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MULTIPLICATION OF MEROZOITES OF SARCOCYSTIS DISPERSA ČERNÁ, KOLÁŘOVÁ ET ŠULC, 1978 AND SARCOCYSTIS CERNAE LEVINE, 1977 IN THE BLOOD STREAM OF THE INTERMEDIATE HOST

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Abstract. Merozoites of sarcosporidia (*Sarcocystis dispersa* and *S. cernae*) were found to multiply in the blood stream, probably by endodyogeny, prior to the intramuscular multiplication in the intermediate host. The parasites were detected both in the cells of macrophages and outside them. *S. dispersa* infection could be transmitted from donor mice to recipient mice by means of intraperitoneal inoculation of blood of donors from day 7 to day 14 p.i. when the experiment was terminated. The significance of blood stages in spreading of infection in the intermediate host is discussed.

During the studies of the extramuscular development of *S. dispersa** in the mouse liver, Černá (1977) observed merozoites in impression smears from various organs (liver, lungs, spleen) occurring either inside or outside the cells of macrophages which were supposed to transfer them through the blood stream into the muscles of the intermediate host. Fayer and Leek (1979) managed to demonstrate the possibility of transmission of sarcosporidia (*S. bovicanis*, *S. ovicanis* and *S. suihominis*) by blood transfusion. Fayer (1979) noted that *S. bovicanis* (*S. cruzi*) merozoites are capable of multiplying during the transport in blood. The same phenomenon was observed by Černá (1981) in *S. dispersa* in the peripheral blood and impression smears from organs of experimentally infected mice. The present paper summarizes the results obtained while studying the circulating stages of *S. dispersa* and *S. cernae*. (The species *S. cernae* was recorded by Černá and Loučková (1976, 1977) in *Microtus arvalis* as intermediate host and *Falco tinnunculus* as definitive host. The specific name was given by Levine (1977)). The possibility of transmission of parasites circulating in the blood to another intermediate host by means of intraperitoneal inoculation is also studied.

MATERIAL AND METHODS

Sarcocystis dispersa. Seven white laboratory SPF mice (Mm_1 — Mm_7) were inoculated with 8×10^5 sporocysts each. The sporocysts were isolated from the intestine of *Tyto alba*. The blood

* *Sarcocystis dispersa* was first reported by Černá (1976) as a sarcosporidium, the life cycle of which involves *Tyto alba* and *Mus musculus*. One year later (Černá 1977), the asexual development of the parasite was studied in detail in the mouse intermediate host. The parasite was named *S. dispersa* in the paper by Černá et al. (1978) which included the description of the life cycle in the intermediate host (*Mus musculus*) and in the definitive hosts (*Tyto alba* and *Asio otus*). In 1977, Černá and Sénaud published in C. R. Acad. Sci. Paris a paper dealing with electron microscopical studies of a part of the asexual cycle of *S. dispersa*. This was probably the reason why Levine and Tadros (1980) in their paper on sarcosporidia erroneously mentioned Černá and Sénaud as authors of the species *S. dispersa*.

of the donor mice was collected daily in the following way: from mice Mm_1 — Mm_5 on days 6—10, from mouse Mm_6 on day 12 and from mouse Mm_7 on day 14 p.i. Each blood sample was injected intraperitoneally into two recipient mice. The blood smears and impression smears from liver, lungs and spleen of donors were fixed with methanol and stained by Giemsa for further studies. The recipients were inoculated with 0.2 ml of blood of donors diluted with saline in the ratio of 1 : 2.

The infection of recipients was controlled by homogenization of their muscle tissue in saline on days 92—142 p.i. (Černá and Loučková 1977). Seven mice of the same group were not injected and served as controls.

Sarcocystis cernae. Only the morphology of circulating stages was studied in this species. Two specimens of *M. arvalis* (laboratory generation) were inoculated with the sporocysts (8×10^7 per mouse) isolated from the intestine of *F. tinnunculus*. The mice were killed on days 5 and 6 p.i. and the blood smears and impression smears from liver and lungs were studied for the presence of sarcosporidia.

RESULTS

1. MULTIPLICATION OF MEROZOITES OF SARCOSPORIDIA IN THE BLOOD STREAM

A. Sarcocystis dispersa. The merozoites were detected microscopically in blood smears of peripheral blood (20—25 specimens in 15×10 mm of blood smear) and impression smears from organs on days 7—8 p.i. There were two types of merozoites: a) slender, with oval nucleus (Plate I, Fig. 1), measuring $7—9 \times 2$ μm, and b) thick ones ($8—9 \times 4—5$ μm), with the nucleus distinctly dividing or already divided into two nuclei (Plate I, Figs. 2—5; Plate II, Fig. 6). The progeny was already forming in two of them (Plate I, Figs. 4, 5). The dividing merozoites were found either free (Plate I, Figs. 2—5) or in cells of macrophages (Plate II, Fig. 6).

B. Sarcocystis cernae. Like in *S. dispersa*, the dividing merozoites were observed. In this species, the infection was studied on days 5 and 6 p.i., at the time when the multiplication of parasites by multiple endopolygony culminated. Dividing specimens (Plate II, Fig. 7) were also found in the heart blood and impression smears from liver, lungs and spleen. These stages occurred both free and in macrophage cells, like in *S. dispersa*.

2. INTRAPERITONEAL TRANSMISSION OF SARCOSPORIDIA VIA BLOOD INJECTION

The recipient mice became infected with the blood of donors from the 7th day of infection. As it is mentioned above, the sarcosporidia were detected microscopically in the peripheral blood of donors. From day 9 p.i., however, no merozoites could be demonstrated in the blood smears of donors, but their presence was confirmed by the fact that the recipients could be infected with this blood. The recipients could be infected with the blood of donors even 14 days after injection, when the experiment was terminated.

Sarcosporidial infection of recipients was demonstrated by the finding of cysts in muscles on days 92—142 after intraperitoneal injection. No sarcosporidia were found in the muscles of 7 control mice killed at the same time.

DISCUSSION AND CONCLUSIONS

It was demonstrated that the intensive multiplication of *S. dispersa* and *S. cernae* by multiple endopolygony in parenchymal cells of liver was followed by further multiplication of the parasites during their circulation in blood. They divided into two daughter cells, which apparently resembled the multiplication by endodyogeny described first by Goldman et al. (1958) in *Toxoplasma gondii* and detected later in the cyst of all heteroxenous coccidia (Sénaud 1967, 1979, Kepka and Scholtyseck 1970). The dividing parasites were found both in macrophage cells and free in the blood smears and impression smears from organs. Similarly Fayer (1979) observed dividing forms of *S. bovicanis* occurring in blood smears both in the host cells and outside them. It is possible that the parasites occurring outside the host cells were originally in the macrophage cells and were released during the preparation. It is surprising, however, that these "free" dividing merozoites were often more numerous than those which remained in the cytoplasm of macrophage cells. This question requires further studies, as it is not known that the coccidia would be capable of multiplying in the host without the host cells. In any case, our results indicate that after an intensive multiplication by multiple endopolygeny (Černá and Sénaud 1977), which was confirmed by many authors in various species of sarcosporidia (Pacheco and Fayer 1977, Heydorn and Mehlhorn 1978, Aryeetey et al. 1980, Dubey et al. 1980, Simpson and Mayhew 1980), the sarcosporidia may multiply in another manner, most probably by endodyogeny. This way of multiplication occurs at the time when the parasites are transported from the organs into muscles. The multiplying merozoites may circulate in the blood still at the time when the others already produce cysts in the muscles of the intermediate host. This was confirmed also by our experiments with *S. dispersa*. The zoites of this species were found in the muscles of experimentally infected mice from day 10 p.i. (Černá 1977), but after intraperitoneal transmission they were detected in the blood still on day 14 p.i., when the experiment was terminated. These merozoites probably occur in the blood of mice still longer.

It was demonstrated by intraperitoneal injection of blood of mice infected with *S. dispersa* that the merozoites occurring in the peripheral blood of the intermediate host are capable of infecting a further intermediate host. This was confirmed also by the results of Fayer and Leek (1979), who induced by blood transfusion acute sarcosporidiosis in cattle (with *S. bovicanis*), sheep (with *S. ovicanis*) and pig (with *S. suihominis*). The transfer of these stages between intermediate hosts in the laboratory was demonstrated also by Tadros and Laarman (1979). They injected intraperitoneally the homogenized liver of *Apodemus sylvaticus* with schizonts of *S. sebeki* into other specimens of *A. sylvaticus* and 4 months later demonstrated the presence of sarcosporidia in the muscles of the host.

The existence of multiplying sarcosporidia in the blood of intermediate host may be of a great importance also from the veiwpoint of epizootology. It is very probable that a transplacental transmission of sarcosporidia may occur in females if they became infected during pregnancy. Of course, the possibility of this transmission must be verified experimentally.

РАЗМНОЖЕНИЕ МЕРОЗОИТОВ САРКОСПОРИДИЙ *SARCOCYSTIS DISPERSA* ČERNÁ, KOLÁŘOVÁ ET ŠULC, 1978 И *SARCOCYSTIS CERNAE* LEVINE, 1977 В КРОВИ ПРОМЕЖУТОЧНОГО ХОЗЯИНА

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Резюме. Было обнаружено, что саркоспоридии *Sarcocystis dispersa* и *S. cernae* размножаются в крови промежуточного хозяина разделением, вероятно эндодиогонией, прежде чем они размножаются в мышцах. Паразиты встречались или свободно или внутри клеток макро-

фагов. Заражение саркоспоридией *S. dispersa* можно было перенести от мыши-донора на мышь-реципиента при помощи интраперитонеального введения крови донора от 7-го до 14-го дня после заражения, когда эксперимент окончился. Обсуждается значение размножения саркоспоридий в крови для распространения инфекции в промежуточном хозяине.

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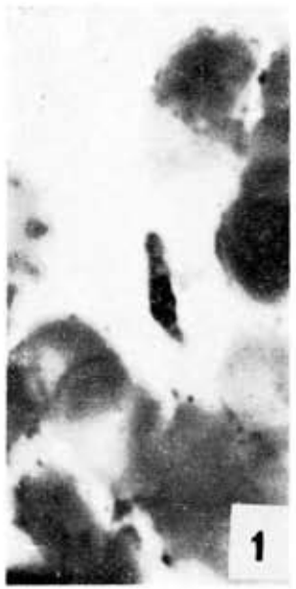
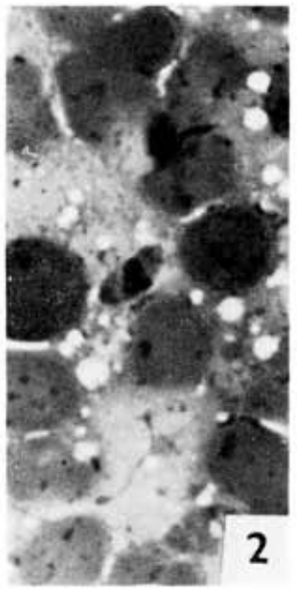
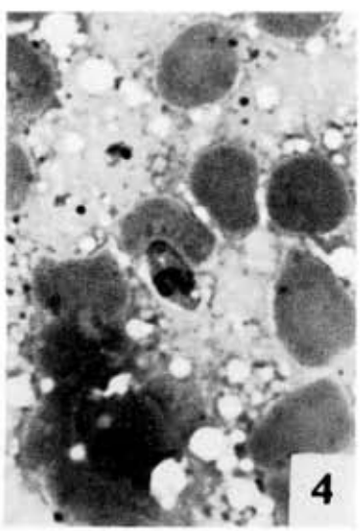
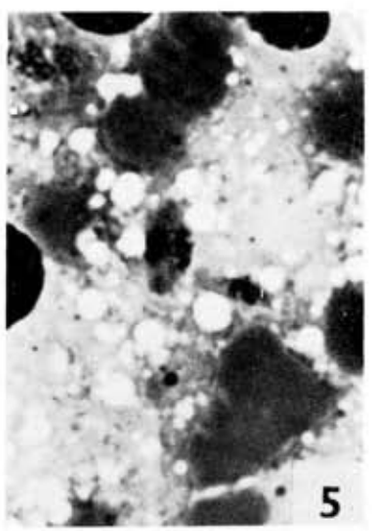
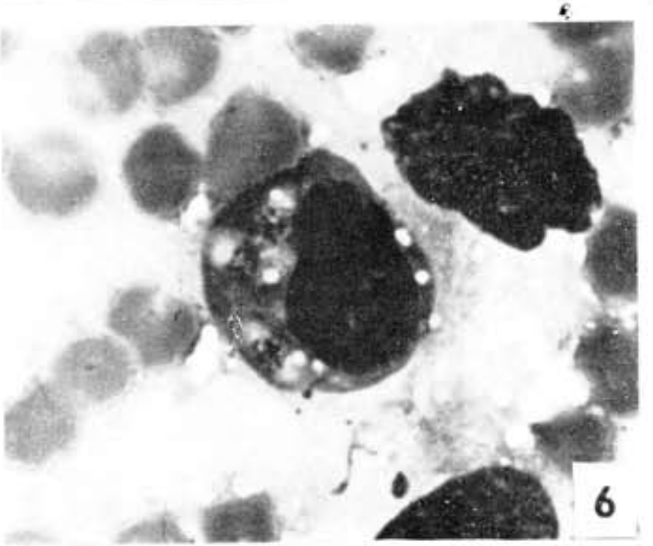








Figs. 1—5. Merozoites of *S. dispersa*. Giemsa (× 750). **Fig. 1.** Slender merozoite with oval nucleus; blood smear on day 8 p.i. **Fig. 2.** Thick merozoite with irregular nucleus; impression smear from liver on day 8 p.i. **Fig. 3.** Thick merozoite with a nucleus possessing two distinct processes; impression smear from lung onday 8 p.i. **Fig. 4.** Merozoite with separating nuclei; impression smear from liver on day 8 p.i. **Fig. 5.** Two daughter merozoites lying still close to one another and one free merozoite; impression smear from liver on day 8 p.i.



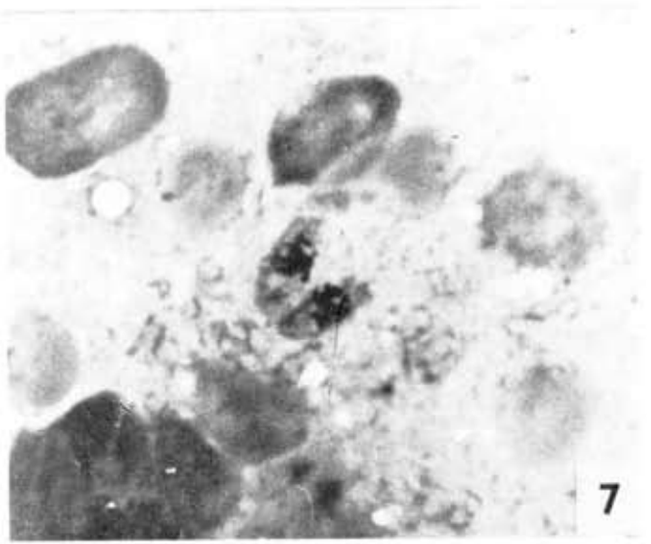


Fig. 6. Dividing merozoites of *S. dispersa* in macrophage cell; impression smear from lung on day 8 p.i. Giemsa (× 750). **Fig. 7.** Dividing merozoite of *S. cernae;* impression smear from liver on day 6 p.i. Giemsa (× 750).