insect, some oocysts had ruptured inward and sporozoites occurred in the anterior midgut as well as in the salivary glands. Air-dried sporozoites stained with Giemsa stain were considerably shorter (4 to 6 by 0.8 μm) than sporozoites of avian or mammalian *Plasmodium* species and appeared similar to erythrocytic and exoerythrocytic merozoites seen in the lizard.

Hatchling and mature fence lizards were inoculated intraperitoneally with sporozoites from 40 sandflies, but patent infections have not yet been produced. Two hatchling lizards fed upon by infected sandflies died before infections could have developed. Seasonal reduction of the sandfly population and the difficulty of rearing California sandflies preclude further experiments until next spring.

If sandflies do transmit *P. mexicanum* in nature, infection of the lizard probably occurs when the insect takes a blood meal. Infection by ingestion is unlikely because the activity periods of lizards and sandflies do not overlap. Oocysts mature when the female sandfly has laid her first batch of eggs and is ready to take a second blood meal. We frequently observed sporozoites escaping from the mouthparts of sandflies dissected 11 days or more after feeding.

Although transmission has still to be demonstrated, these findings suggest that sandflies transmit lizard malaria. It is a general finding with mosquito-borne malaria that only insect species capable of transmitting the parasite will support its complete development to the point of invasion of the salivary glands by sporozoites. Phlebotomines occur in nearly all areas of the world from which lizard malaria has been reported, but little is known about the species that attack cold-blooded vertebrates.

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Toxoplasma gondii: The Oocyst, Sporozoite, and Infection of Cultured Cells

Abstract. *The infective form of* Toxoplasma gondii *found in cat feces is an oocyst which, when sporulated, resembles that of the genus* Isospora *in having two sporocysts. Sporozoites obtained by artificial excystation of the oocyst are infective for monkey kidney cell cultures. Ultrastructural characteristics of sporozoites resemble those seen previously in proliferative stages of* Toxoplasma gondii.

Toxoplasmosis can be produced in mice by feeding them infective forms of *Toxoplasma gondii* collected from feces of experimentally infected cats (*1, 2*). Work and Hutchison identified the infective form as a "new cystic form" of *T. gondii* (*3*). In freshly passed feces this form had a central granular mass which divided into two spherical bodies after incubation.

This cystic form, when mature, is morphologically similar to a coccidian oocyst, specifically, that of the genus *Isospora*. It is ovoid, it measures about 9 by 12 μm, and it is bordered by a thin wall. When passed in the feces, it is almost spherical and has a central granular mass (Fig. 1). After sporulation it contains two ovoid sporocysts (Fig. 2). Within each sporocyst we have tentatively identified four sporozoites and a refractile mass that possibly represents a residual body.

The infective form, the sporozoite, can be artificially released by fracture of the oocyst wall and subsequent incubation of the sporocysts in excystation fluid. Cats were infected by feeding them *T. gondii* cysts in mouse brain (strain M-7741 or C-56), and oocysts were collected from cat feces by flotation with zinc sulfate (*2*). Sporulated oocysts were then suspended in Melnick's A medium and broken by grinding them in a Teflon tissue grinder. The preparation was sedimented and then incubated for 30 minutes at 37°C in Melnick's A medium with the addition of 5 percent cat bile and 0.5 percent trypsin. After the bile and trypsin were removed by washing the pellets with stock medium, the sporozoites were observed by light microscopy, processed for electron microscopy, or inoculated into tissue cultures.

The sporozoites were fixed in bulk with 5 percent glutaraldehyde, smeared on a cover slip, and stained with Giemsa; microscopically they appeared crescent shaped and measured approximately 7 by 1.5 μm (Fig. 3). They had a dark central area separated from the anterior and posterior ends by areas of lighter density. Specimens fixed in glutaraldehyde and osmium viewed by electron microscopy had the following characteristics (Fig. 4). The organism had a pellicle consisting of two layers and an underlying system of microtubules. A conoid was situated at the anterior end. Most of the anterior portion was filled with micronemes and several rhoptries. Glycogen bodies and one or two mitochondria with tubular cristae were located near the anterior edge of the nucleus. The nucleus was posterior to the middle of the organism and had chromatin distributed in clumps around its periphery. Refractile bodies were not seen by either light or electron microscopy.

Excysted sporozoites, inoculated into Leighton tubes containing primary cultures of monkey kidney cells on cover slips, were incubated in Melnick's A medium at 37°C for periods up to 31 days. When cultures were fixed with Bouin's solution and stained with hematoxylin and eosin, many intracellular organisms were seen in isolated areas of the cell layer (Fig. 5). The organisms usually occurred in pairs or rosettes. Cells in the centers of isolated areas of infection were often destroyed by rupture that resulted from proliferation of organisms. Freed organisms were recovered from the medium; when inoculated intraperitoneally into mice, they caused toxoplasmosis. No organisms resembling *Toxoplasma* were observed in cultures inoculated with material processed from feces passed prior to infection of the cat with *T. gondii* nor when oocysts were inoculated directly into cultures without excystation.

Feces collected 1 day after *T. gondii*

infection of one cat contained oocysts of *Isospora felis* (approximately 35 by 43 μm) and *I. rivolta* (approximately 15 by 23 μm). These oocysts were sporulated, excysted, and their sporozoites were inoculated together into tissue cultures. No organisms were observed after incubation for various times. Three days after infection of the cat with *T. gondii*, the feces still contained oocysts of both *Isospora* species as well as those of *T. gondii*. After sporulation and excystment, this mixed sample was inoculated into cultures, and a typical *T. gondii* infection resulted.

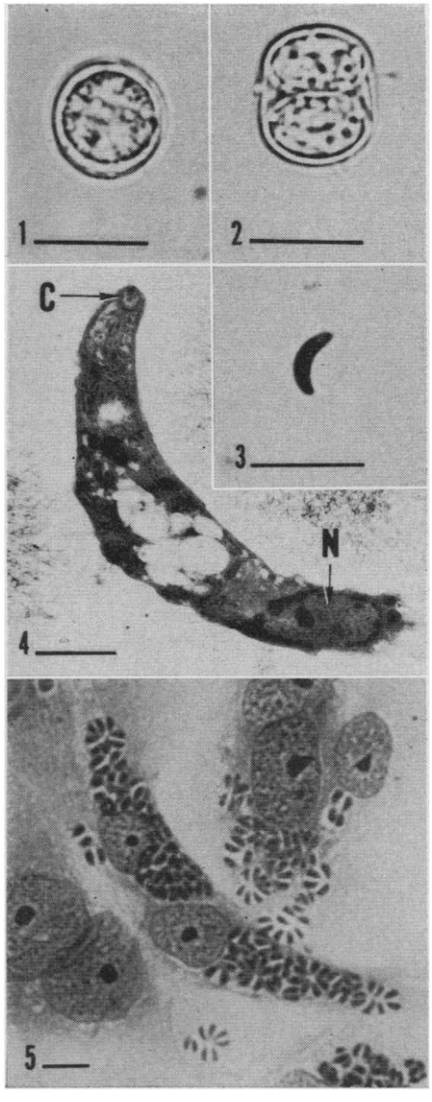

Fig. 1. Freshly passed unsporulated oocyst of *Toxoplasma gondii*; scale, 10 μm. Fig. 2. Sporulated oocyst containing two sporocysts; scale, 10 μm. Fig. 3. Artificially excysted sporozoite. Stained preparation; scale, 10 μm. Fig. 4. Longitudinal thin section of sporozoite showing conoid (*C*) at anterior end and nucleus (*N*) near posterior end. The two holes in glycogen bodies are embedding artifacts; scale, 1 μm. Fig. 5. Cultured monkey kidney cells infected with *T. gondii*, 25 days after inoculation with excysted sporozoites; scale, 10 μm.

The oocyst of *Toxoplasma gondii* probably has been observed by others but may have been identified as *Isospora bigemina* in which the oocyst of the small race measures 10 to 16 μm by 7.5 to 10 μm (*4*). There are several reasons for designating the oocysts in our study as those of *T. gondii*.

When feces were examined prior to infection of cats with *T. gondii*, no oocysts in the size range of 9 by 12 μm were seen. After infection, they usually appeared in the feces on day 3 and were no longer seen after days 7 to 10. Oocysts collected during this period from eight of nine cats produced toxoplasmosis in mice. No oocysts were observed in feces of the remaining cat nor was transmission successful.

Infectivity of the fecal material to mice corresponded with the presence of oocysts, except in a few cases where no oocysts were seen possibly because of their low numbers. In studies of the "new cystic form" of *T. gondii* (*3*), there was a positive correlation between number given and severity of infection in mice. Diagnosis of toxoplasmosis in our mice was confirmed by the demonstration of dye-test antibodies, by the identification of *T. gondii* in the lungs of dying mice, or by the presence of cysts in the brains of survivors.

Recognition of an oocyst stage in the life cycle of *Toxoplasma gondii* confirms previous acceptance of this parasite as a sporozoan. Inasmuch as electron microscope studies have established its close relationship to the merozoites of *Eimeria bovis* and other species of *Eimeria* (*5*), it is conceivable that the other stages of the life cycle may be found in the intestinal tissues of the cat.

A reinvestigation of the life cycle of *Isospora bigemina* is indicated owing to the existence of two different sites of development in the cat and in the sizes of the oocysts (*4*). This supports the idea that *T. gondii* oocysts have been previously identified as those of the small race of *I. bigemina*.

More importantly, a new effort in epidemiological studies is indicated. Infection through fecal contamination provides a simple route for dissemination of the organism and may account partly for its widespread existence in man. Thus far, only the cat has been a successful host. However, the possibility of low host specificity with complete development of the organism in other animals including man must not be overlooked.

Note added in proof: Typical coccidian schizonts and gametocytes, probably of *T. gondii*, have been observed in the intestinal epithelium of an infected cat in association with oocysts (*6*).

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Toxoplasma gondii in Cats: Fecal Stages Identified as Coccidian Oocysts

Abstract. Isospora-*type oocysts were excreted by cats following the ingestion of* Toxoplasma *from infected mice. Oocysts appeared 3 to 5 days after cysts were ingested and 8 to 10 days after trophozoites were ingested, and also 21 to 24 days after the administration of infective fecal suspensions from cats. A close quantitative and biologic correlation between oocysts and* Toxoplasma *infectivity of the feces was observed which could not be separated by density gradient centrifugation and filtration methods.* Toxoplasma *is an intestinal coccidian of cats which is fecally spread. It has evolved to multiply in brain and muscle and in other species, making it possible for carnivorism to become another means of transmission.*

Toxoplasma has been isolated repeatedly from the feces of cats that had eaten mice infected with *Toxoplasma* (*1*). The infectivity seemed to be associated consistently with the eggs of the nematode *Toxocara cati*. Since the fecal infectivity persisted in water upon storage at room temperature,