

УДК 576.36:593.193

*Ats METSIS*

## A LIGHT AND ELECTRON MICROSCOPIC STUDY OF DEGENERATING *SARCOCYSTIS BOVICANIS* TISSUE CYST FROM BOVINE HEART

*Ats METSIS.* DEGENEREERUVA *SARCOCYSTIS BOVICANIS*'E KOETSÜSTI VALGUS- JA ELEKTRONMIKROSKOOPILINE UURIMINE

*4тс МЕТЧИС.* СВЕТО- И ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ДЕГЕНЕРИРУЮЩЕЙ ЦИСТЫ *SARCOCYSTIS BOVICANIS* ИЗ СЕРДЦА КОРОВЫ

*Sarcocystis bovicanis* is a tissue cyst-forming protozoan parasite. Tissue cysts play the role of persisting stages in the life cycle of *Sarcocystis* preserving in the muscles until consumed by the carnivores, in whose intestine the cyst merozoites (-gamonts) give start to the sexual process (gamogony) without any preceding asexual cell division. In literature the question about the fate of tissue cysts in the intermediate hosts has been open for a long time. It has been indicated only that the cysts preserve for many years (Dubey, 1977). Besides that it has been mentioned that the older the infection in the intermediate host, the greater the amount of glial structures in the cysts (Федосеевко, Левит, 1979), and that in the case of species with the so-called macrocysts the presence of degenerative processes in the central part of the tissue cysts is very common (Грикенеене, 1983). The tissue cyst necrosis has been shown for *Sarcocystis* species from goats and cattle after oral vaccination with sporocysts (Dubey, 1983; Fayer, Dubey, 1984). The present paper deals with a degenerating *Sarcocystis* tissue cyst from bovine heart.

### Material and methods

Little pieces of bovine heart with tissue cysts were fixed with 3% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3) immediately in the slaughterhouse. Later on the material was postfixed in the laboratory in 1% OsO<sub>4</sub> in a 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-Araldit. The sectioning was carried out on a LKB-III ultramicrotome (LKB, Sweden). For light microscopy 1 μm thick sections were cut and mounted on glass slides. The sections were stained with 1% pyronin and then with 1% methyl blue at 80 °C for 1 min, and embedded in Canadian balsam.

For electron microscopy ultrathin sections were cut, stained with uranyl acetate and lead citrate and examined on a Jem-100 C-X (JOEL, Japan) electron microscope.

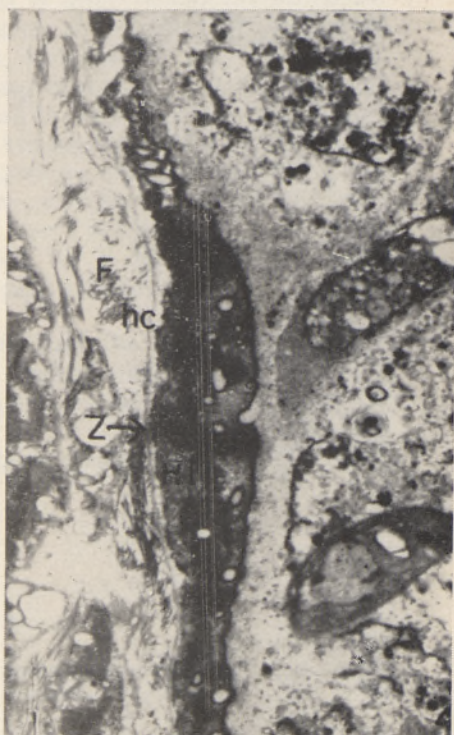
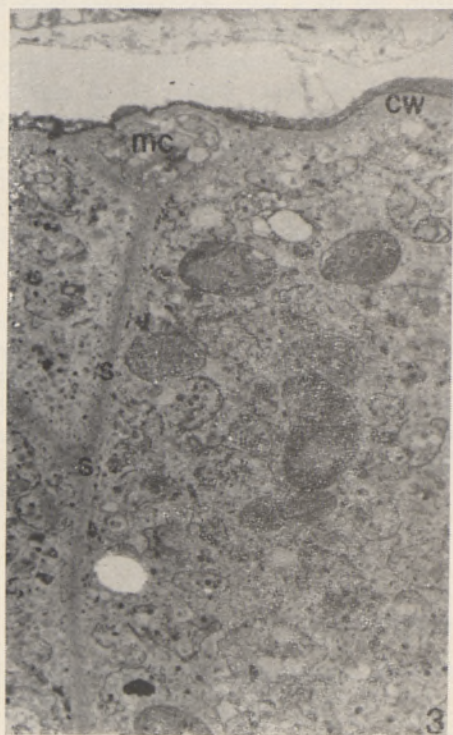
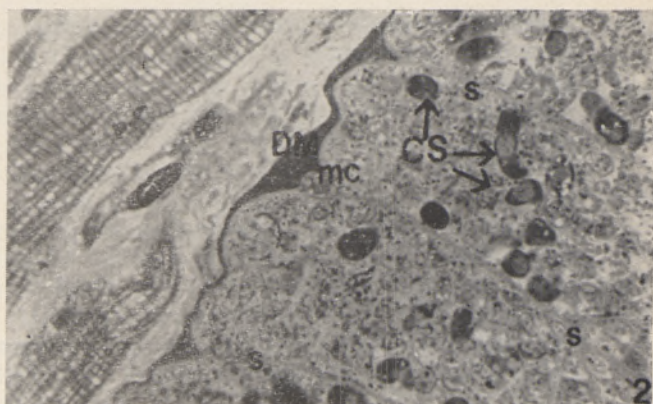
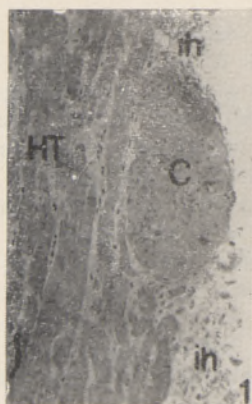


Fig. 1. A degenerating tissue cyst of *Sarcocystis bovicanis*. On the poles of the cyst (C) the infiltration of host cells (ih) into the muscle tissue (HT) is seen. Magnification  $10 \times 3.2 \times 2.5$ .

Fig. 2. A fragment of a tissue cyst divided by septa (s) to chambers where some cyst stages (CS) can be seen including the metrocytes (mc). The cyst is surrounded by a layer of dense material (DM). Magnification  $100 \times 3.2 \times 2.5$ .

Fig. 3. The cyst wall (cw) is covered by a layer of dense material. The material of the cyst wall and the septa (s) have become diffuse. A group of degenerating metrocytes (mc) can be seen. Magnification  $2000 \times$ .

Fig. 4. Around the cyst the remainders of degenerating myofibers (HT) can be seen with the proposed Z-discs (Z) and the covers of the host cell (hc). A lot of collagen fibers (F) are seen in the host tissue. Magnification  $3500 \times$ .

## Results and discussion

On semithin sections the infiltration of the host cells could be mentioned (Fig. 1). It must be said that in the case of *Sarcocystis bovicanis* the degeneration process embraces at once the entire cyst distinguishing the given species from the species with the macrocysts where the degradation processes are usual in the central part of a vital cyst (Грикене, 1983). During the process of degeneration it is characteristic that a layer of dense substance is formed around the cyst (Figs. 2, 3). Electron microscopy suggests that it is formed from degenerated myofibers as the material has maintained some kind of striated structures which resemble the Z-discs (Fig. 4). The cyst wall itself and the septa have become somewhat diffuse (Figs. 2, 3, 4). Although inside the cyst a little amount of intact cyst stages is still preserved (Fig. 2), the cyst is mainly filled with membranous and granular structures resulting from the destructed cells. This is also seen on the ultrastructural level (Fig. 3). It should be mentioned that besides the cyst also the host cell is undergoing degeneration. Around the cyst many degenerating structures of muscle cell, numerous membrane vesicles, degenerating myofibers, mitochondrions, etc. can be seen (Figs. 2, 3). This layer of degenerating material is surrounded by a supplement shell (Fig. 4) which is supposed to consist of the remainders of the covers of the parasitized host cell. Analogous structure has been described in the case of *Sarcocystis hardangeri* as external lamina of the host cell (Gjerde, 1985). Around the cyst the process of connective tissue forming is observed as a lot of collagen fibers are seen (Fig. 4). Analogous processes have also been mentioned for *S. hardangeri* (Gjerde, 1985). This indicates that after a parasite degradation a cicatrice is left in the host tissue.

The results of the present study indicate that the persisting potency of a tissue cyst in the intermediate host is limited and if the cyst is not consumed by a final host it is finally destroyed by the immune system of the intermediate host. This corresponds also to the statement of Dubey and Fayer (1983), concerning the sarcosporidia of goats, that the old cysts are eliminated by the host.

## REFERENCES

- Dubey, J. P. *Toxoplasma*, *Hammondia*, *Besnoitia*, *Sarcocystis* and other tissue cyst-forming *Coccidia* of man and animals. In: *Parasitic Protozoa*. III., New York; San Francisco; London, 1977, 101—237.
- Dubey, J. P. Immunity to sarcocystosis: modification of intestinal coccidiosis, and disappearance of sarcocysts in dairy goats. — *Veterin. Parasitol.*, 1983, **13**, 23—34.
- Dubey, J. P., Fayer, R. Sarcocystosis. — *Brit. Veterin. J.*, 1983, **139**, 371—377.
- Fayer, R., Dubey, J. P. Protective immunity against clinical sarcocystosis in cattle. — *Veterin. Parasitol.*, 1984, **15**, 187—201.
- Gjerde, B. Ultrastructure of the cysts of *Sarcocystis hardangeri* from skeletal muscle of reindeer (*Rangifer tarandus tarandus*). — *Can. J. Zool.*, 1985, **63**, 2676—2683.
- Грикене Я. С. Цитологическое исследование цистных стадий *Sarcocystis ovis* Heydorn et al., 1975. — Автореф. дис. канд. биол. н. Л., 1983.
- Федосенко В. М., Левит А. В. Электронно-микроскопическое изучение цист *Sarcocystis muris* в скелетной мускулатуре белых мышей. Токсоплазмиды. — *Протозоология*, 1979, № 4, 106—110.