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 sarcocystidia (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 sarcocystidia (S. bovisi, S. ovicani and S. suihominis) by blood transfusion. Fayer (1979) noted that S. bovisi (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 Loušková (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. — Mm-) were inoculated with 8 x 10⁶ sporocysts each. The sporocysts were isolated from the intestine of Tyto alba. The blood

*Sarcocystis dispersa was first reported by Černá (1976) as a sarcocystidium, 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 Arce 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 sarcocystidium 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 on days 6–10, from mouse Mm on day 12 and from mouse Mm on day 14 p.i. Each blood sample was separated microscopically 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 1:2.

The infection of recipients was controlled by homogenization of their muscle tissue in saline on days 92–142 p.i. (Černá and Longkóva 1977). Seven mice of the same group were not infected 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 spocyst (8 x 10^12 per mouse) isolated from the intestine of F. tinnunculus. The mice were killed on days 5 and 8 p.i. and the blood smears and impression smears from liver and lungs were studied for the presence of sarcocystis.

RESULTS

1. MULTIPICATION 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 x 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 x 2 μm, and b) thick ones (8–9 x 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 endopolygon process 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 macrophages, 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 sarcocystis 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.

Sarcocystis infection of recipients was demonstrated by the finding of cysts in muscles on days 92–142 after intraperitoneal injection. No sarcocystis 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, Kerka and Scholte- biek 1979). 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. bovis in the blood smears of bovines occurring in both 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 cytoplasma 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 endopolygony (Černá and Sénnaud 1977), which was confirmed by many authors in various species of sarcocystis (Pacheco and Fayer 1977, Heydorn and Møhlhorn 1978, Arreyetey et al. 1980, Dubey et al. 1980, Sjöstrand and Nilsson 1980), the sarcocystis 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 observed in the jeans of S. dispersa occurring in blood both in 50 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 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 sarcocystiosis in cattle (with S. bovisnianus), sheep (with S. ovicanus) and pig (with S. atheromus). The transfer of these stages between intermediate hosts in the laboratory was demonstrated also by Tadros and Laarmann (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 sarcocystis in the muscles of the host.

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


Received 5 February 1982.
Translated by: M. Duková
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 on day 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. cernua*; impression smear from liver on day 6 p.i. Giemsa (× 750).