasite consisted of activated histiocytes organized in palisades in the vicinity of the parenchymal portion of *C. bovis* (Plate V, Fig. 2). Sometimes, the layer of histiocytes covered with a fine, fibrinous, membrane, formed a wide, pseudo-epithelial, rim together with fibroblasts, eosinophil- and neutrophil polynucleates and an occasional plasmocyte. It inner part, in which fibroblasts and reticular cells increased in number, passed into a layer of newly formed granulation tissue. Focal changes of a necrotic appearance, similar to those described for a spontaneous infection, were found in 5 of the 8 cysts of this group.

Younger developmental stages of *C. bovis* (incompletely enclosed cysticerci) were found in 2 of the 10 cysts collected on day 83 p.i. The parenchymal portion of the parasite was not yet enclosed in the bladder, the scolex was evaginated. The whole parasite, changed by necrosis, was collapsed. The two cysticerci differed from those of the foregoing group in their location. A complete series of histological sections confirmed that the two parasites were located in the initial branches of the lymphatic capillary. Their bladder portion occupied the blind start of the lymphatic capillary, the parenchymal portion was extended into the capillary bed. An inflammatory reaction similar to that observed in the foregoing group of cysts, developed as a wide border; it was most noticeable around the parenchymal portion. Its inner layer composed of resorbing histiocytes and multinucleate giant cells entered from the outside the granulation tissue. The inflammatory reaction barred the only communication of the initial lymphatic capillary with the beginning of the lymphatic vessel system. The discontinuation of this connection was responsible for the death of the parasite. It was accompanied by a marked exudation of eosinophil and neutrophil leukocytes into the cavity of the cyst. The exudate started to necrotize and became dystrophically calcified.

We evaluated the tissue reaction in histological sections of 46 cysts at 102 days of an experimental infection. Completely differentiated parasites with their parenchymal portion enclosed by the bladder were present in 37 of these cysts. The scolecies of two cysticerci were growing into the spiral canal. Almost completely enclosed parasites, one with a scolex outgrown into the spiral canal, were found in 7 cysts. One cyst contained a *C. bovis* immediately before the complete enclosure of its parenchymal portion in the bladder. In one case we found a *C. bovis* with an evaginated parenchymal portion.

Even after 102 days of infection, the location of *C. bovis* in the lymphatic capillaries of the skeletal muscles was accompanied by an inflammatory reaction. However, it was less extensive than that observed on day 83 p.i. The inflammatory rim was much thinner and did mostly not reach the poles of the ovoidly elongate cyst. The pseudo-epithelial rim described for cysts at 83 days of infection was reduced to a thin layer, its inner side was composed of flattened histiocytes. Thinner was also the cellular granulation layer. On the other hand, the peripheral layer of mature granulation tissue containing numerous aggregations of lymphoid cells was more prominent. Also in these cases we observed focal changes in the pseudoepithelial rim which appeared to be necrotic (Plate VI, Figs. 1, 2).

An identical situation was observed in the tissue reaction to two cysts containing completely enclosed parasites with scolecies outgrown into the spiral canal.

In 7 cysts collected on day 102 p.i. for an evaluation of the tissue reaction, the parasite was almost completely enclosed in the bladder. The extent of the tissue reaction to these cysts was wider than that to cysts with completely enclosed larvae. The inflammatory rim surpassed the poles of the ovoid cyst as it did in an 83 day-old infection. The pseudoepithelial rim was more prominent and contained an appreciable number of eosinophil leukocytes concentrating mainly in the vicinity of the opening of the spiral canal of the parenchymal portion. Small numbers of eosinophil leukocytes.
were also present in the homogeneous proteinaceous exudate surrounding the parasite. Focal changes in the pseudoepithelial rim similar to those observed in a spontaneous infection, were regularly found in this group of cysts (Plate VI, Figs. 1, 2). Mast cells, either singly or in minute aggregations, were diffusely dispersed in the vicinity of these foci. The cellular component of the layer of granulation tissue was particularly marked, infiltrating cells were represented mainly by lymphocytes and plasmocytes. In two of the 7 cysts of this group, the location of C. bovis in the initial lymphatic capillary could be confirmed. The tissue reaction to a C. bovis with a scolex outgrown into the spiral canal was identical to that in our other findings.

In one of the 46 cysts collected on day 102 p. i., the scolex portion of a life cysticercus was evaginated. In our opinion, the evagination of the scolex was associated with the location of C. bovis in a nodule of the lymphatic network, where projections of connective tissue (described by Recklinghausen 1871, and later by Slais 1967 for lymphatic capillaries of skeletal muscles in swine) protruded into the lumen of the lymphatic capillary. The part of the capillary wall with a projection of connective tissue was considerably less distended by the growing parasite than the remaining parts of the lymphatic capillary. The projection pressed into the growing bladder, deformed it and forced the parenchymal part to “evaginate”, which, under normal conditions, occurs during the development of C. bovis in its definitive host. Contrary to all other observations, a tissue reaction occurred mainly near the poles of the cyst; it was negligible in the vicinity of the evaginated scolex portion. The relatively wide, inflammatory rim at the poles was infiltrated with numerous lymphocytes forming nod-like aggregations along the periphery. Focal changes in the pseudoepithelial rim were remarkably big. They were surrounded by multinucleate giant cells, diffusely dispersed mast cells and an occasional eosinophil leukocyte.

Four cysts collected on day 102 p. i. from the muscles of the tongue, contained enclosed cysticerci. Also these cysts were located in lymphatic capillaries. Neither the character nor the extent of the tissue reaction differed from that of other cysts containing enclosed cysticerci.

**DISCUSSION**

Tissue reaction to a developed cysticercus. Our material enabled studies on the development of inflammatory changes occurring after day 83 of infection with C. bovis. Developed cysticerci were always located in dilated lymphatic capillaries. The exact mode of migration of larvae to the lymphatic system, the origin of the reaction and its initial phase are still not fully understood. However, work in this field has been initiated by Silverman and Hulland (1961). These authors observed a weak host reaction in the liver suggestive of the initial stage of an inflammation, on day 17 of infection. A marked reaction in the heart muscles was found on day 28 p. i. Another study on early phases of tissue changes in cattle infested with cysticercosis was made by Romboli et al. (1965a, b), but a complex picture of the origin and development of tissue changes could not be obtained from any of these studies.

At the time of finding developed cysticerci in dilated lymphatic capillaries, the endothelium of the capillaries was swollen and it passed into a layer of pseudoepithelial-like organized histiocytes. Both lympho- and plasmocytes appeared on the periphery. The pseudoepithelial rim originated in a topical relationship to the scolex portion of the cysticercus, evidently under the influence of the production of antigens, the location of which has been demonstrated by Machnicka et al. (1977) by means of the immunofluorescent test. Eosinophil leukocytes entered the pseudoepithelial rim, which thickened and projected into the lumen of the dilated lymphatic capillary. A prolonged influence
of antigens from the scolex portion was followed by an apical necrosis of cells of the pseudoepithelial rim. Individual cells of the exudate and a fibrinoid substance became attached to their surface. Subsequent changes in the pseudoepithelial rim resulted in its dystrophic calcification affecting even the finest reticular fibres. These started to fragmentize and to coarsen. The changes caused a marked basophilia in reticular fibres which formed a system of "cavities" containing histiocytes with a large, light, nucleus. Generally, the cytoplasm of the histiocytes retracted during fixation. When the parasite changed its position, these sites were no longer exposed to the effect of antigens and became covered with a thin layer of connective tissue. Thus, even older foci could be demonstrated with Gomori's staining method in cross- and longitudinal sections, because they were always covered by a layer of coarser, dystrophically calcified, connective tissue.

Apart from changes in the reticulum, we observed a thickening of the mucoid-like intracellular substance in several foci. These foci were chondroid in appearance. Mostly, reticular fibres were masked by the mucoid substance, because also this layer was affected by calcification.

The origin of new foci of the dystrophically changed pseudoepithelial rim initiated by the movement of the parasite, displaced the existing foci to the periphery of the cyst. They decreased gradually in size and became enveloped in connective tissue. The cellular nuclei decreasing in number were pyenotic. Sometimes, the process of calcification affected the lymphoid-infiltrated granulation tissue. In these instances, cells of a lymphoid type were enclosed in the calcified skeleton of reticular fibres.

The local effect of antigens released by the cysticercus, and changes in the tissues associated with it are typical of a C. bovis and define the site of local pathogenic activities of a life cysticercus. The relatively prolonged survival of cells in dystrophically changed foci of the pseudoepithelial rim might be explained by the fact that the nutrition of the cells is accomplished by tissue diffusion.

A component part of tissue reactions to life, dying and dead cysticerci (C. bovis) is also another type of necrosis, which can be characterized as a focal necrosis and a calcification of collagenous fibres. This type originated in the maturing connective tissue of the granulation tissue, and was observed mainly in the heart and the liver (Šťrba and Dyková, in press). Changes in these organs were particularly marked, and the quantity of granulation tissue was considerably increased in comparison with that in the skeletal muscles. A dystrophic calcification of coarse, collagenous fibres of the maturing connective tissue appeared also on the periphery of the tissue reaction.

An advanced tissue reaction and the appearance of the first signs of death of the parasite. The development of inflammatory changes started anew in connection with the first signs of necrotic changes in the cysticercus. A starting necrobiosis could always be seen in calcareous corpuscles in the parenchymal portion of the cysticercus. It was difficult to distinguish these corpuscles in a life cysticercus. An eosinophil staining could be obtained with haematoxylin eosin, and they stained faintly (mainly in their centre) with Kossa's reaction for calcium. The eosinophilia of these corpuscles changed into a marked basophilia under the effect of the necrobiosis, and an intensive red colour was obtained with Kossa's reaction.

The development of a tissue reaction associated with the death of the cysticercus, was characterized by an exudation into the cyst. The number of eosinophil leukocytes increased in the exudate which, together with the abutting inflammatory rim and part of the granulation tissue, became affected by a local, colliquative, necrosis close to the dying or dead cysticercus. Soon, these foci succumbed to a dystrophic calcification, and multinucleate giant cells appeared in their vicinity.

A dominant, characteristic sign of inflammatory changes in the heart was the pro-
duction of a thick, pseudoepithelial, rim and a prominent layer of granulation tissue. The pressure exerted by both the rim and the layer of granulation tissue constituted a mechanical barrier preventing the normal development of the cystickercus. Particularly at first, the exudative component of the inflammation was very weak.

An advanced reaction in the skeletal muscles was characterized by inflammatory changes originating in a topical relationship to the invaginated portion of the parasite. Attention to this fact has been given in earlier studies by Melhose (1909) and Nieberle Cohrs (1952). The pseudoepithelial rim contained numerous eosinophil and neutrophil leukocytes, which entered sites in the immediate vicinity of the parasite and produced an exudate. The pseudoepithelial rim widened and exudation became more marked in connection with a progress in necrotic changes in the parasite.

Under the influence of products of decomposition originating after the death of the cystickercus, both the exudate and the granulation tissue succumbed to a focal necrosis. A new demarkation of activated histiocystes was formed in the vicinity of necrotic foci. They calcified dystrophically and multinucleate giant cells appeared in their vicinity.

Resorptive processes after the death of the cystickercus. The death of the cystickercus caused either by a violent development of inflammatory changes or by overage, was followed by the resorption of both the exudate and remnants of the parasite. Secondary reaction centres originated in the vicinity of dystrophically calcified fragments of the parasite. The final phase was a cicatrization of the foci.

The length of time required for a completion of resorptive processes could not be estimated either in our own material or assessed from information in the literature available.

ТКАНЕВАЯ РЕАКЦИЯ В СКЕЛЕТНОЙ МУСКУЛАТУРЕ КРУПНОГО РОГАТОГО СКОТА ПРИ СПОТАННОМ И ЭКСПЕРИМЕНТАЛЬНОМ ЗАРАЖЕНИИ ЦИСТИЦЕРКОМ

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Резюме. Изучали тканевую реакцию на спонтанное и экспериментальное заражение цистицерком Cysticercus bovis материала из крупного рогатого скота. Обследовали в общем 232 цист из скелетной мускулатуры. C. bovis вызвал тканевую реакцию, которая имеет характер воспаления и возникает всегда в месте выхода инвагинированного сколекса. Для этих изменений характерно возникновение псевдоэпителиального ободка и зоны гранулярной ткани, сопровождающей на периферии. В более поздней стадии, совпадающей с началом некробиотических изменений цистицерка, снова возникает воспаление, провождаемое приступанием. Объясненные структуры возбуждают реакцию резорбции. С резорбцией наразита и экссудата совпадает последовательное заживление. Обнаружены два вида некрозов: 1. фокальный некроз экссудата и воспалительного ободка с последующим дистрофическим объяснением и 2. фокальный некроз и объяснение коллагеновых волокон и их групп. В воспалительном ободке типичные для реакции на C. bovis очаги некротического вида. Эти очаги, подчиняющиеся несовершенному дистрофическому объяснению, различаются от одновременно встречающихся некрозов при помощи специальных методов окрашивания.
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Fig. 1. *C. bovis* without a marked inflammatory reaction in the skeletal muscles. HE (×20). Fig. 2. Inflammatory reaction at the opening of the spiral canal of *C. bovis* HE. (×45).
Fig. 1. Extensive inflammatory reaction in the affected sector of the lymphatic capillary. HE (x 90).
Fig. 2. Vascular granulation tissue of the inflammatory rim with foci of lymphoid infiltrates. HE (x 130).
Fig. 1. A focus necrotic in appearance, lined with fibrotizing granulation tissue. HE (×100). Fig. 2. A focus necrotic in appearance, changed by metaplasia into bone. HE (×130).
Fig. 1. Inflammatory reaction in the whole periphery of the cyst. A concentration of eosinophil leukocytes surrounds the cysticercus. HE (×15).

Fig. 2. A cyst containing incompletely resorbed remains of the parasite, and exudate in the heavily, lymphoid-infiltrated granulation tissue. HE (×14).
Fig. 1. Unresorbed remnants of a dystrophically calcified parasite, and necrotic exudate. Gomori (×80). Fig. 2. Inflammatory rim in the vicinity of the parenchymal portion of *C. bovis* with histiocytes organized in palisades. HE (×50).
Fig. 1. Foci necrotic in appearance in the inflammatory rim HE (×300). Fig. 2. Calcified foci necrotic in appearance, in the pseudoepithelial rim. HE (×70).