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CONTRIBUTION TO THE PROBLEM OF CYST-PRODUCING COCCIDIANS

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Abstract. The problem of life cycles of cyst-producing coccidians, mainly those of the genus *Sarcocystis*, from carnivorous birds has been discussed. Oocysts of the "sarcocystic" type have been recovered from 9 species of birds of prey and owls from Czechoslovakia. The course of experimental infection in a group of birds consisting of the species *Tyto alba*, *Asio otus*, *Strix aluco*, *Buteo buteo* has been described. We inoculated these birds with cystic stages of the genus *Sarcocystis* obtained from white laboratory mice (*Mus musculus*) infected previously with oocysts from *Tyto alba*. The nomenclature of heteroxenous coccidians of the genus *Sarcocystis* has been discussed and suggestions have been made for the use of simple, one-word specific names. A description is given of a new species of the genus *Sarcocystis* — *Sarcocystis dispersa* sp. n. Its asexual development and cyst production in *Mus musculus* (intermediate host), and cyst production in *Tyto alba* and *Asio otus* (definitive hosts) have been studied.

Repeated findings of oocysts of the "sarcocystic" type in owls and birds of prey prompted our intention of obtaining knowledge of the intermediate hosts of these parasites. It has been indicated by the results of recent studies on materials from beasts of prey and man (Rommel and Heydorn 1972, 1974, Rommel et al. 1972, Heydorn and Rommel 1972 a, b, Wallace 1973, Tadros and Laarman 1975, Powell and Mac Carey 1975, Ruiz and Frenkel 1976) that they all belonged to the life cycle of the genus *Sarcocystis*. We disclosed for two coccidians of the genus *Sarcocystis* that partly carnivorous birds partly small rodents were involved in their life cycles: bi-sporocystic oocysts isolated from the intestine of *Falco tinnunculus* produced muscle cysts in the common vole (*Microtus arvalis*) after having multiplied in the liver of this animal species (Černá and Loučková 1976, 1977). Although this parasite did not develop in the white laboratory mouse (*Mus musculus*), the mouse was found to act as an intermediate host for another sarcosporidian species from birds; its oocysts described originally as *Isospora buteonis* Henry, 1932 were isolated from *Tyto alba* (Černá 1976, 1977). We failed to produce infection with these sporocysts in the common vole (*Microtus arvalis*).

The present study surveys all knowledge available on the incidence of oocysts of the "sarcocystic" type in birds of prey and owls examined in the years 1975, 1976, and reconsiders the problem of life cycles of the genus *Sarcocystis* from *Tyto alba* and *Mus musculus* and questions of the nomenclature of heteroxenous coccidians.

MATERIALS AND METHODS

By courtesy of the members of the preparatory shop in Prague Bráník, the intestinal contents of birds of prey and owls have been made available for our present study. The intestinal contents of birds were examined with Faust's flotation method. In positive cases, oocysts - sporocysts were removed from the material by decantation with water, and stored at 4 °C for infection experiments.

Methods used for the inoculation of rodents with oocysts and sporocysts isolated from birds, and

further procedures with these experimental animals are essentially similar to those described in earlier papers (Černá 1977, Černá and Loučková 1977).

Birds from the quarantine department of the Prague Zoo selected for experimental infection were kept separately in cages. Prior to the experiment, we examined coprologically a 4-day faecal sample of each bird. The results were negative, none of the birds was infected with sarcosporidian oocysts. The group of birds examined consisted of the species *Tyto alba*, *Asio otus*, *Strix aluco*, *Buteo buteo*. The birds were infected with muscle tissue of white latoratory mice which had been infected previously with a dose of 200 000 sporocysts from *Tyto alba* to mouse. Infection of the birds followed 4 months after the inoculation of the mice with sporocysts: at that time, a massive infection with muscle cysts was visible in the mice. Each bird was given one infected mouse. On day 16 p. i., two owls (*Tyto alba* and *Asio otus*) were examined in necropsy in order to determine the presence of developmental stages of sarcosporidians in their guts. The gut tissue was treated histologically and stained with Harris'hematoxylin. In addition, the intestinal contents of both birds were washed out in order to obtain oocysts-sporocysts for control infection. Two of the SPF mice (Mm_1 and Mm_2) received a dose of approximately 600 000 sporocysts isolated from the experimental *Tyto alba;* two other SPF mice (Mm_3 and Mm_4) received each a dose of approximately 7 000 sporocysts from the experimental *Asio otus*. Mm_1 and Mm_2 were killed on day 5 and day 6 p. i. respectively, Mm_3 on day 5 p. i. Mm_4 on day 20 p. i. For control purposes, we infected 4 common voles (*Microtus arvalis* Ma_1 to Ma_4) with a dose of approximately 1.5 million sporocysts from *Tyto alba*. Similar to the mice, these animals were killed on day 5 and 6 p. i. and examined for a possible liver infection.

RESULTS

Entire examination of owls and birds of prey

Intestinal contents of owls and birds of prey from Czechoslovakia were examined in the years 1975, 1976. Findings of oocysts-sporocysts of the "sarcosporidian" type in these carnivorous birds are surveyed in Table 1. The table provides evidence that cyst-producing coccidians are frequent parasites of carnivorous birds. They were present in the intestinal tract of all owls and birds of prey examined.

Henry (1932) and Scholtyseck (1954) described from birds findings of isospores different from *Isospora lacazei* (Labbé, 1893) which they called *Isospora buteonis*. This parasite was recorded from *Buteo buteo*, *Tyto alba*, *Strix aluco* and *Asio flammeus*. The lack of records on findings of oocysts of the "sarcocystic" type from other carnivorous

Table 1. Oocysts of the "sarcocystic" type from the intestinal contents of owls and birds of prey

Species	Number of examined	Number of positive	% positive	Oocyst described as
Asio otus	46	9	19.5	not described
Athene noctua	5	1	—	not described
Strix aluco	19	1	5.2	*Isospora buteonis* Henry, 1932
Tyto alba	71	24	33.8	*Isospora buteonis* Henry, 1932
Accipiter gentilis	34	14	44.1	not described
Accipiter nisus	14	3	—	not described
Buteo buteo	57	20	35.1	*I. buteonis* Henry, 1932
Circus cyaneus	2	1	—	not described
Falco tinnunculus	18	7	38.8	not described
Total	266	80	30.0	

Note: The percentage has been given only if the number of birds of the individual species was higher than 15

birds may partly be ascribed to difficulties in obtaining material from these birds, partly to the fact that solitary sporocysts may escape attention in an examination of these birds. So far, we succeeded in three cases only to determine the intermediate hosts of cystic stages of coccidians released from carnivorous birds (Table 2).

Table 2. Asexual development of cyst-producing coccidians of owls and birds of prey in small rodents

Sporocyst isolated from	cystic stages found in	Organ attacked by cysts	Organ of precystic reproduction	Parasite placed in genus	Authors
Buteo buteo	*Clethrionomys glareolus*	brain	liver	*Frenkelia*	Rommel and Krampitz 1975
Falco tinnunculus	*Microtus arvalis*	muscles	liver	*Sarcocystis*	Černá and Loučková 1976
Tyto alba	*Mus musculus*	muscles	liver	*Sarcocystis*	Černá 1976

Experimental infection of birds with muscle cysts from mice

In earlier experiments we succeeded repeatedly in producing infection with oocysts-sporocysts from the intestine of *Tyto alba* in white laboratory mice (*Mus musculus*). Inoculation with a large dose of sporocysts enabled a detailed study on the very interesting asexual reproduction of the parasite in the intermediate host up to the production of muscle cysts (Černá 1977b). In the present study, we infected a group of birds with muscle cysts from mice (Plate I, Figs. 6, 7). In addition to *Tyto alba* (sporocysts from this species were used for the infection of the mice) the group contained two other owl species — *Asio otus* and *Strix aluco* — and another bird of prey — *Buteo buteo*. The course of infection in the birds, traced coprologically, is shown in Table 3.

Conform to our expectations, infection was readily acquired by *Tyto alba*. The release of oocysts started on day 8 of infection and continued until day 16 p.i. when the bird was killed for examination in necropsy (Table 3). Sporulated oocysts were present in the subepithelium of the entire small intestine, mainly in its central and posterior part, with an occasional oocyst in the caecum (Plate II, Figs. 11, 12).

The white laboratory mice Mm_1 and Mm_2 were inoculated repeatedly with sporocysts from this *Tyto alba*. The asexual development of the parasite in the liver of these animals was essentially similar to that described earlier for experimental infection of mice (Plate I, Figs. 1—5). We did not succeed in producing infection with this material in the liver of 4 common voles (*M. arvalis*).

Throughout the course of our experiment, infection was not acquired by either *Strix aluco* or *Buteo buteo*, but we found an occasional oocyst in the droppings of *Asio otus* from day 10 p. i. until day 16 p. i. when the bird was killed and examined in necropsy for the presence of oocysts in its intestinal tissue. An occasional sporulated oocyst was found in cells of the intestinal epithelium in the posterior part of the small intestine. Sporocysts inside the oocysts released from *Asio otus* were conform in both their morphology and size to sporocysts from *Tyto alba*. Size of sporocysts from *Tyto alba* (40 sporocysts measured):

12.9—13.7 × 10.8—12.9 μm

Size of sporocysts from *Asio otus* (20 sporocysts measured):

12.9—13.7 × 10.8 μm

Table 3. Coprological examination of birds fed with mice with infected muscles

Days p. i.	*Tyto alba*	*Asio otus*	*Strix aluco*	*Buteo buteo*
1	neg	neg	neg	neg
2	neg	neg	neg	neg
3	neg	neg	neg	neg
4	neg	neg	neg	neg
5	neg	neg	neg	neg
6	neg	neg	neg	neg
7	neg	neg	neg	neg
8	+	neg	neg	neg
9	(+)	neg	neg	neg
10	+	(+)	neg	neg
11	+	neg	neg	neg
12	+	(+)	neg	neg
13	+	neg	neg	neg
14	++	(+)	neg	neg
15	++	(+)	neg	neg
16	++	(+)	neg	neg

Note: (+) — an occasional oocyst in the viewing field at a magnification of 200
+ — 1—5 oocysts in each viewing field (× 200)
++ — more than 5 oocysts in the viewing field (× 200)

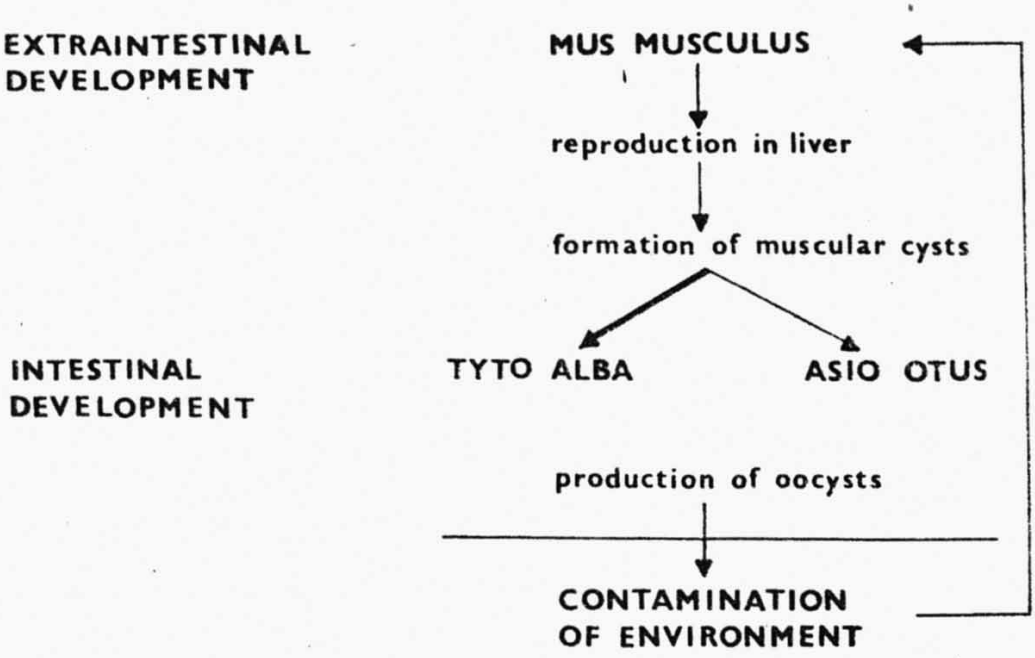

Fig. 1. Schematic illustration of the life cycle of *Sarcocystis*.

Also sporocysts from the contents of the intestine of *Asio otus* were used for the inoculation of two laboratory mice (Mm_3 and Mm_4). Mm_3 was killed on day 5 p.i. A schizont (25.5 × 17 μm) was found in an impression smear from its liver; Mm_4 was killed on day 20 p.i. An occasional cystic zoite was present in the muscle mixture. Fig. 1 gives a schematic illustration of the life cycle of *Sarcocystis* from mice and owls.

It emerges from our results that *Sarcocystis* from *Tyto alba* — *Mus musculus* may produce a light intestinal infection in *Asio otus*.

Sporocysts from a natural infection of *Tyto alba* and sporocysts from an experimental infection

Sporocysts from both inside and outside the oocysts obtained from our original material (intestinal contents of owls from the preparatory shop) contained relatively large residual granules of a different size (up to 4 μm in diameter, see Plate II, Figs. 9, 10). As the material had been stored in the freezer at temperatures of up to —20 °C, we were doubtful at first whether the sporocysts isolated from it would still be able to produce infection. However, their infectiousness was confirmed in earlier studies (Černá 1976, 1977). These sporocysts inoculated repeatedly into mice produced infection in these intermediate hosts.

A reverse experimental infection of *Tyto alba* from the mouse provided evidence that sporocysts inside the oocysts released from *Tyto alba* with a fresh infection possessed fine residual granules of identical size (approximately 1 μm in diameter) (Plate II, Fig. 8). Also these sporocysts were able to produce infection in the mouse as confirmed by a reverse infection of these animals (see above).

DISCUSSION AND CONCLUSIONS

Of the coccidians producing cysts in their intermediate hosts attention has so far been given mainly to those infecting beasts of prey and of these particularly dog and cat. The cat has been determined as the definitive host of both *Toxoplasma gondii* (Hutchinson et al. 1968, Sheffield and Melton 1969, Frenkel et al. 1969), and *Hammondia hammondi* (Frenkel and Dubey 1975). Wallace and Frenkel (1975) regarded the cat as the definitive host of members of the genus *Besnoitia* from mice and rats, Ruiz and Frenkel (1976) suggested that this animal was the host of intestinal stages of sarcosporidians isolated from the muscles of mice. Earlier, Heydorn and Rommel (1972 a, b), Rommel et al. (1972) regarded both cat and dog as the host of *Sarcocystis* from sheep and cattle. Tadros and Laarman (1975) identified the weasel as the definitive host of cystic stages of sarcosporidians from the common vole (*Microtus arvalis*). In addition to carnivores, man was regarded as the definitive host of sarcosporidians from cattle (Rommel and Heydorn 1972). Rzepzyk (1974) examined in rats cystic stages of the genus *Sarcocystis* from snakes.

Apart from a study by Rommel and Krampitz (1975) on the development of the genus *Frenkelia* from *Buteo buteo* in *Clethrionomys glareolus*, carnivorous birds have been left aside any attention. The results of the mentioned study indicated a possible participation of birds of prey in the life cycles of cyst-producing coccidians.

Repeated findings of oocysts of the "sarcocystic" type in various species of owls and birds of prey (Table 1) instigated our study on the role played by small rodents in the nutrition of carnivorous birds, and this resulted in the disclosure of two developmental cycles of the genus *Sarcocystis* as mentioned in the introduction to this paper.

Reverse experimental infections of birds with muscles from mice inoculated with sporocysts from *Tyto alba* provided evidence for the fact that yet another owl species — *Asio otus*—was utilized by this parasite as its definitive host under conditions of the laboratory. Although infection in *Asio otus* was much weaker than that in *Tyto alba*, sporocysts isolated from the former species produced infection in the intermediate host (mouse). There is evidently the possibility that apart from the "typical" definitive host, other "atypical" hosts may acquire infection if they have similar feeding habits and ingest muscles harbouring sarcosporidian cysts. However, the course of infection in the latter may be less severe than that in the "typical" host.

So far, all cyst-producing coccidians described from the definitive host have been

identified as members of the genus *Isospora*, and their cystic stages in the intermediate hosts have been placed in the genera *Toxoplasma*, *Besnoitia*, *Frenkelia*, *Sarcocystis*. A character common to all oocysts of these genera and enabling their differentiation from the "typical" genus *Isospora* is the absence of a Stieda body in the sporocyst. The identification of members of the genus *Sarcocystis* (and also *Frenkelia*) is facilitated in that sporulated oocysts are released from the host (sporulation itself occurs in the intestinal tissue of the host) (Plate II, Figs. 11, 12). In an attempt to solve the taxonomy of the genus *Sarcocystis*, Heydorn et al. (1975) proposed the use of a combined name for the individual species, ie, that of the intermediate and the definitive host participating in the life cycle. For sarcocysts for which at least some knowledge of their life cycle was available, they proposed these names: *Sarcocystis ovi-felis*, *S. bovi-felis*, *S. ovi-canis*, *S. bovi-canis*, *S. bovi-hominis*. Their concepts emerged from the hypothesis of a two-host life cycle, and was supported by the finding of morphologically different cystic stages in the muscles of the intermediate hosts completing their life cycles in different definitive hosts. We found an analogous situation in e.g., a comparison of sarcosporidians from *Microtus arvalis* in our material (Černá and Loučková 1976) with those found by Tadros and Laarman (1975) in the same intermediate host. In the first case, the life cycle was completed in the intestine of *Falco tinnunculus*, in the second case in *Mustela nivalis*. Our experiments with a transmission to birds indicated that several definitive hosts may acquire infection after ingesting muscle cysts. This has also been suggested by Heydorn et al. (1976, 1976) who found *Sarcocystis bovi-hominis* in the monkey species *Macaca rhesus* and *Papio cynocephalus*. Also man may apparently be one of the definitive hosts of sarcocysts from cattle, and if we compared data on the morphology of muscle cysts of *Sarcocystis bovi-felis*, these did not appear to be very different from those on the morphology of muscle cysts of *Sarcocystis bovi-hominis* (Mehlhorn et al. 1976). For our sarcosporidians utilizing carnivorous birds and small rodents in their life cycles, the suggestion in the nomenclatorial proposition that priority should be given to the name of the epidemiologically more important host does not appear to be acceptable.

In our opinion, species of the genus *Sarcocystis* should be given simple, one-word names similar to those used for other heteroxenous sporozoans.

We suggest the name *Sarcocystis dispersa* for the parasite whose asexual life cycle in the mouse has been described in an earlier paper (Černá 1977), and whose definitive host is mainly *Tyto alba*, but also *Asio otus*.

Description of *Sarcocystis dispersa* sp.n.:

(syn. *Sarcocystis muris* Blanchard, 1885 pro parte
Isospora buteonis Henry, 1932 pro parte)

Intermediate host: *Mus musculus*

First asexual reproduction in the liver only. Formation of cysts in skeletal muscles, diaphragm, heart, tongue. Zoites in cysts subtle, 8—9 × 4 μm, metrocytes scarce, measurements 4 × 3 μm.

Definitive hosts: *Tyto alba*, *Asio otus*

Sporulated oocysts in subepithelium of small intestine. Sporocysts without Stieda body. Measurements of oocysts: 17—22 by 10—14 μm. Measurements of sporocysts: 11—14 × 8—12 μm. Prepatent period: 8—10 days.

Terra typica: Bohemia and Moravia (no details could be obtained on the places of origin of the owls).

Type material: Deposited in the collection of the Department of Parasitology, Faculty of Natural Sciences, Charles University, Prague.

МАТЕРИАЛЫ К ИЗУЧЕНИЮ ОБРАЗУЮЩИХ ЦИСТЫ КОКЦИДИЙ

Ж. Черна, И. Коларжова и И. Шулц

Резюме. В работе обсуждается вопрос циклов развития образующих цисты кокцидий, особенно принимая во внимание род *Sarcocystis* у плотоядных птиц. Приведены находки ооцист типа „саркоцисты" у 9 видов сов и хищных птиц (всего 266 птиц) из территории ЧССР. Дано описание экспериментального заражения стадиями рода *Sarcocystis* от белых лабораторных мышей *Mus musculus* зараженых ооцистами от совы *Tyto alba* у следующих видов птиц: *Tyto alba*, *Asio otus*, *Strix aluco*, *Buteo buteo*. Обсуждается вопрос номенклатуры гетероксенных кокцидий рода *Sarcocystis* и рекомендуется также у этих протозойных применять простые видовые названия, выраженные одним словом. Вид рода *Sarcocystis*, бесполое развитие и образование цист которого обнаружены у *Mus musculus* (промежуточный хозяин) и образование ооцист у *Tyto alba* и *Asio otus* (окончательные хозяева), описан как *Sarcocystis dispersa* и дана его характеристика.

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FIRST RECORD OF "ISOSPORA HOMINIS" IN CZECHOSLOVAKIA

First human coccidiosis in Czechoslovakia caused by the species *Isospora belli* was diagnosed in 1973 (Giboda M., Čatár G., Bratisl. lek. Listy 2: 229—233, 1973). We give a record on finding of *Isospora hominis* in 12-year old girl from eastern Slovakia.

One coccidian oocyst and one sporocyst were found during the routine stool examination. The oocyst was fully sporulated in 2 sporocysts, each of which containing 4 sporozoites and a coarse-grained residual body (Fig. 1). The membrane of the oocyst could not be clearly seen in the whole periphery (Fig. 2). In the course of next 50 days excretion of the sporocysts without negative phase was proved in 9 examinations. Intensity of the infection was very weak and often only one sporocyst was found in the slides. Sporocysts were much more frequent than oocysts in slides and they seemed to be resistent osmotically because of their shape persistence in hypertonic solution. Sporocysts measured 12.35×8.91 μm in diameter (taken 50 measurements). According to the epidemiological anamnesis the patient stated tasting a crude sausage mixture a year before. Finding of isospores in other three members of the family where also mother tasted the sausage mixture proved to be negative. Of domestic animals investigated, the dog was also negative. The cat excreted sporocysts measuring 12.9×8.91 μm with the sporozoites and a residual body similar in appearance to those excreted by the girl.

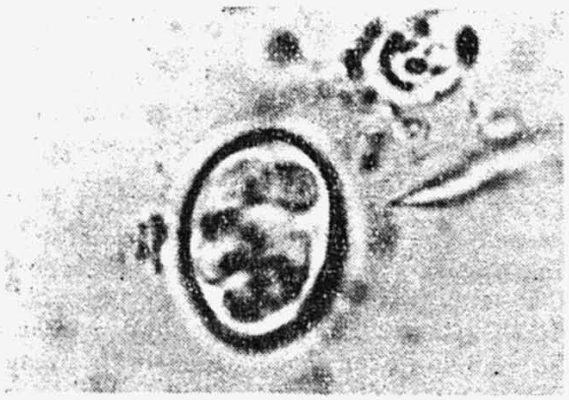

Fig. 1. Sporocyst of *I. hominis* with sporozoites.

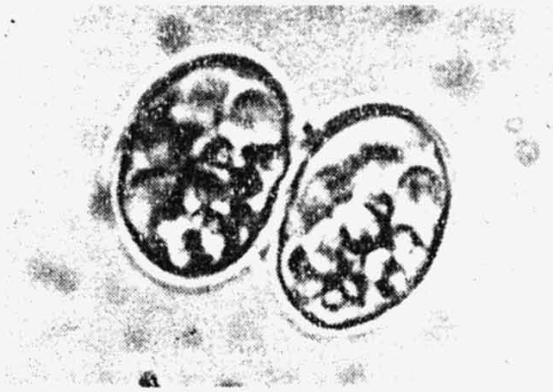

Fig. 2. Oocyst with 2 sporocysts joined by a common membrane.

According to Rommel and Heydorn (Berl. Münch. tierärztl. Wschr. 85: 141—145, 1972) who recorded that "*Isospora hominis*" should be included in the life-cycle of sarcosporidia of the genus *Sarcocystis*, we suggest that our patient was infected by the sarcosporidia from the sausage mixture. Since the patient was hospitalized for tuberculosis of intrathoratic lymphatic nodes, finding of isospores cannot be put in the connection with the clinical condition of the patient. We failed to prove schizogony in the enterocytes by a bioptic examination of the jejunal mucous membrane. Biochemical tests and blood examination were within the bounds of normal. But increased values of immunoglobulines: IgG 18.80 mg %, IgA 2.40 mg %, IgM 1.48 mg % were recorded. This fact may give evidence for the antibodies formation against the sarcocystic antigen as observed by Tadros et al. (Z. Parasitenk. 3: 221—224, 1974).

Treatment with pirimethamin (Daraprim) for 5 days in a dose of 37 mg a day, next 10 days in a dose of 25 mg a day and with Sulfisoxazol for 14 days in a dose of 3 g a day was not successful. By reason of her primary disease, the patient was treated at the same time with STM, Nitrazid, Oxacilin and with vitamins.

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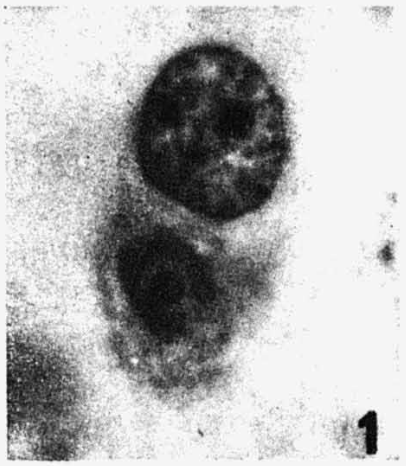

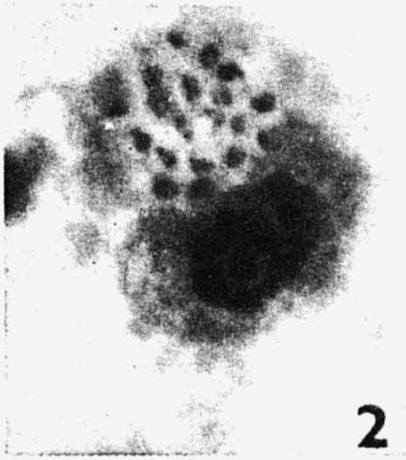

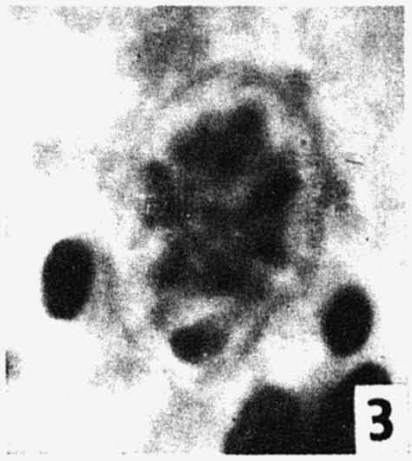

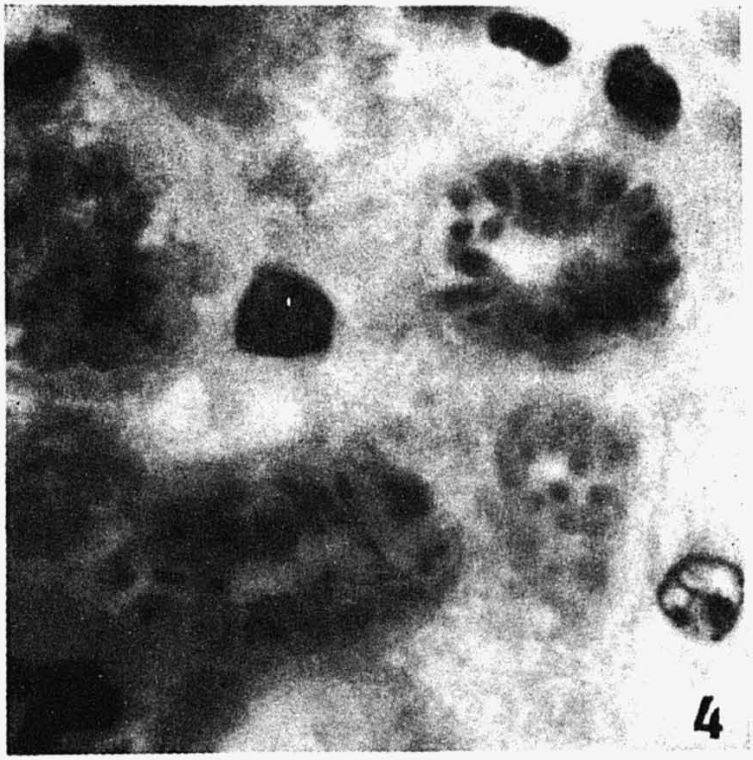

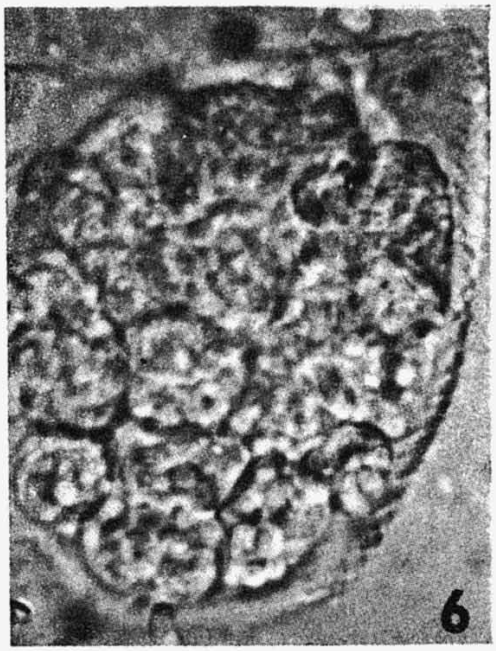

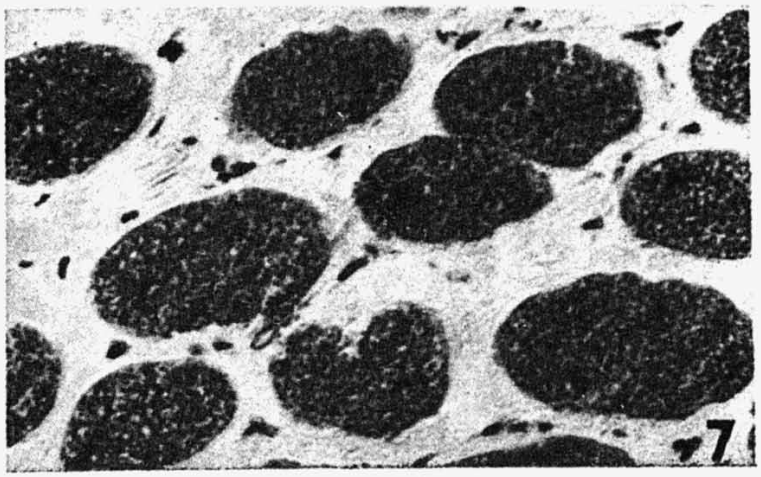

Figs. 1—4. Production of merozoites in the liver of mice after inoculation with oocysts from *Tyto alba* (4—6 days p. i.) Harris'haematoxylin (× 1 000). 1: uninucleate stage with large nucleolus. 2: dividing stage with two nucleoli in the nucleus, 3: multinucleate schizont (separation of nuclei not complete), 4: schizonts starting to produce merozoites.

Fig. 5. Two merozoites in macrophage cell, one free merozoite (6—7 days p. i.). (Impression from the liver, Giemsa (× 2 000).

Figs. 6—7. Muscle cysts in inoculated mice. 6: part of a septate cyst with zoites extracted from the muscle (× 2 400), 7: cysts in transverse section through the muscle Harris'haematoxylin (× 1 200).