Sarcocystis in Mice Inoculated with Toxoplasma-Like Oocysts from Cat Feces

Abstract. *Sarcocysts morphologically similar to* Sarcocystis muris *were observed in mice after inoculation with* Toxoplasma*-like oocysts found in feces of a stray cat. Cats that were fed mice infected with the oocysts shed similar oocysts in their feces. Sarcocysts were found histologically in about 50 percent of mice inoculated with 40,000 or more oocysts and examined 42 days or longer after exposure. Most inoculated mice developed low* Toxoplasma *dye-test antibody titers 3 to 4 weeks after exposure, but* Toxoplasma *antibody was usually not detectable in infected cats.*

In the course of research on the prevalence of *Toxoplasma gondii* oocysts in feces of naturally infected cats in Hawaii, oocysts morphologically similar to *Toxoplasma* were found which could not be identified according to the criteria used (demonstration of *Toxoplasma* antibody and typical trophozoite or cyst stages in mice inoculated with oocysts) (*1*, *2*). Mice that did not develop *Toxoplasma* antibody in their serums (screened at a 1 : 16 dilution in the dye test) after inoculation with some of these oocysts were eventually fed to a cat that began shedding *Toxoplasma*-like oocysts in its feces 7 days later. Examination of tissues from several mice inoculated orally with feces from this cat revealed the presence of a sarcocyst stage similar to *Sarcocystis muris*, as described by Smith (*3*) (see Fig. 1). Cats fed carcasses of some of these mice (not including the brain or gastrointestinal tract) shed *Toxoplasma*-like oocysts in their feces, and cat-mouse-cat cycles were established. Information on this parasite is of interest because of its similarities to both *Toxoplasma* and *Sarcocystis* and since the life cycle of *Sarcocystis*, which is known to infect man, is obscure.

Cats used in experiments were purchased as 8- to 10-week-old kittens from pet stores on the island of Oahu, Hawaii. They were maintained in the laboratory on a diet of commercially prepared dry cat food for at least 3 weeks before use. None had *Toxoplasma* dye-test antibody ($< 1 : 4$). *Toxoplasma*-like oocysts were not seen in two or three fecal specimens examined before experimental infection, but *Isospora felis* or *I. rivolta* oocysts were present in some cats.

Oocysts in cat feces were collected, processed, and inoculated into mice by methods described previously (*1*, *2*). The mice we used came from colonies known to be free of *Toxoplasma*, and reported to be free of *Sarcocystis*, at the National Institutes of Health, Bethesda, Maryland. Mouse serums were tested for antibody in a modified dye test (*4*) and in an indirect fluorescent antibody test (IFAT) (*5*). Antigens used in the IFAT included *Toxoplasma* trophozoites (RH strain) from peritoneal exudate of mice infected with *Toxoplasma* and "trophozoites" liberated from sarcocysts found in muscles of mice infected with the parasite in question, which will be referred to as WC1170. Mouse tissues were fixed in neutral, buffered 10 percent formalin, sectioned, and stained (with hematoxylin and eosin) according to standard histopathological procedures for microscopic examination. Unfixed brain tissue, usually about one half of the brain, from most exposed mice was suspended in normal saline and about one third of this was examined microscopically for cysts.

Dye tests were done on serums from 129 mice inoculated orally with 10 to 10^6 WC1170 oocysts from feces of ten different experimentally infected cats. Eighty-eight mice (68 percent) had *Toxoplasma* dye-test titers of 1 : 4 or higher 3 to 4 weeks after exposure. Of the mice with *Toxoplasma* antibody, 67 percent had titers of only 1 : 32 or lower. The single highest titer observed was 1 : 512. Only 1 of 12 mice inoculated with 10 to 100 oocysts developed *Toxoplasma* antibody. Mice that were dye-test negative and that had been given small numbers of oocysts were found to be infective for a cat.

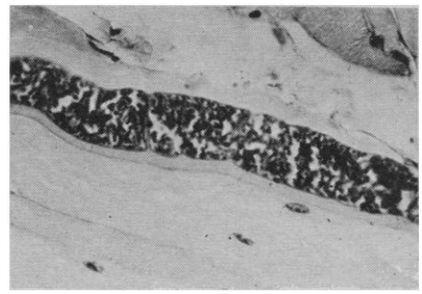

Fig. 1. Sarcocyst in diaphragm of mouse 60 days after exposure to oocysts. Hematoxylin and eosin stain. (× 200)

Indirect fluorescent antibody tests, in which trophozoites harvested from sarcocysts were used as antigen, were done on serums from 14 mice. Results indicated that antibody to this stage became detectable in mouse serum about 30 days after exposure to oocysts and in some instances reached titers in the thousands. Such serums, however, reacted only in low dilutions to *Toxoplasma* trophozoites in the same test. Serums with high dye-test and *Toxoplasma* IFAT titers ($\geq 1 : 2000$) from mice with chronic *Toxoplasma* infections did not react at a 1 : 4 dilution with the *Sarcocystis* trophozoites in the IFAT.

Sarcocyst will be used hereafter for the morphologically distinct entity presumed to be a stage of *Sarcocystis muris* or a similar species. Cyst will be used collectively in reference to either sarcocysts or *Toxoplasma*-like cysts. Stained sections were examined microscopically from diaphragm muscle, muscles from the ventral abdominal wall, or from the thigh (or from all three locations) of 38 mice killed between 20 to 140 days after exposure to various doses (4×10^2 to 10^6) of WC-1170 oocysts. *Toxoplasma*-like cysts, probably an early form in the development of the sarcocyst, were found in one or more muscles from 15 mice, and typical *S. muris* sarcocysts were found in muscles from 8 mice. Both types of cysts were found together in muscle in two of the eight mice. Sarcocysts were not found in mice sooner than 42 days after exposure and were found in only one mouse inoculated with fewer than 40,000 oocysts. Of 14 mice inoculated with 40,000 or more oocysts and examined 42 days or longer after exposure, sarcocysts, located in one or all three of the muscle groups examined, were found in seven mice. Fully mature sarcocysts were not found before 70 days, although it appeared that there was considerable variation in their time of development. Forty-four days after infection, sarcocysts as large as 26 by 185 μm were found, whereas at 69 days some sarcocysts were more than 220 μm in length. In 1 mouse killed 112 days after exposure, there were grossly visible sarcocysts occupying entire muscle fibers in most skeletal muscles. These cysts measured 65 to 80 μm in width. Trophozoites in the older sarcocysts were crescent-shaped and rounded at both ends. They aver-

aged 4.2 ± 0.4 μm by 12.0 ± 0.8 μm in size (based on measurement of 12). They appeared to be compartmentalized into groups within the cyst; however, septa arising from the cyst wall were not usually obvious.

Cysts resembling *Toxoplasma* were seen in mice as early as 14 days after exposure and seemed to be most prevalent in muscle between 22 and 40 days after exposure. In contrast to the mature sarcocysts, they were spherical to elliptical and measured from about 15 to 35 μm in diameter. The wall was much thinner and the contents were more homogeneous. There were numerous basophilic granules, possible nuclei of parasites, but individual parasites were not apparent.

Examination of suspensions of fresh brain tissue from individual mice or groups of two to four mice 1 month or longer after exposure revealed one to five *Toxoplasma*-like cysts in the brains of at least 18 of 40 mice examined. Cysts were present in brains of 4 of 7 mice with sarcocysts and in 8 of 13 mice with *Toxoplasma*-like cysts in muscles.

Myositis was observed in most of the mice examined and was apparent before cysts developed and in exposed mice in which no cysts were found. Myocarditis was also observed, but sarcocysts were not found in the hearts of 30 mice examined, including those with cysts in other muscles and in the brain. Inflammatory reaction was not observed in the immediate vicinity of the cysts. Only eight mice became overtly ill following infections with WC1170 oocysts, and four of these died. Each had been inoculated with 144,000 oocysts.

Attempts to infect mice by inoculating them (intraperitoneally or orally) with muscle tissue or brain tissue from 14 groups of mice infected with WC-1170, and killed between 21 and 62 days after exposure, were all unsuccessful. The absence of infection in five groups of mice inoculated with tissue from infected mice was confirmed by feeding them to susceptible cats, which failed to result in a patent infection. Also, antibody to *Toxoplasma* or *Sarcocystis* was not detectable in mice inoculated with tissue from mice known to be infected.

Of 15 cats fed carcasses of one or more mice infected with WC1170 oocysts, 14 developed a patent infection. Prepatent periods ranged between 4 and 7 days (average, 5 days), and patent periods ranged between 4 and 18 days (average, 13 days). The only evidence of disease in cats infected with WC-1170 was watery feces a day or two before patency. In contrast to the ease of initiating patent infections in cats by feeding them mice infected with oocysts, two cats that were inoculated orally with approximately 200,000 to 400,000 oocysts, from a pool known to be infectious for mice, failed to develop patent infections within 51 days after exposure. Serums from all except two exposed cats, which were collected and tested before exposure and approximately once a week thereafter for several weeks, were negative for *Toxoplasma* antibody. One cat fed carcasses and one inoculated with oocysts had transitory low dye-test titers. Observations on cross immunity in cats infected with WC1170 or strains of *Toxoplasma* are summarized in Table 1. Infection with WC1170 conferred little or no immunity to *Toxoplasma* and vice versa, whereas a relatively solid immunity to WC1170 or *Toxoplasma*, respectively, was observed.

There were no obvious morphological differences between the WC1170 oocyst and the *Toxoplasma* oocyst described by Dubey *et al.* (*6*). Oocysts in freshly passed feces were unsporulated, and sporulation occurred after 2 to 4 days at room temperature (25° ± 1°C) under aerobic conditions. Fully developed oocysts averaged 10.8 μm (standard deviation, ± 1.1 μm) in width (range, 9.9 to 13.2 μm) and 13.0 ± 0.5 μm in length (range, 11.6 to 13.2 μm) (based on measurements of 62 oocysts). Sporocysts (28 measured) averaged 6.8 ± 0.7 μm in width (range, 6.6 to 8.3 μm) and 8.1 ± 1.1 μm in length (range, 6.6 to 9.9 μm). Sporozoites were not measured.

The WC1170 parasite has been consistently transmitted in the laboratory from cat feces to mice, with the occurrence of a sarcocyst stage in some of the infected mice. Four cat-mouse-cat passages have been completed. Possibilities exist that some of the mice had been naturally infected with *S. muris* or that the WC1170 parasite is an unusual strain of *Toxoplasma*. However, sarcocysts were not found in mice examined earlier than 42 days after exposure, and antibody to *Sarcocystis* trophozoites was not detected in mice until 30 days or longer after exposure. Also, the following characteristics of the parasite tend to differentiate it from *Toxoplasma*: (i) lack of transmission between mice at least within 2 months after infection, (ii) very low virulence and low *Toxoplasma* titers in mice, (iii) usually no *Toxoplasma* antibody in serum of infected cats, and (iv) little or no cross immunity in cats to at least three strains of *Toxoplasma*. Nevertheless, the dye-test findings in mice indicate that WC1170 and *Toxoplasma*

Table 1. Cross challenge studies in 13 cats infected with WC1170 or *Toxoplasma*. ND, Not determined; —, no control used.

Parasite in initial infection*	Challenge parasite*	*Toxoplasma* dye-test titer		Patency (days)			
				Prepatent period		Patent period	
		On day of challenge*	20 to 30 days after challenge	Exp.	Control†	Exp.	Control
WC1170	*Toxoplasma*						
WC1170	M7741	<4‡	16	3	—	ND	
WC1170	M7741	<4	128	5	—	5	
WC1170	WC1847	<4	16	4		9	
Toxoplasma	WC1170						
HH1	WC1170	32	8	5	5	17	14
WC409	WC1170	128	128	6	5	15	14
WC1170	WC1170						
WC1170	WC1170	<4	<4	0	5		14
WC1170	WC1170	<4	<4	0	5		14
WC1170	WC1170	<4	<4	0	5		16
WC1170	WC1170	<4	<4	20	7	4	3
Toxoplasma	*Toxoplasma*						
WR154	M7741	4	4	0	—		
WR169	M7741	8	4	0	—		
M7741	M7741	16	4	0	—		
M7741	M7741	64	32	0	—		

* Cats were challenged between 27 and 74 days following an initial patent infection. For exposure to WC1170, cats were fed carcasses, not including brain or other viscera, from infected mice. Only one strain was available. For exposure to *Toxoplasma*, cats were fed brains containing several hundred to several thousand *Toxoplasma* cysts. Six strains were used; isolated originally from sheep (M7741), from stray cats (WC409 and WC1847), from wild rats (WR154 and WR169), and from man (HH1). † Control cats not previously exposed to the parasite were fed the same material at the same time as the challenged cats. ‡ Reciprocal of serum dilution.

share some antigens. In this regard, it is of interest that certain ultrastructural features of the trophozoites of *Sarcocystis* and *Toxoplasma* are similar (*7*).

The following characteristics of WC-1170 infection in mice are remarkably similar to those described for *S. muris* (*3*): (i) failure to transmit the parasite between mice by mouth, or parenterally, before 75 to 90 days after exposure, (ii) the finding of *Toxoplasma*-like cysts before sarcocysts are apparent, (iii) the appearance of morphologically typical sarcocysts some time between 40 to 60 days after exposure, but not before, (iv) absence of sarcocysts in the myocardium of infected mice, and (v) the morphologically distinctive trophozoite stage contained in the mature sarcocyst.

The *Toxoplasma*-like oocyst apparently initiated the sarcocyst stage in mice, although this was not definitely established. An alternative hypothesis is that we have been dealing with a mixed infection—an unusual strain of *Toxoplasma* shed in cat feces simultaneously with an unrecognized stage of *Sarcocystis*. This is unlikely since the *Toxoplasma*-like oocyst has been the only parasite found consistently in the feces of cats in transmission experiments. Furthermore, the experiments of Rommel *et al.* (*8*) and of Heydorn and Rommel (*9*), apparently demonstrating that an oocyst stage of *S. Tenella* and *S. Fusiformis* develop in carnivorous animals, supports the assumption that there is both an oocyst stage and a sarcocyst stage in the life cycle of the WC1170 parasite. Fayer's (*10, 11*) observations on cell cultures inoculated with organisms from sarcocysts found in the musculature of wild grackles suggested a coccidial type of life cycle for that parasite.

Since the discovery of *Sarcocystis* in mice 130 years ago, species of this parasite have been found in a variety of vertebrates including man. In spite of numerous investigations, however, the life cycle or cycles of *Sarcocystis* sp. have remained obscure. Of particular interest is the potential for human infection with the WC1170 parasite, which might ensue after exposure to cat feces, as appears to be the case with *Toxoplasma*. About 20 cases of human infection with *Sarcocystis* have been referred to (*12*), but some of these, in retrospect, were probably *Toxoplasma* infection (*13*). Human muscle is infrequently examined microscopically and most human infections have been an incidental postmortem finding. It is also possible that man could become infected without the development of a typical sarcocyst, as apparently happens in some mice. In such cases, antibody to *Toxoplasma* might develop in low titer.

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14. I gratefully acknowledge the technical assistance and help in identifying the sarcocysts from Dr. Jack Frenkel, Professor of Pathology, University of Kansas Medical Center; Dr. Eunice Larson, Chief of Pathology, Children's Hospital, Honolulu, Hawaii; and Drs. Edward Howard and Thomas Sawa, Veterinary Pathologists, Hawaii State Department of Agriculture.

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Wood-Boring Bivalves, Opportunistic Species in the Deep Sea

Abstract. *Wood exposed for 104 days at a depth of 1830 meters at the permanent station of the research submersible D.S.R.V.* Alvin *was completely riddled by two species of bivalve wood borers (subfamily Xylophagainae, family Pholadidae). Their high reproductive rate, high population density, rapid growth, early maturity, and utilization of a transient habitat classify them as opportunistic species, the first recorded from the deep sea.* Xylophaga *is shown to be the most important species involved in decomposing woody plant material in the deep sea.*

During a dive of the D.S.R.V. *Alvin* (*1*) on 14 June 1972, panels of wood 36 by 16 by 2 cm were pushed 12 to 15 cm into the bottom sediment at a depth of 1830 m at the *Alvin* experimental site (39°46′N; 70°41′W), about 180 km south of Woods Hole, Massachusetts (Fig. 1). Two panels were removed by the *Alvin* on 25 September 1972, after an exposure of 104 days. The wood was so weakened as a result of the activity of wood-boring bivalve mollusks (Xylophagainae, Pholadidae) that it began to fall apart while being picked up by the mechanical arm of the *Alvin* (Fig. 2). The minute openings of their burrows covered the surface, averaging about 150 per square centimeter (Fig. 2b), and in some areas the surface had fragmented and broken away (Fig. 2c). The boring bivalves penetrated from both sides, meeting in the middle (Fig. 3), and the burrows of the largest specimens were about 20 mm in length. These specimens were stunted (*2*) because their growth was limited by the lack of space, but the gonads were nearly ripe. Removal of all specimens from a 3-cm^2 section showed that two species were present, *Xylophaga* n. sp. and *Xyloredo ingolfia* Turner, in a ratio of about 5 to 1. The larval shell of the latter is brown and that of the former is white, so the two species can be readily separated at a very young stage. Both species were represented by newly attached or recently metamorphosed specimens as well as specimens which had penetrated the wood to a depth of over 5 mm, which indicated two settlements for each. Examination

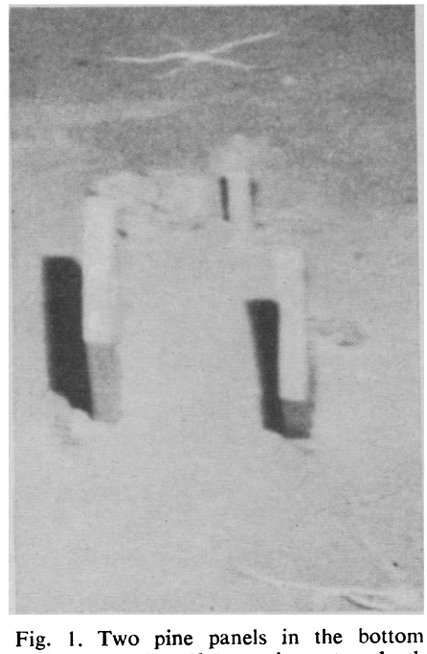

Fig. 1. Two pine panels in the bottom sediment at the *Alvin* station, at a depth of 1830 m.