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Ats METSIS

A CYTOCHEMICAL STUDY OF SOME PHOSPHATASES IN THE TISSUE CYSTS OF *SARCOCYSTIS BOVICANIS* FROM BOVINE HEART

Sarcocystis bovicanis is a Coccidian parasite with a two host life cycle. For this parasite the canines serve as final hosts, the sexual part of the cycle taking place in their intestine, and cattle serve as intermediate hosts, the asexual part of the cycle developing in the endothelial cells of their vascular system and bloodstream, and terminating with the formation of tissue cysts in their muscles (Heydorn, Rommel, 1972; Fayer, Johnson, 1973; Fayer, 1979; Dubey, 1982). The present paper treats the tissue cyst stage of this intracellular parasite. The distribution of acid phosphatase (3.1.3.2. AcPase), alkaline phosphatase (3.1.3.1, APase), adenosinetriptospatase (3.6.1.3, ATPase; optimum pH 9, the so-called myosine ATPase), and glucose-6-phosphatase (3.1.3.9, G6Pase) were studied.

Material and methods

Material. The bovine hearts were received from a slaughterhouse, and 1 cm^3 pieces of myocard were instantly frozen in liquid nitrogen for cryosectioning. For electron microscopy the material was fixed in the slaughterhouse instantly after butchering.

Methods. *Enzyme cytochemistry.* Sections of 1 to 5 μm thickness were cut at −22 °C with a Bright-5030 cryostat (England). The sections mounted on glass slides were desiccated at room temperature under a stream of dry air. In the case of G6Pase the cytochemical reaction was carried out on unfixed sections. In other cases the sections were prefixed in 4% paraformaldehyde (Serva, FRG) in 0.09% sodiumchloride solution. While carrying out the cytochemical reactions the methods given in the lab manual of Lojda et al. (1979) were used. The AcPase was tested according to Gomori (1950; see Lojda et al., 1979) with the help of the metal salts method, and according to Burstone (1962; see Lojda et al., 1979) with the help of naphtol-AS-BI-phosphate (Sigma, USA) method. For APase the reaction of Gomori (1952; see Lojda et al., 1979) with metal salts was used. Besides that APase was tested according to Burstone (see Lojda et al., 1979) with naftol AS-BI (Sigma, USA). The calcium-cobalt method of Padykula and Herman (1955; see Lojda et al., 1979) was used to determine the ATPase distribution. In the case of G6Pase the method with heavy metal salts after Chiquoine, 1953, 1955; and Wachstein, Meisel, 1956 in modification (see Lojda et al., 1979) was used. Control sections were incubated with media lacking the appropriate substrate. In the case of AcPase incubation with 5 mM NaF served as a supplement control. Each reaction was performed several times with the material from different animals. All the reactions were carried out simultaneously on the sections of bovine liver mounted on the same glass slides in order to check the quality of reaction media.

Electron microscopy (EM). The only enzyme distribution studied on the ultrastructural level was that of AcPase. The material was fixed at 4 °C for 2—4 hours in 3% paraformaldehyde in Sjostrand's acetate-veronal buffer (pH 7.3) or in 0.1 M sodium cacodylate buffer (pH 7.3). The reaction was carried out according to Mehlhorn et al., 1974. After fixation the specimens were washed three times in a pH 7.3 buffer and twice in 0.1M acetate buffer (pH 5.0). The incubation media consisted of two stock solutions [(a) 110 mg β-glycerophosphate (Sigma, USA) in 30 ml of 0.1M acetate buffer (pH 5.0); and (b) 50 mg of $Pb(NO_3)_2$ in 20 ml of the same buffer] which were mixed, incubated for 30 min at 37 °C and filtrated before the specimen incubation. The reaction was carried out at 37 °C for 30 min to 1 hour or at room temperature for 1.5 to 2 hours. Next the specimens were washed in two portions of the acetate buffer (pH 5.0) and three portions of the respective pH 7.3 buffer, postfixed in 1% OsO_4 in 0.1 M cacodylate buffer containing glucose (1.6 ml of 5.4% solution in 10 ml of fixation media) dehydrated in increasing concentrations of alcohol, infiltrated with 1 : 1 mixture of propylene oxide/embedding media, and embedded in Epon 812 or Epon-Araldit. The sections were cut with a LKB-III ultratome (LKB, Sweden), stained with uranyl acetate, and examined on a Jem-100 C-X (JOEL, Japan) electron microscope. In all cases the specimens were examined during the period of two weeks after embedding. The control specimens were incubated with only one of the two stock solutions of the incubation media.

Results

In the case of testing AcPase and APase on cryo-sections of frozen bovine myocard with the help of azo-coupling and naphtol-phosphate methods the distribution of enzymes in tissue cysts could not be followed. Positive results were obtained only when using the Gomori reaction with heavy metal salts. Although both the enzymes gave a weak reaction, in the case of AcPase the staining was more adequate. The APase could be localized in the cyst wall, and less in the septa (Figs. 1, 2).

The testing of ATPase by means of the calcium-cobalt method gave positive results. The staining intensity of tissue cysts was comparable with that of the host tissue (Fig. 3). The enzyme activity could be localized in the septa and cyst wall as well as in the cyst stages. Although it was quite difficult to localize the reaction product inside the cell, it seemed to be localized polarly.

The enzyme activity proved to be the highest in the case of G6Pase. On the cryo-sections the reaction product could be followed only inside the tissue cyst, in the surrounding host tissue it was absent. The staining was seen in the septa and cyst wall as well as in the cyst stages (Fig. 4). In the cyst stages the granular deposit of reaction product was seen in the cell cytoplasm.

The AcPase reaction product could be followed mainly in the cyst wall and in the septa (Fig. 5). AcPase could not be localized in the cyst stages by means of light microscopy. In addition AcPase distribution was studied on the ultrastructural level. The results received made it possible to follow the enzyme distribution in the cell organelles of different cyst stages (metrocytes and merozoites) as well as in the cyst wall and the septa. The highest activity of AcPase could be seen on the outer membrane of the cyst wall (Figs. 6, 7). In the septa a positive reaction to this enzyme was also received, although the enzyme activity was much lower than expected after the light-microscopic studies. In the cyst stages the

reaction could be followed on the outer surface of the inner membrane complex (Figs. 6, 8) while the greatest amount of the reaction product deposit was seen in the apical part of the merozoites (Fig. 8). Although inside the micronemes enzyme activity could not be followed, lead salt deposits were seen around these organelles in the cell cytoplasm (Figs. 7, 8). A very inintensive reaction was received in the ducts of rhoptries (Fig. 8). In the rhoptries themselves the reaction was followed only in the upper part, just opposite the ducts and on the membranes of these organelles (Fig. 8). In the metrocytes the reaction product was seen besides the cell covers on the membranes of vacuolar structures and some deposit granules in the cytoplasm (Fig. 6). In uncellular structures of the tissue cysts the reaction product was followed in the places of proposed degenerative processes (Fig. 6). Somewhat confusing is the fact that the reaction product occurred also in the mitochondrions of the cyst stages (Fig. 9).

Discussion

Although carried out on the material received from spontaneously infected animals, it may be said that the study deals with a monoinfection as it is considered that only one species of *Sarcocystis* (*S. bovicanis*) parasitizes the myocard of cattle (Markus, 1978; Joyner, 1982; Метсис, 1987).

The light-microscopic studies of APase and AcPase distribution in the tissue cysts show that both these enzymes are localized in the same structures, i. e. the cyst wall and the septa. These findings prove the importance of the structures mentioned in the nutrition processes. The EM studies showed that AcPase was located also in the pellicule of cyst stages. Relying on the analogy with *Leishmania donovani*, for which it has been shown that the AcPase located on the cell membranes has the role of inhibiting the toxic effect of the oxidative burst of the host cell (see Mauel, 1984) on the parasite (Remaley et al., 1985), such a location on the structures which are or will be in contact with the cytoplasm structures of the host cell may indicate on the same role of the *Sarcocystis* AcPase when surviving inside the host cell.

Besides the mentioned structures the AcPase was found in the apical part of the ampoules and ducts of the rhoptries. Although inside these organelles the reaction was not observed, the salt deposits were seen on the rhoptry membrane. Such a different localization on several parts of the organelles mentioned may be explained with the help of the assumptions that the rhoptry excreta change their chemical characteristics during the extrusion process, or that the enzyme itself plays a concrete role in the extrusion process. The first hypothesis could be checked with the help of cytochemical studies of the extrusion processes themselves. The AcPase location in the mitochondrion cristae is too complicated to be explained. It should be only noted that analogous results are mentioned also in an earlier paper on the same subject (Mehlhorn et al., 1974), and we cannot exclude some kind of an artefact either.

Quite a high activity of the so-called "myosine" ATPase was detected. Keeping in mind that for a related parasite *Toxoplasma gondii* the presence of myosin at the anterior end of the parasite has been shown (Schwartzman, Pfefferkorn, 1983), the polar localization of ATPase in *Sarcocystis* merozoites may indicate on a certain role of this enzyme in the motility of the apical organelles or the whole parasite cell.

The high activity of G6Pase in the cyst stages of *Sarcocystis bovicanis* indicates on the presence of glyconeogenesis processes (see Огородникова,

1986). The presence of NAD-dependent malat dehydrogenase and lactate dehydrogenase (Jesus Fihlo, Miraglia, 1977; Казакаускайте, 1980b; Метсис, 1988) and the high concentration of polysaccharides shown in the cell cytoplasm of several species (Fayer, Thompson, 1975; Jesus Fihlo, Miraglia, 1977; Казакаускайте, 1980a; Dubey, 1983; Gjerde, Bratberg, 1984; Метсис, 1987) also support this assumption.

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Institute of Experimental Biology

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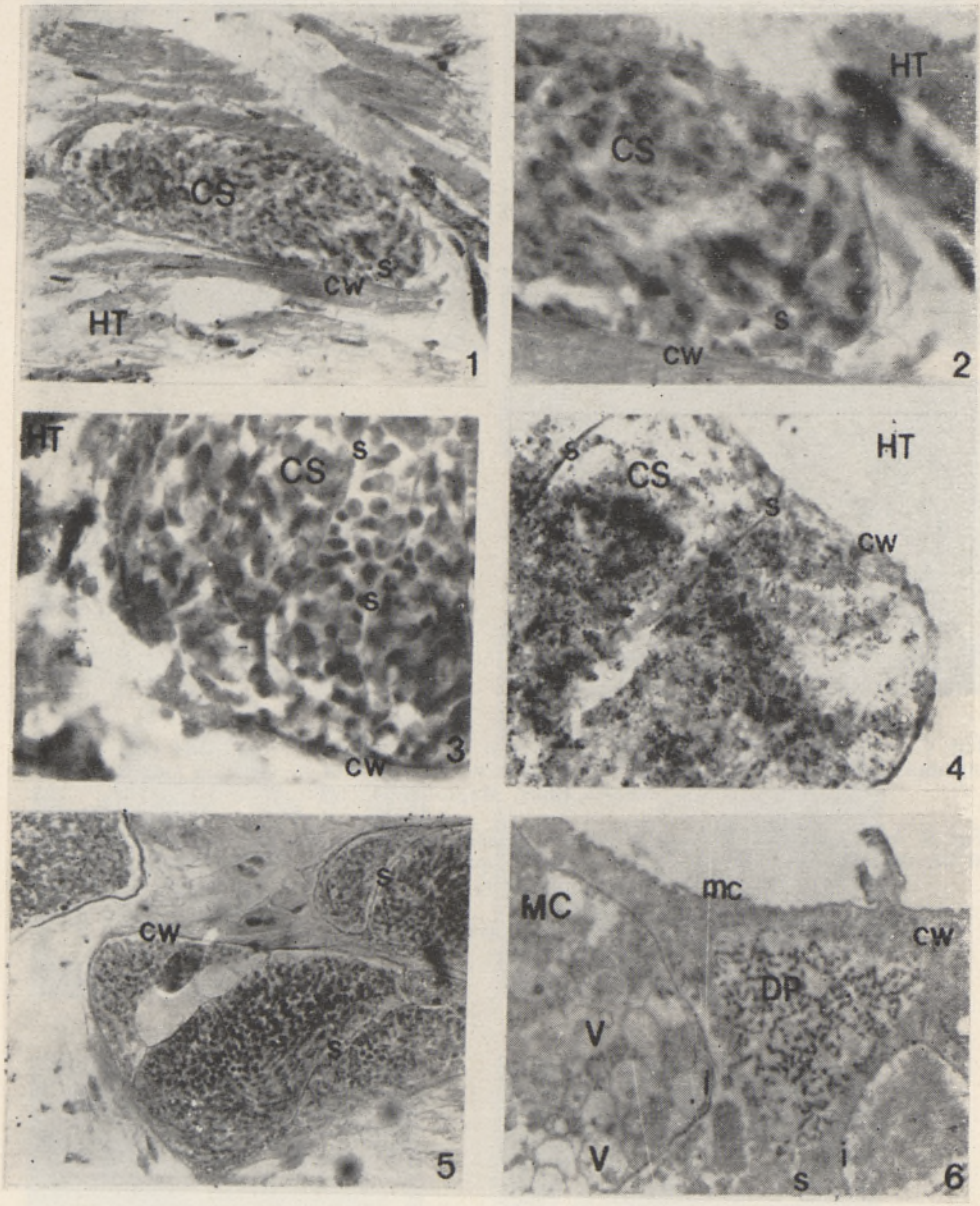


Fig. 1. Reaction to APase after Gomori in tissue cysts of *S. bovicanis*. A weak reaction can be seen in the cyst wall (cw) and septa (s). The staining of the host tissue (HT) and cyst stages (CS) is caused by light green understaining. Magnification 40×3.2×2.5.

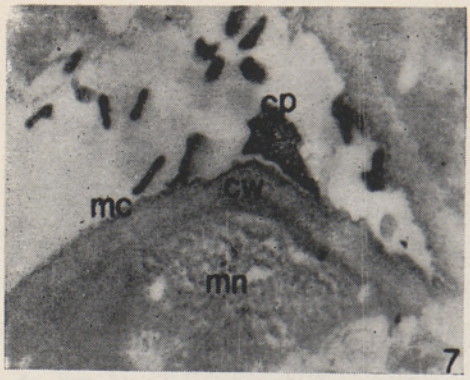
Fig. 2. Same as Fig. 1. Magnification 100×3.2×2.5.

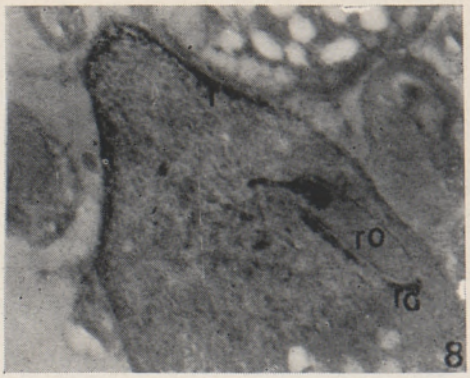
Fig. 3. Reaction to ATPase in cyst stages (CS), septa (s) and cyst wall (cw) is comparable to that in the host tissue (HT). Magnification 100×3.2×2.5.

Fig. 4. Reaction to G6Pase is seen in the cyst stages (CS), septa (s) and cyst wall (cw). Reaction in the host tissue (HT) is extremely weak. Magnification 100×3.2×2.5.

Fig. 5. The Gomori reaction to AcPase can be seen in septa (s) and cyst wall (cw). The staining of cyst stages and host tissue is caused by light green understaining. Magnification 40×3.2×2.5.

Fig. 6. Reaction to AcPase is seen in the outer membrane of the cyst wall (mc), in the cyst wall (cw) and septa (s). In cyst stages the reaction is seen on the membranes of the inner membrane complex (i). In the metrocyte (MC) the reaction is seen also in the vacuolar structures (V). The reaction is also seen in the places of degenerative processes (DP). Magnification 7500×.





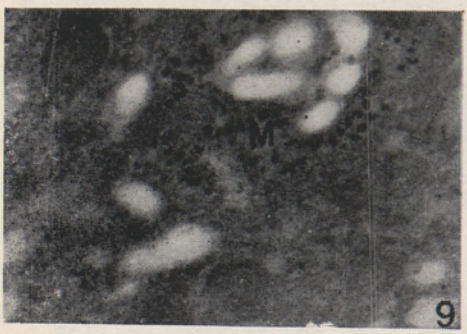


Fig. 7. The reaction product of AcPase is seen in the cyst wall protrusions (cp), on the membrane of the cyst wall (mc), in the cyst wall (cw), and in the cyst stages around the micronemes (mn). Magnification 6000×.

Fig. 8. A reaction on the inner membrane complex (i) of the apical part of a merozoite, in the ducts of the rhoptries (rd), on the rhoptry (ro) surface and around micronemes. Magnification 7000×.

Fig. 9. Reaction in the mitochondrion cristae (M). Magnification 13000×.

Рис. 1—23. Пыльцевые зерна.

Figs 1—23. Pollen grains.

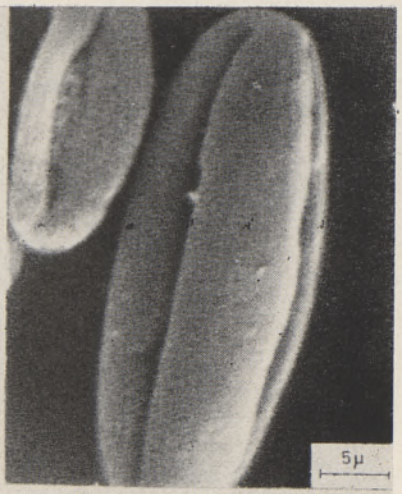


Рис. 1. *P. subarenaria.*

Рис. 2. *P. argentea.*

Рис. 3. *P. supina.*

Рис. 4. *P. argentea.*

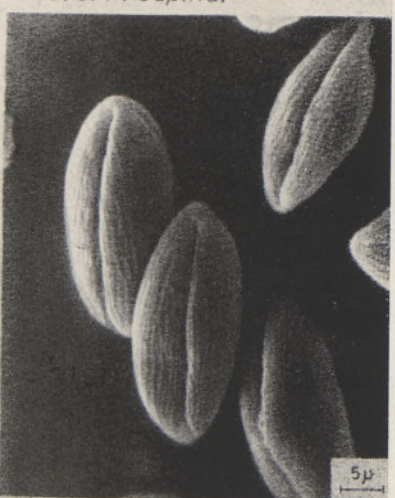
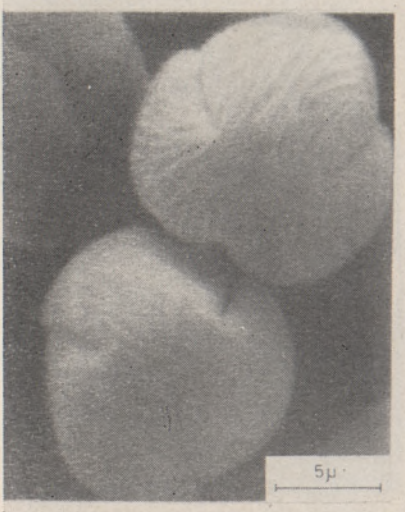


Рис. 5. *P. fruticosa.*

Puc. 6. *P. leucopolitana.*

Puc. 7. *P. erecta.*



Puc. 8. *P. arenaria.*

Puc. 9. *P. subarenaria.*

Puc. 10. *P. crantzii.*

Puc. 11. *P. bifurca.*

Puc. 12. *P. anserina.*

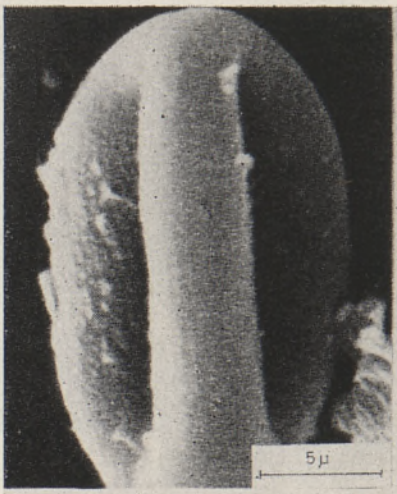
Puc. 13. *P. canescens.*

Puc. 14. *Geum rivale.*

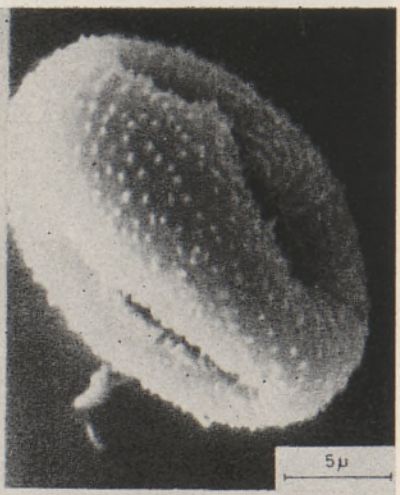
Puc. 15. *Fragaria vesca.*

Puc. 16. *Comarum palustre.*

Puc. 17. *Rubus chamaemorus.*

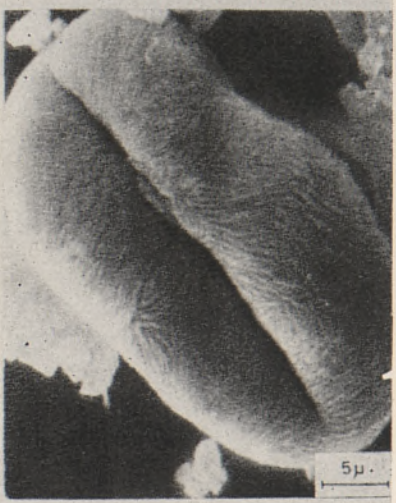


Puc. 18. *Alchemilla wichurae.*

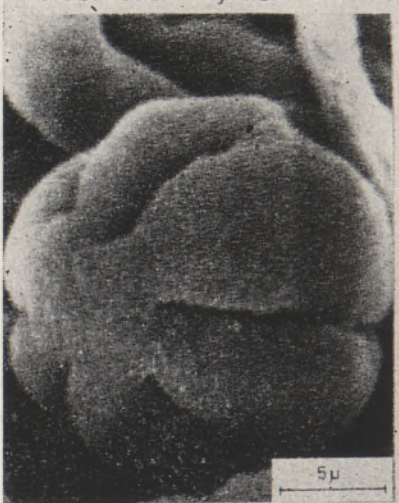


Puc. 19. *Filipendula ulmaria.*

Puc. 20. *Rosa majalis.*



Puc. 21. *Agrimonia eupatoria.*



Puc. 22. *Sanguisorba officinalis.*

Puc. 23. *Poterium sanguisorba.*

Рис. 24–34. Семена.

Figs 24–34. Seeds.

Рис. 24. *P. goldbachii.*

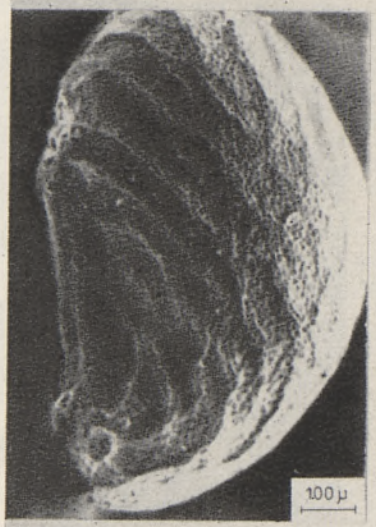


Рис. 25. *P. argentea.*

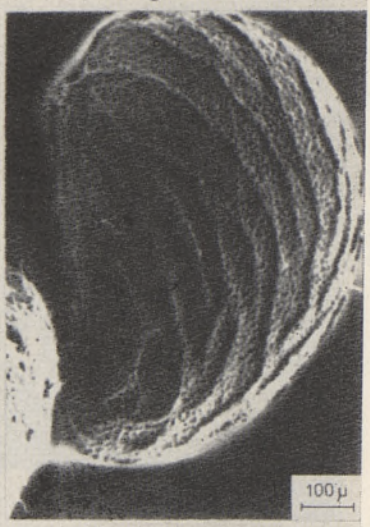


Рис. 26. *P. arenaria.*

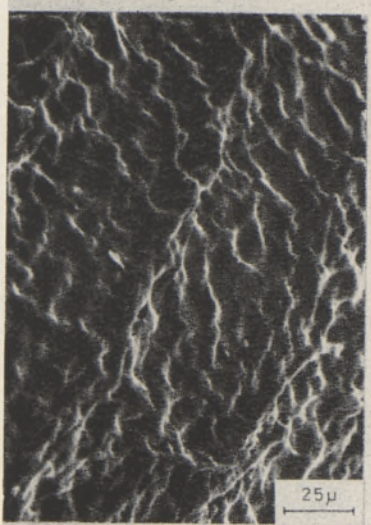


Рис. 27. *P. argentea.*

Рис. 28. *P. argentea.*

Рис. 29. *P. goldbachii.*

Рис. 30. *P. crantzii.*

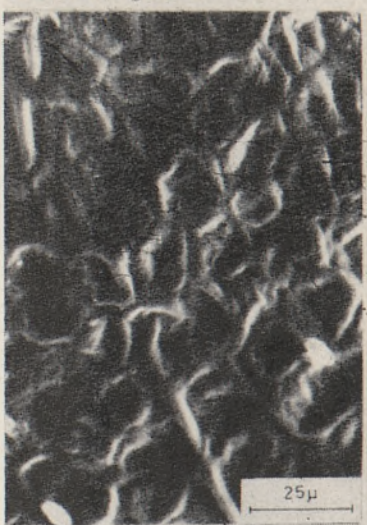


Рис. 31. *P. tabernaemontani.*

Рис. 32. *P. erecta.*

Рис. 33. *P. anserina.*

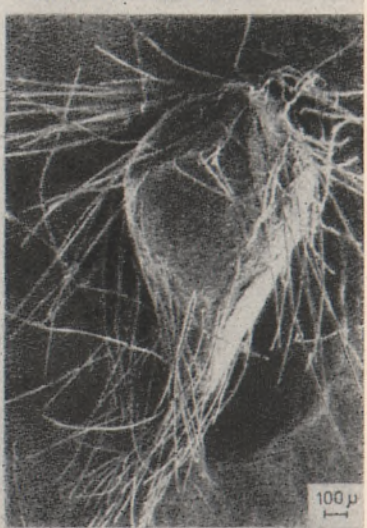


Рис. 34. *P. fruticosa.*

Ats METSIS

MÕNEDE FOSFATAASIDE TSÜTOKEEMILISED UURINGUD LEHMA SÜDAMEST PÄRINEVATES *SARCOCYSTIS BOVICANIS*'E KOETSÜSTIDES

Tsütokeemilisi meetodeid kasutades on valgusoptiliselt uuritud happelise ja leeliselise fosfataasi, ATPaasi (pH optimum 9) ja glükoos-6-fosfataasi paiknemist *Sarcocystis bovicanis*'e koetsüstides. Happelise fosfataasi lokalisatsiooni on uuritud ka elektronmikroskoopiliselt. Gomori reaktsiooni abil valgusoptiliselt teostatud happelise ja leeliselise fosfataasi uuringud andsid suhteliselt nõrga resultaadi. Sellest hoolimata võis mõlemaid ensüüme täheldada tsüsti seinas ja septides. Reaktsioonid ATPaasile ja glükoos-6-fosfataasile andsid parasiitide väga intensiivse värvumise. Viimase ensüümi olemasolu *Sarcocystis bovicanis*'e koetsüstides osutas glükoneogeneesi protsesside olemasolule parasiidi elutsükli vaadeldud etapil. Elektrontsütokeemiliste uuringute tulemused osutasid happelise fosfataasi olemasolule tsüstistaadiumide erinevates organellides, tsüsti seinas ja septides. Saadud andmete alusel on esitatud hüpotees happelise fosfataasi rollist parasiidi tungimisel peremeesrakku.

Атс МЕТСИС

ЦИТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ НЕКОТОРЫХ ФОСФАТАЗ В ТКАНЕВЫХ ЦИСТАХ *SARCOCYSTIS BOVICANIS* ИЗ СЕРДЕЦ КРУПНОГО РОГАТОГО СКОТА

Проведено исследование распределения кислой и щелочной фосфатаз, миозиновой АТФазы (pH оптимум 9) и глюкозо-6-фосфатазы (Г6Фазы) на светооптическом уровне на криостатных срезах тканевых цист. Показано также распределение кислой фосфатазы и на электронно-микроскопическом уровне. На светооптическом уровне реакции, проведенные по методу Гомори, дали в случае кислой и щелочной фосфатаз относительно слабые результаты, но несмотря на это, оба энзима прослеживались как в стенке цисты, так и в септах. Реакции на АТФазу и на Г6Фазу были выражены значительно сильнее. Наличие Г6Фазы указывает на присутствие процессов глюконеогенеза в цистных стадиях *Sarcocystis bovicanis*. В результате электронно-цитохимических исследований было выявлено наличие кислой фосфатазы в различных органеллах цистных стадий, в стенке цисты и в септах. Сделано предположение о возможной роли кислой фосфатазы в процессе проникновения зоитов паразита в клетки хозяина.