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SOME ASPECTS OF HISTOCHEMICAL STUDIES ON THE PARASITE AND TISSUE REACTION IN BOVINE CYSTICERCOSIS

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Abstract. Histochemical studies on the cysticercus and surrounding tissue reaction were performed at various intervals after experimental infection. It was found that acid mucosubstances and proteins with SH- and SS-groups appeared first in the granulation tissue around the cysticercus (on about day 14 p.i.) and only later (on day 28 p.i.) in the tegument of the cysticercus where they were localized in the rim of microtriches. This envelope consisting of mucosubstances and proteins seems to be identical with the electron-dense substance found on the surface of developing cysticercus during electron-microscopical studies. It is considered to be a mimicry enabling the cysticerci to survive even in an immunologically unfavourable environment. Phospholipides were found in activated fibroblasts and in some cells of macrophage type on days 21 and 30 p.i. and in a large number in subtegumental cells of cysticercus on days 28—34 p.i. This phenomenon seems to be correlated with the increased activity of subtegumental cells of the larva in this period. In morphologically fully differentiated cysticerci, the reaction for phospholipides in subtegumental cells and distal cytoplasm was only feebly positive. Phospholipides were not detected in the rim of microtriches at any time after infection.

Some of our previous papers dealt with the pathogenicity of *Taenia saginata* larva during its development (Blažek et al. 1981, 1982b, Blažek and Schramlová 1981), ultrastructure of its tegument (Schramlová and Blažek 1981), ultrastructure of the tissue reaction (Blažek and Schramlová 1980), and immune response of the intermediate host (Blažek et al. 1980a, b). It was found that the cellular reaction to the presence of cysticercus is very strong at the early stages of infection and gradually decreases with increasing morphological differentiation. Electron-microscopical studies revealed electron-dense granules or droplets in the plasma of cells and an electron-dense substance on the surface of some types of granulation tissue cells and on the surface of cysticercus. This tissue was tentatively regarded as a product of interaction between the parasite and host tissue, probably of immunocomplex character. In this paper we present the results of histochemical studies aimed at the elucidation of the character of processes and substances occurring in the cysticercus and surrounding host tissue. The available histochemical studies of other authors deal with the cysticercus and tissue reaction at the time when the cysticercus is already morphologically differentiated (Žďárská 1973, 1975) or with the detection of enzymatic activity in the larva and tissue reaction (Žďárská and Machnicka 1978, Žďárská et al. 1981, Gustowska and Pawlowski 1981). This approach, however, does not enable to evaluate the results with regard to the dynamics of the pathological process and to their significance in the system of defence mechanisms.

MATERIAL AND METHODS

The material was recovered from calves perorally infected with *Taenia saginata* eggs at the age of 4 days or 3—4 months. The method was described in detail in previous papers (Blažek et al. 1980, 1982).

Foci from skeletal muscles on days 14, 21, 23, 28, 34, 42, 55, 112, 168 and 250 after infection were used for histochemical studies. The material was fixed with neutral formol and Baker's fluid and embedded in paraffin.

The following tests were used for the detection of mucosubstances and their distribution: PAS reaction combined with acetylation and desacetylation after Pearse (1968) for neutral mucosubstances; Alcian blue (Alcianblau 8GS, Fluka), pH 2.6, after Quintarelli et al. (1964) for acid mucosubstances; Alcian blue, pH 2.6, combined with methylation and demethylation after Fisher and Lillie (1954) and Spicer and Lillie (1959) for a detailed differentiation of acid mucosubstances; Mowry's modification of AB—PAS reaction for a simultaneous demonstration of acid and neutral mucosubstances; DDD (2,2-dihydroxy-6,6-dinaphthyldisulphide) for sulphydryl (SH) groups of proteins; DDD reaction combined with thioglycolic acid after Müller and Chytil (1962) for disulphidic (SS) groups; DMAB (dimethylaminobenzaldehyde) method after Adams (1959) for tryptophan; tetrazonium copulation reaction after Müller and Chytil (1962) for a group of amino acids, tyrosine, histidine and tryptophan; PFA—AB after Pearse (1968) for SS-groups and cystine. The presence of lipides was detected by the method of Luxol blue staining after Vacek (1961).

RESULTS

On day 14 p.i., the cysticercus lesion appeared like a solid nodular focus with a larva in its centre. The focus contained a small amount of acid mucosubstances among the cells around the zone of necrosis surrounding the larva. The thin tegument of larva contained neutral mucosubstances. The necrosis itself contained proteins with SH- and SS-groups. Cells containing tryptophan, but not only eosinophiles, were found in the granulation tissue at the periphery of necrosis.

On days 21 and 23 p.i., the amount of acid mucosubstances in the zone of granulation tissue in the node somewhat increased (Plate I, Fig. 2). The tegument of the parasite, in which only microvilli were formed at that time, contained neutral mucosubstances (Plate I, Fig. 1). A small amount of proteins with SH- and SS-groups was detected in macrophages in the vicinity of larva. Single cells with granules in the plasma, positive for cystine, occurred at the periphery of the node. The tegument of cysticercus was negative for cystine, but feebly positive for phospholipides (the rim of microvilli, however, was negative). The granules in the plasma of cells of tissue reaction of the type of activated fibroblasts were markedly positive for phospholipides.

On days 28 and 34 p.i., cysticerci with scolex and sucker anlages were found in the centre of the node. Microtriches were already formed on their tegument. The region of microtriches was positive for acid mucosubstances (Plate II, Fig. 1), whereas neutral mucosubstances were demonstrated in the distal cytoplasm of tegument. The tegument was feebly positive for cystine. Acid mucosubstances were found among disintegrating macrophages surrounding the larva (Plate II, Fig. 2) and vacuoles with neutral mucosubstances were present in macrophages. The granulation zone at the periphery contained acid mucosubstances among the cells in droplets. The reactions for the detection of SH- and SS-groups were more intensive than in the previous time period and were localized in sigle cells of granulation tissue and near the surface of cysticercus. Tryptophan was again demonstrated in some cells dispersed in the granulation zone. A strongly positive reaction for cystine was observed in fibroblasts and histiocytes in the peripheral and intermedial zone of granulation tissue. At this period, there was a strong positive reaction for phospholipides in subtegumental cells of cysticercus and a more feeble reaction in distal cytoplasm; the rim of microtriches remained unstained. A positive reaction was demonstrated also in the newly formed collagen in fibroplastic granulation tissue and in granules in macrophages accumulated mainly in the region near the opening of spiral canal on the bladder surface.

On days 42 and 55 p.i., accumulation of acid mucosubstances was observed in the immediate vicinity of the parasite (Plate III, Fig. 1). They appeared like flowing from the surrounding tissue towards the cysticercus (Plate III, Fig. 3). Acid mucosubstances were also demonstrated in the microtriches of bladder and spiral canal. Mast cells positive for acid mucosubstances were dispersed in more distant parts of tissue reaction. The reactions for the detection of proteins with SH- and SS-groups were much less intensive than on days 28 and 34 and decreased proportionally to the disintegration of cells which in the previous stages (14 and 28 days) were dispersed in the granulation tissue and sometimes accumulated around the parasite. The reaction for cystine, however, was strongly positive in the tegument of cysticercus, in cells of fibroblast type, and in cells with spherical nuclei, which occurred mostly near the cysticercus and some of them also at more distant sites. These cells were accumulated also in structures resembling migratory canals near the focus with cysticercus. The tissue reaction contained also single free granules positive for cystine. Cells containing tryptophan, mostly eosinophiles, were found in front of the opening of spiral canal.

On days 112, 168 and 250 p.i., the histochemical character of the tissue reaction and parasite did not change in substance. Acid mucosubstances were demonstrated in the microtriches of tegument and around the bladder. The tissue (fine connective tissue walls of the cyst) around the bladder contained only a small amount of acid mucosubstances. A greater amount of them was demonstrated only near the opening of the spiral canal where the accumulation of histiocytes persisted. The tegument of cysticercus (probably the rim of microtriches) was strongly positive for cystine. The connective tissue wall of cyst was only feebly positive, but it contained single spindle-shaped cells, fibroblasts, with strongly positive granules in the plasma; they were more numerous near the opening of the spiral canal. A feeble positive reaction for phospholipides was demonstrated in subtegumental cells and distal cytoplasm (zone of microtriches remained unstained), but a strong positivity was found in the connective tissue of the cyst and in granules of large cells of tissue reaction near the opening of spiral canal. At that time (112 days p.i. and later) some of the cysts contained again a greater number of cells positive for tryptophan (Plate IV, Fig. 1). These were activated fibroblasts and eosinophiles the granules of which were positive for tryptophan. Such cysts contained dying cysticerci. Also the reactions for SH- and SS-groups were more intensive here.

DISCUSSION

A comparison of the results of histochemical studies at various intervals after infection revealed that the tegument of larva at an early stage of development (14 days and about 20 days p.i.) provided with microvilli contains neutral mucosubstances. Four weeks after infection, the tegument of bladder, as well as the tegument of the scolex, which is already formed at that time, possess microtriches containing acid mucosubstances with HSO_3 groups, whereas the distal cytoplasm of tegument contains only neutral mucosubstances. The same was observed in morphologically differentiated cysticerci in our own material and it was also described by other authors in *C. bovis* (Žďárská 1973) and larva of *Multiceps endothoracicus* (Hulínská et al. 1978). A question arises, however, how these acid mucosubstances get into the layer of microtriches. It is supposed that in adult cestodes, acid mucopolysaccharides in the rim of microtriches may be a product of cells of interproglottidal glands or that they can be derived from neutral mucosubstances of the tegument (Howells and Erasmus 1969). Lumsden (1966) even assumed that the presence of mucopolysaccharides in the layer of microtriches might be related with pinocytosis. In *C. bovis* (and in any of cestode larvae in general), however, the first hypothesis cannot be considered, since there are no glands or solitary gland cells in the bladder wall. Not even pinocytosis is probable. If it occurs at all, then particularly in basal parts of microtriches, as it is

Table 1. Detection of some substances in cysticercus and tissue reaction

Days p.i.	Test for	Cysticercus			Tissue reaction			
		Tegument		Subtegumental cells	Necrosis	Zone of macrophages	Granulation tissue	Wall of differentiated cyst
		Microtriches Microvilli	Distal cytoplasm					
14	Acid mucosubstances	–		–	–	0	+	0
	Neutral mucosubstances	++		–	+	0	–	0
	Proteins with SH- and SS-groups	+		–	++	0	+	0
	Proteins with SS-groups — cystine	NT						
	Tryptophan	–		–	–	0	+++ single cells	0
	Phospholipides	NT						
21, 23	Acid mucosubstances	–	–	–	0	–	++	0
	Neutral mucosubstances	++	++	+	0	+++	++	0
	Proteins with SH- and SS-groups	++	++	++	0	+	+++	0
	Proteins with SS-groups — cystine	–	–	–	0	+	+++	0
	Tryptophan	–	–	–	0	–	+++ single cells	0
	Phospholipides	–	++	–	0	–	+++ single fibroblasts	0
28, 34	Acid mucosubstances Neutral mucosubstances	+++	+	+	0	++	+++ droplets among fibroblasts	0
	Proteins with SH- and SS-groups	–	++	++	0	+++	–	0
	Proteins with SS-groups — cystine	++	++	++	0	+++	+++	0
		++	–	–	0	–	++++	0
	Tryptophan	–	–	–	0	–	+++ single cells	0
	Phospholipides	–	++	++++	0	++	++	0

Table 1 (continued)

Days p.i.	Test for	Cysticercus			Tissue reaction			
		Tegument		Subtegumental cells	Necrosis	Zone of macrophages	Granulation tissue	Wall of differentiated cyst
		Microtriches Microvilli	Distal cytoplasm					
42, 55	Acid mucosubstances	++++	++	++	0	0	++++	–
	Neutral mucosubstances	–	++	++	0	0	++	–
	Proteins with SH- and SS-groups	+	+	+	0	0	++	–
	Proteins with SS-groups — cystine	++++	–	–	0	0	++++	–
	Tryptophan	–	–	–	0	0	++++	–
	Phospholipides	–	+	++	0	0	–	++++
112, 168, 250	Acid mucosubstances	++++	++	++	0	0	0	+—++
	Neutral mucosubstances	–	++	++	0	0	0	–
	Proteins with SH- and SS-groups	+	+	+	0	0	0	+
	Proteins with SS-groups — cystine	++++	+	–	0	0	0	++ single fibroblasts
	Tryptophan	–	–	–	0	0	0	++++ +++ single cells
	Phospholipides	–	+	++	0	0	0	++++ ++++ granule of fibroblasts

+ — slight positivity, ++ — medium positivity, +++ — marked positivity, ++++ — strong positivity, – — negative, 0 — not formed, NT — not tested.

indicated by electron-microscopical studies. Consequently (according to the hypothesis by Howells and Erasmus 1969), the neutral mucosubstances of distal cytoplasm of tegument might have been changed to acid ones and pressed into microtriches, but we exclude even this possibility. The amount of neutral mucosubstances in the distal cytoplasm of tegument (if their amount can be measured by the intensity of the respective histochemical reactions) does not suggest that such a large amount of acid mucosubstances could appear in the rim of microtriches without any substantial effect on the amount of "mother" neutral mucosubstances. In our opinion, it is of importance that acid mucosubstances in the granulation tissue around a young cysticercus can be detected earlier than on the surface of tegument and that these substances, probably also in the complex with proteins (as indicated by reactions for SS-groups of proteins and cystine), are released from the cells of inflammatory reaction towards the surface of larva. This relation escapes attention while evaluating the histochemical reactions in larvae isolated from the host tissue. The extrusion of a part of cytoplasm of large macrophages accumulating around the larva at an early stage of infection was demonstrated also in an electron microscope (Blažek and Schramlová 1980). On about 30th day p.i., the cysticercus is already enveloped with substances of the nature of acid mucopolysaccharides (mucosubstances) and the rim of microtriches is positive for acid mucosubstances (as well as for cystine) even in fully differentiated cysticerci at the late stage of infection, despite the fact that the bladder surface is not fully metabolically active at that time (Žďárská et al. 1981). The formation of acid mucosubstances, proteins with SS-groups and cystine around the larva is marked in the cellular reaction not only in the period around 30 days after infection, but also on day 42 and partly also on day 55 p.i. At the late stage of infection it is only weak and is limited to the region of the cellulizate near the opening of spiral canal on the bladder surface, with which the metabolic activity (although only low) is associated even at that time (Žďárská et al. 1981). An increased amount of mucosubstances was detected also in the tissue around the dying cysticercus, which agrees with the release of a great amount of antigens from the dystrophically changed or disintegrating cysticercus (Walther 1978, Walther and Grosskalus 1979).

We assume that the envelope consisting of acid mucosubstances and proteins represents a certain antigenic mimicry which makes it possible that a morphologically differentiated cysticercus survives even in an immunologically unfavourable environment. It seems to be identical with the electron-dense substance detectable on the surface of cells of inflammatory reaction already about 20 days p.i. and on the surface of cysticercus somewhat later (Blažek and Schramlová 1980).

While evaluating the whole process it should be also noted that the positive reaction for proteins with SH- and, particularly, SS-groups was demonstrated in tissue reaction (except necrosis) in fibroblasts and cells of macrophage type already on day 20 p.i. and that this reaction was most strong on days 28 and 34 p.i. It was also confirmed that the activation of fibroblasts detected by us in the electron microscope at the early stage of infection is the result of a live proteosynthesis (Blažek and Schramlová 1980). Also the reactions for phospholipides were strongest at that period (28—34 days p.i.), particularly in subtegumental cells of larva, tissue reaction around cysticercus, newly formed collagen and granules in plasma of macrophages accumulating in scolex region. The strong positivity of this reaction in the subtegumental cells is undoubtedly associated with their increased secretory activity at the time when the scolex is formed and differentiated (Schramlová and Blažek 1981) and gives evidence that their secretion contains a lipoid complex.

ГИСТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ ПАРАЗИТА И ТКАНЕВОЙ РЕАКЦИИ ПРИ ЦИСТИЦЕРКОЗЕ КРУПНОГО РОГАТОГО СКОТА

Я. Шрамлова и К. Блажек

Резюме. Изучали гистохимию цистицерка и окружающей тканевой реакции в разных промежутках времени после экспериментального заражения. Было обнаружено, что кислые мукосубстанции и белки с SH- и SS-группами встречаются сначала (на 14-й день после заражения) в грануляционной ткани вокруг цистицерка и только позже (на 28-й день после заражения) в тегументе цистицерка, где они локализованы в кайме микротрихов. Эта оболочка, состоящая из мукосубстанций и белков, по-видимому сходна с электронно-плотным веществом, находящимся на поверхности развивающегося цистицерка и видимым при помощи электронного микроскопа. По нашему мнению эта оболочка представляет собой мимикрию, которая делает возможным переживание цистицерка даже и в иммунологически неподходящей среде. Фосфолипиды были обнаружены в активированных фибробластах и некоторых клетках типа макрофагов на 21-й и 30-й дни после заражения. Большое количество фосфолипидов встречалось в субтегументальных клетках цистицерка от 28-го до 34-го дня после заражения. Кажется, что это связано с повышенной активностью субтегументальных клеток личинки в этом периоде. У морфологически вполне дифференцированных цистицерков реакция на фосфолипиды в субтегументальных клетках и дистальной цитоплазме была только слабо положительна. В кайме микротрихов фосфолипиды не обнаруживались.

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On the occasion of 150 years of its existence (see Folia parasit. (Praha) 29: 278, 1982) the Zoological Institute of the USSR Academy of Sciences issued a volume devoted to the history of this research centre and of its different laboratories and biological stations since the very beginning of its activities until the present day. In five comprehensive chapters the reader will find a wealth of information on the scientific activities of the Institute's departments and its specialists, on the results achieved and on the most important work published to date. The parasitologist's interest will be primarily attracted to the chapter entitled "Parasitological and protozoological studies", including subchapters Parasitological laboratory (Yu. S. Balashov), Group for helminth investigations (A. V. Gussev) and Protozoological laboratory (M. V. Krylov). There is a supplement, containing brief biographies of academicians and corresponding members of the USSR Academy of Sciences who have been active at the Zoological Institute, and a list of monographs published in the series Fauna of the USSR (154 titles) and Keys to the fauna of the USSR (133 titles) based mainly on the Institute's collections or published by the Institute. At the end of the volume there is a list of most important bibliography referring to the history of the Institute. This is a very useful book which provides many valuable data to anyone interested in the advances of the Russian and Soviet science.

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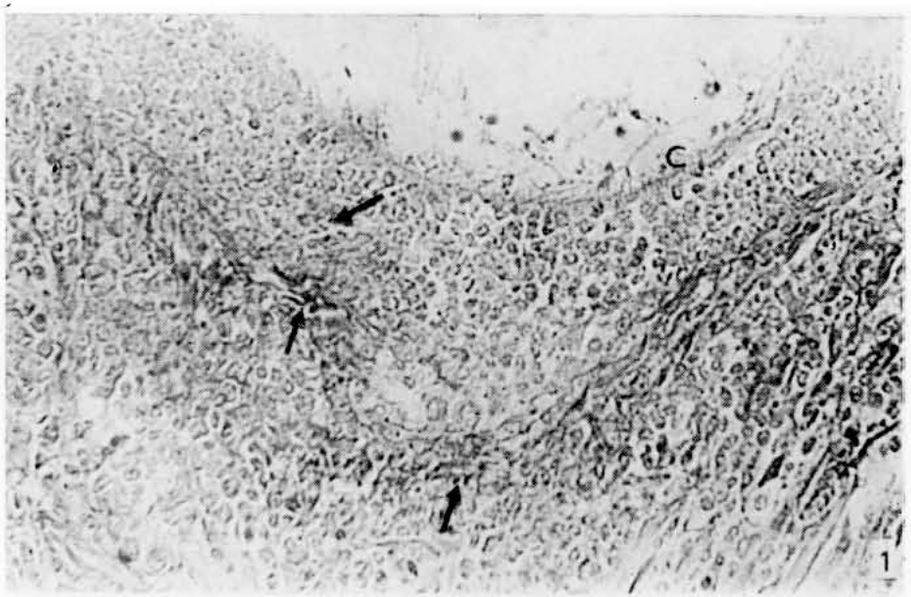


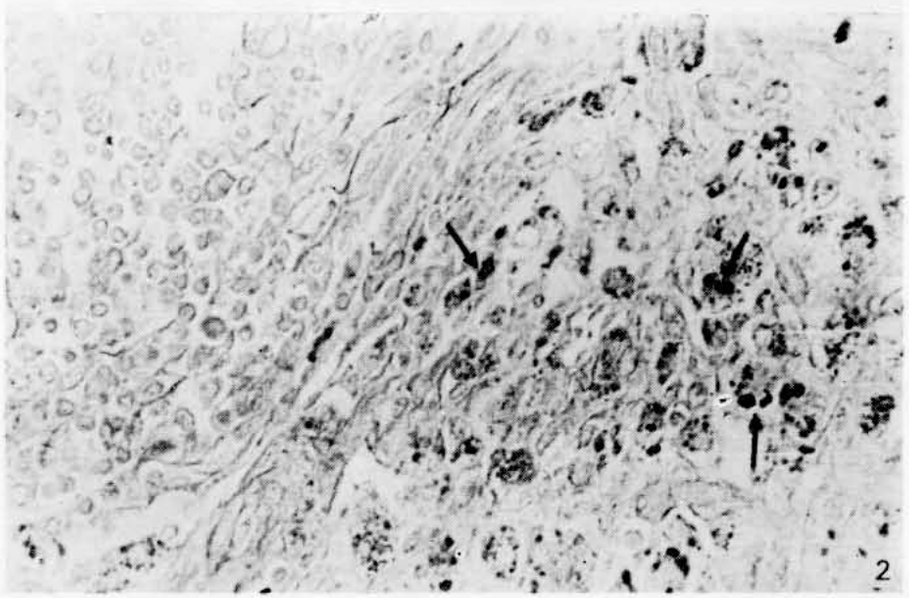
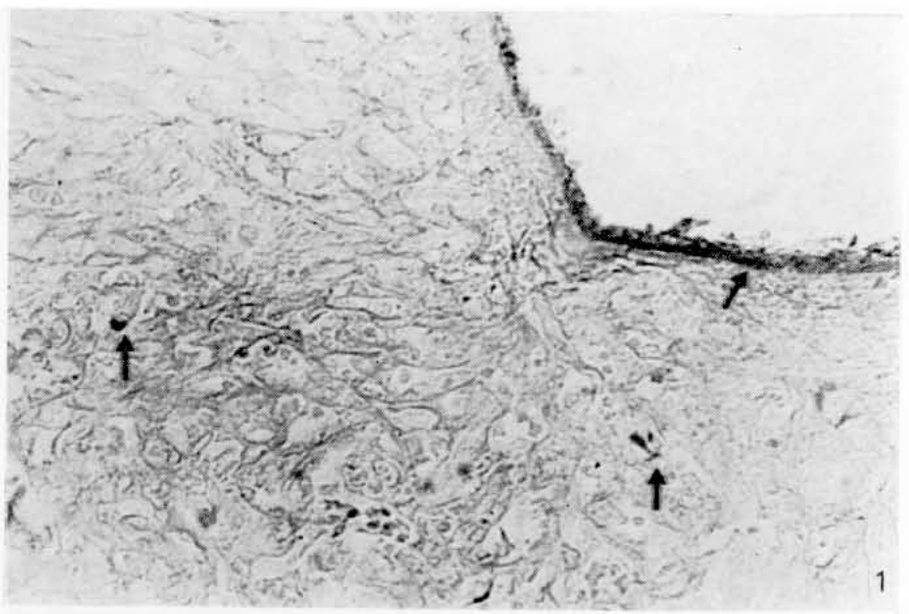


Fig. 1. Acid mucosubstances (arrows) in a node with *Cysticercus bovis* (C) on day 21 p.i. AB + PAS (300×). **Fig. 2.** Acid mucosubstances (arrows) in cells at the periphery of node on day 21 p.i. AB + PAS (600×).



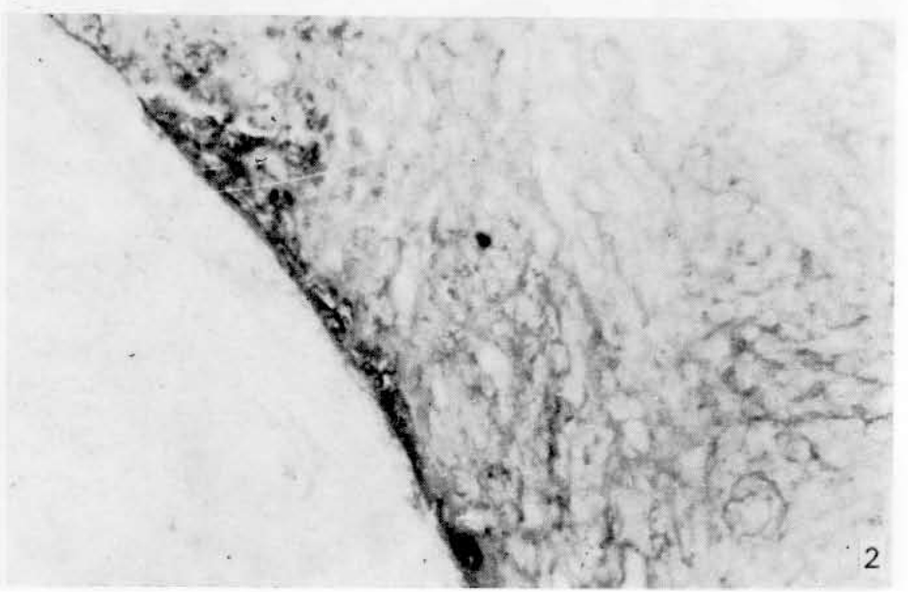
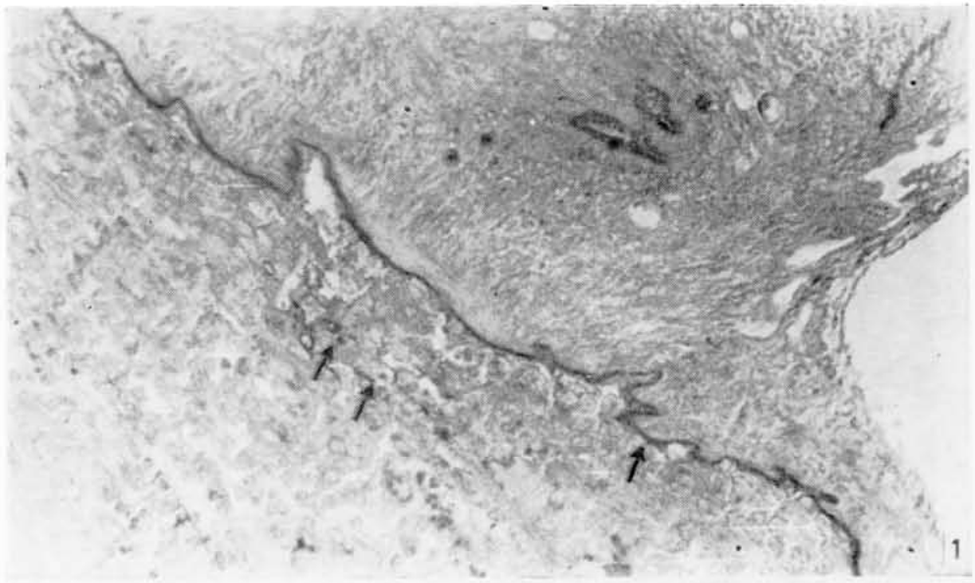
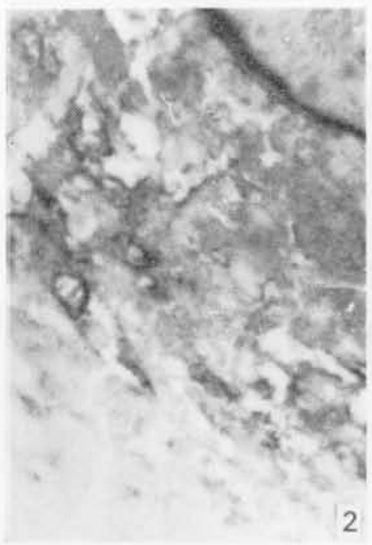


Fig. 1. Acid mucosubstances in region of microtriches (arrows) and among cells of tissue reaction (arrows) on day 28 p.i. AB + PAS (125×). **Fig. 2.** Accumulation of acid mucosubstances in the host tissue around the cysticercus on day 28 p.i. AB + PAS (115×).





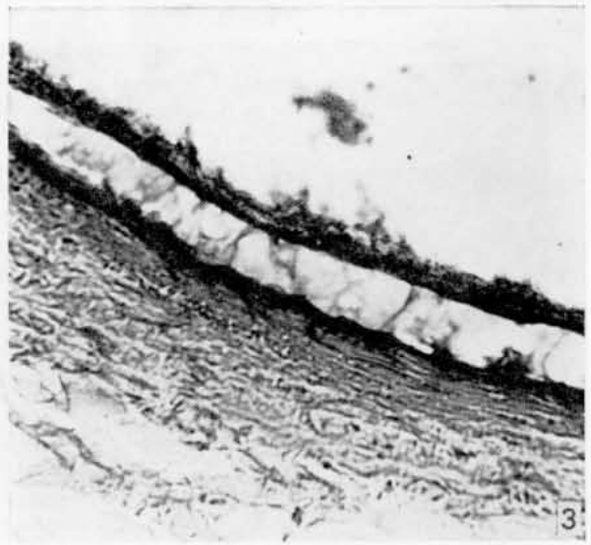


Fig. 1. Acid mucosubstances in microtriches of *C. bovis* and in tissue reaction (arrows) on day 42 p.i. SC — scolex part of *C. bovis*. AB + PAS (125×). **Fig. 2.** Acid mucosubstances in plasma of some cells and among them (detail from Fig. 1). (700×). **Fig. 3.** Acid mucosubstances in tissue reaction accumulated in a close vicinity of the parasite on day 42 p.i. AB + PAS (125×).

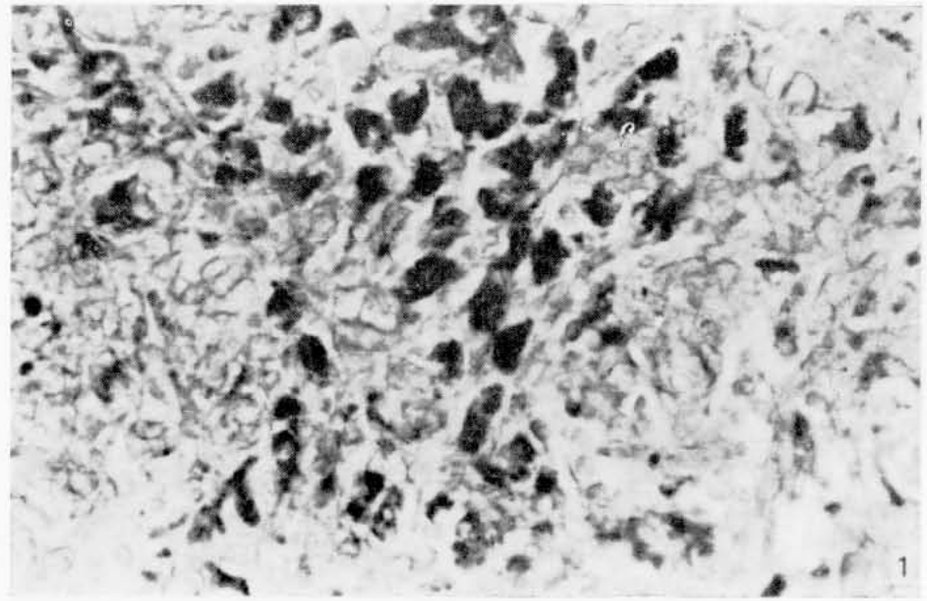


Fig. 1. Cells containing tryptophan. DMAB (400×). **Fig. 2.** Acid mucosubstances in a dystrophic focus in the cyst wall (arrow). Section through the wall of cysticercus bladder (C) on day 112 p.i. AB + PAS (200×).