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.

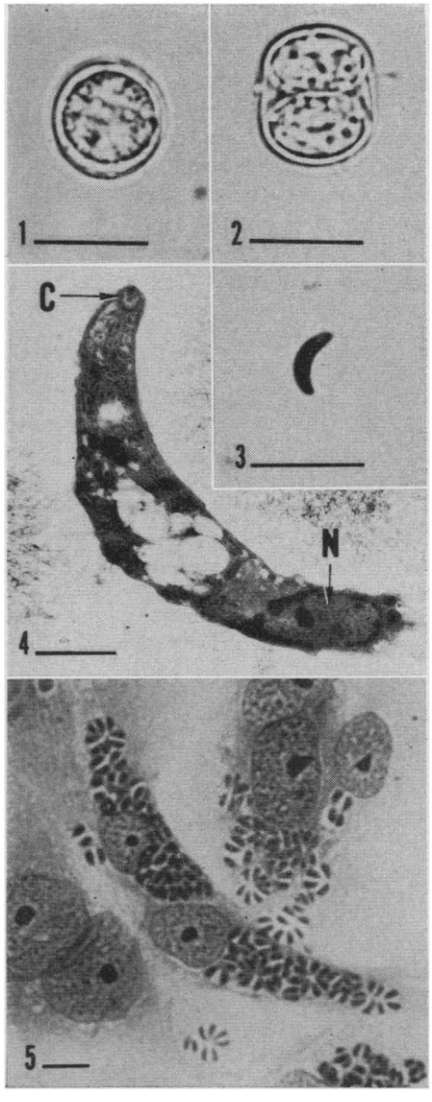

Fig. 1. Freshly passed unsporulated oocyst of *Toxoplasma gondii*; scale, 10 μm. Fig. 2. Sporulated oocyst containing two sporocysts; scale, 10 μm. Fig. 3. Artificially excysted sporozoite. Stained preparation; scale, 10 μm. Fig. 4. Longitudinal thin section of sporozoite showing conoid (*C*) at anterior 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,

Table 1. Filtrability of oocysts and *Toxoplasma* infectivity. Each of two mice were fed 40 percent of the filtrate. An earlier time of death reflects a higher number of organisms inoculated than does either a later time of death or development of positive (+) serology. S, survivor.

Filter system	Pore rating (μm)	Oocysts in 10 percent of filtrate (No.)	Infectivity to mice	
			Time of death (days)	Serology
Electrofoil*	15	79	9, 9	
Electrofoil	10	77	9, 9	
115 μm, Micules†	18	40	7, 12	
60 μm, Micules	9	3	12, 12	
50 μm, Micules	8	0	S	−
Mitex‡	10	4	12, 14	
Mitex	5	3	S	+
Duralon‡	14	0	S	−
Duralon	7	2§	S	−
Nucleopore‖	8	1§	S	−
Nucleopore	5	0	S	−
None		214	8, 8	

* Nickel foil (Perforated Products, Brookline, Mass.). † Spherical copolymer particles (Sondell Scientific, Palo Alto, Calif.). ‡ Millipore Corporation (Bedford, Mass.). § Unsporulated, collapsed. ‖ General Electric (Pleasanton, Calif.).

unlike other known forms of *Toxoplasma*, it was suggested that *Toxoplasma* might be inside the nematode egg (*1*). However, we separated *Toxoplasma* from the nematode eggs in the feces of cats and isolated *Toxoplasma* from the feces of worm-free cats fed *Toxoplasma* (*2*). Similar results were reported by others (*3, 4*). The morphologic equivalent of fecal *Toxoplasma* infectivity was suggested to be a cystic structure containing first a granular mass and later two separate organisms without definite internal structure (*5*).

Toxoplasma strain M-7741 was fed to nematode-free cats (*2*). We concentrated infectivity in cat feces by flotation at a specific gravity of 1.180 either in zinc sulfate (*2*) or in sucrose. We determined infectivity in mice by demonstrating *Toxoplasma* in impression smears or histologic sections or by demonstrating the development of antibody to the standard RH strain of *Toxoplasma*.

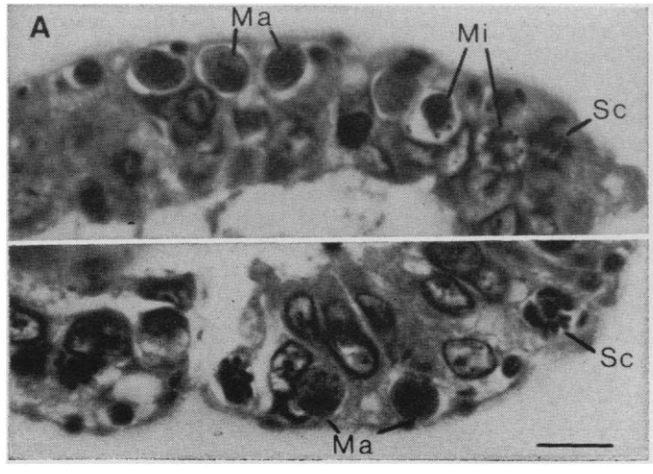
Fecal *Toxoplasma* infectivity had been shown to pass through 37-μm wire mesh sieves (*2*). We therefore examined fecal suspensions for structures smaller than 37 μm and larger than trophozoites (which measure about 2 or 3 by 6 or 7 μm). Numerous forms were examined for fluorescent staining by the indirect method after they were exposed to *Toxoplasma* antibody. Several fungi and oocysts resembling those of *Isospora bigemina* and *I. felis* were stained.

Oocysts morphologically resembling those of *Isospora bigemina* appeared in feces 3 to 5 days after the cats had ingested mice with chronic toxoplasmosis and continued to be shed for 1 to 2 weeks. Fresh feces did not transmit toxoplasmosis to mice (*2, 4*); feces became infectious simultaneously with oocyst sporulation during 2 to 3 days at room temperature.

We repeated these experiments in newborn kittens to rule out the possibility of activation of a latent coccidial infection. Nine 1- to 2-day-old kittens from three litters were fed with suspensions of mouse brain that contained *Toxoplasma* cysts, and their feces were examined at autopsy. *Toxoplasma* infectivity was shown after 2 days in none of one, after 3 to 4 days in one of one, after 5 days in three of three kittens and after 6 to 9 days in four of four kittens. This was paralleled exactly by the finding of oocysts like those of *Isospora bigemina*. Two kittens did not excrete oocysts, nor was *Toxoplasma* isolated from their feces or organ passages.

Quantitative comparisons were made on independent specimens. The oocysts (like those of *I. bigemina*) were enumerated in a hemocytometer where 1/2500 of a milliliter was counted. Sporulation percentage was determined from 50 or 100 oocysts between a thin slide and cover glass. Numbers of sporulated oocysts were compared with intraperitoneal injection of tenfold dilutions to groups of two to six mice. Oocysts and infectivity were closely correlated in all of ten specimens. Titration of the specimens by feeding showed wider discrepancies, since more than 10 percent of the inocula regularly passed through the gut of mice. Hence oral infectivity titers were always less than the number of oocysts fed, but plotting of values showed parallelism. For example, a counted $10^{5.55}$ sporulated oocysts yielded a 50 percent infectious titer of $10^{5.24}$ after feeding but of $10^{5.67}$ after intraperitoneal administration.

Whether we were dealing with real or chance associations had to be decided by independent determinations. Filtrability of oocysts and fecal *Toxoplasma* infectivity coincided closely (Table 1). Centrifuged in a linear den-



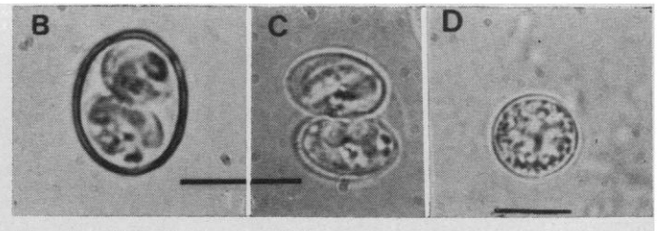


Fig. 1. (A) Schizonts (*Sc*), macrogametocytes (*Ma*), and microgametocytes (*Mi*) in epithelium of ileum of a kitten fed cysts of *Toxoplasma* in mouse brain 8 days earlier. The residual mass of schizonts at 4 o'clock is clearly shown. Helly fixation, hematoxylin and eosin stain, × 1000. Scale, 10 μm. (B to D) *Toxoplasma* oocysts, fresh, unfixed, sporulated in 2.5 percent potassium dichromate. (B) Double oocyst wall, × 1600. (C) Single oocyst wall after exposure to 6 percent sodium hypochlorite for 30 minutes, × 1600. B and C scale, 10 μm. (D) Unsporulated oocyst, × 1000. Scale, 10 μm.

sity gradient between 0.5 and 1.4*M* sucrose, both oocysts and infectivity were banded and confined to four of ten fractions; peak numbers of both were found in the fraction corresponding to 0.92*M* sucrose (specific gravity, 1.11). By continuous particle electrophoresis, both oocysts and infectivity showed a similar broad peak in 9 of 40 fractions. Sporulation time of oocysts from 2 to 3 days at 24°C was delayed to 5 to 8 days at 15°C and to 14 to 21 days at 11°C, and the development of *Toxoplasma* infectivity was identically delayed. Anaerobiasis likewise delayed sporulation and development of infectivity to the same degree. Thermal death points for both undeveloped oocysts and for infectivity were between 45° and 50°C.

Sections of the small intestine of cats fed *Toxoplasma* cysts showed schizogonic stages, macrogametocytes, and microgametocytes typical for coccidia in the epithelium, especially of the ileum (Fig. 1A). These findings were associated with the production of oocysts (Fig. 1, B–D) that measured 9 to 11 by 11 to 14 μm (mean 10 by 12 μm) and contained two sporocysts that were each about 6 by 8 μm and four sporozoites that were each about 2 by 8 μm. These measurements are similar to those reported for *Isospora bigemina* (*6*). *Toxoplasma* trophozoites were found in mesenteric lymph nodes and other organs of these cats.

Staining with fluorescent antiglobulin indicated that mouse serum containing *Toxoplasma* antibody was bound by oocyst and sporocyst walls in paraffin sections, and sporozoites, schizonts, and macrogametocytes on smears. Absorption of this serum with the standard RH strain of *Toxoplasma* abolished its binding capacity.

Oocysts appeared in cat feces not only after the cats fed on cysts from mice with chronic toxoplasmosis but also after they were fed on trophozoites from mice acutely infected, and after they were fed on cat feces containing oocysts and *Toxoplasma* infectivity.

Cyclic development was indicated by finding a progressively increasing incubation period before the appearance of oocysts (prepatent period). This prepatent period was 3 to 5 days after cysts were fed, with all of eight cats responding; it was 8 to 10 days after feeding trophozoites in two of seven cats; and 21 to 24 days in all of five cats fed fecal oocysts associated with

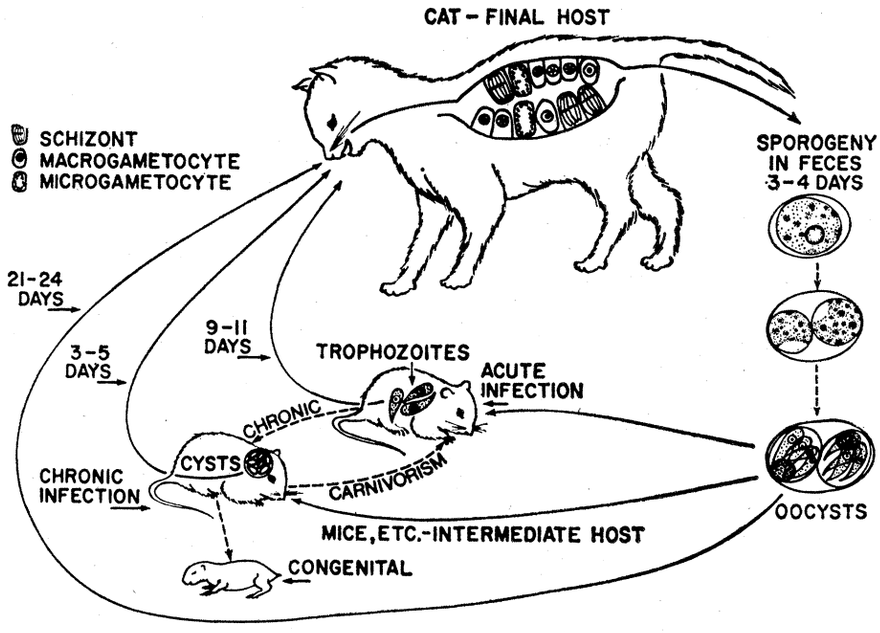


Fig. 2. Postulated life cycle of *Toxoplasma* showing means of transmission by oocysts from cat feces and by ingestion of trophozoites or cysts from intermediate hosts. Days indicated represent time from ingestion of *Toxoplasma* by cat to the excretion of oocysts. Mice are shown to represent the many mammals and birds that can serve as facultative intermediary hosts and among which infection may spread, usually by carnivorism. Congenital transmission may take place during chronic infection as in mice, or during acute infection as in humans.

Toxoplasma infectivity (Fig. 2). *Toxoplasma* infectivity appeared after similar intervals.

In mice, rats, golden hamsters, guinea pigs, dogs, raccoons, skunks, pigs, domestic rabbits, opossums, Japanese quail, chickens, and pigeons, no fecal infectivity was produced during an observation period of 14 to 28 days after they were fed on *Toxoplasma* cysts, although all, except the pigeons, became infected.

On the basis of quantitation, size, density and movement in an electric field, developmental and resistance comparisons, fluorescent antibody staining, and cyclic development, we consider the fecal forms of *Toxoplasma* to be identical with oocysts. We had dismissed the seeming relation between *Toxoplasma* and *Toxocara* as coincidence after separation of *Toxoplasma* from worm eggs and of worm larvae from *Toxoplasma*, and after recovering *Toxoplasma* infectivity from the feces of worm-free cats (*2*). Attempts to separate oocysts from fecal forms of *Toxoplasma* by filtration, density gradients, and electrophoresis have been unsuccessful. That we observed progressively shorter prepatent periods after cats were fed on oocysts, trophozoites, and cysts, respectively (Fig. 2), suggests that these are sequential stages in an obligatory life cycle.

Oocysts of *Isospora* were mentioned in the first publication relating *Toxocara* and *Toxoplasma* (*7*). However, when describing the "new cyst" (*5*) no mention of oocysts like those of *Isospora* was made. Although the sizes of the "new cyst" (9 × 14 μm) and our oocysts (10 × 12 μm) are similar, there is a significant difference between the 3 × 7 μm "internal bodies" and the 6 × 8 μm sporocysts which we observed. We consider the single titration comparison and the four microisolations (*5*) as inconclusive, since the presence of *Isospora* oocysts was not excluded.

The newly postulated life cycle of *Toxoplasma*, including sexual reproduction in the cat intestine, helps to classify the genus *Toxoplasma* as a member of the suborder Eimeriina (*8*) or Eimeriorina (*6*) with the following characteristics: schizogony and gametogony in gut epithelium of cat; oocysts with two sporocysts and four sporozoites developing outside of host; trophozoites multiplying by endodyogeny in many types of cells, leading to the production of cysts with many naked merozoites, mainly in brain and muscle; and facultatively heteroxenous in many mammals and birds in which only the

asexual, extraintestinal cycle has been observed.

Toxoplasma appears to have originated as an intestinal coccidian in cats, with fecal-oral transmission. Of 13 hosts investigated, only cats excreted oocysts in feces. Unlike typical coccidia, *Toxoplasma* multiplies extensively in other tissues and hosts. The formation of a persisting cyst in brain and muscle has made it possible for carnivorism to become an additional means of transmission. Whether infected by oocysts or cysts, other mammals and birds can be regarded as facultative intermediate hosts.

With the newly identified *Toxoplasma* oocysts from cats, the transmission of *Toxoplasma* in the human ecosphere must be reassessed. Congenital transmission plays a small but medically important role. The ingestion of cysts or trophozoites through raw and undercooked meat, whether biologically determined, as in cats, or culturally determined, as in humans, appears important as a means of transmission (*9*). In cats, carnivorism serves not only as an efficient means of infection, but can result in contamination of the environment with large numbers of oocysts. *Toxoplasma* oocysts develop aerobically, and we have found them capable of inducing infection in mice and presumably in man after periods of at least 4 months in water or moist soil.

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Dentin Matrix Transformation: Rapid Induction of Alkaline Phosphatase and Cartilage

Abstract. *An acid-insoluble fraction of rat dentin rapidly initiates a chain reaction in mesenchyma after allogeneic transplantation to the subcutaneous tissues. The tooth matrix induced alkaline phosphatase activity within 24 hours; cartilage appeared within 5 days; bone and bone marrow formed within 14 days. The induced cartilage disappeared within 5 weeks, but bone persisted at least 1 year.*

The stem cells of the connective tissue of animals in postnatal life possess a singular potential for alteration of their phenotype. The following experiments demonstrate the efficiency of dentin matrix as an inductor of differentiation of mammalian cells.

There are two different methods to convert fibroblasts of the mesenchyma into cells of other sorts such as cartilage or bone. In one, the surgical approximation of a transforming epithelium (*1*), for example, the bladder mucosa, and responding mesenchyma induces bone in autologous and allogeneic (*2*) transplants in dog and certain other animals, whereas cartilage is not observed. In the second method, both bone and cartilage are produced by amnion cells (*3*) with placenta and hard tissues acting as transformants of mesenchyma.

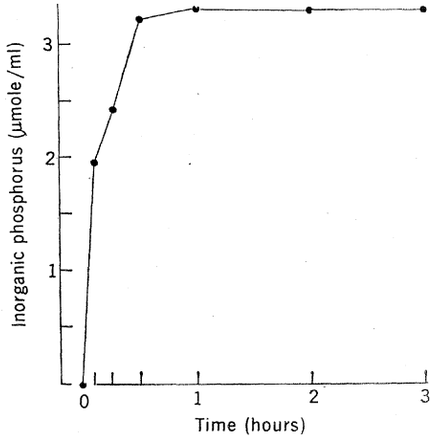

Fig. 1. Rate of extraction (μmole/ml) of inorganic phosphorus (*6*) from tooth in 0.5*N* HCl at 30°C.

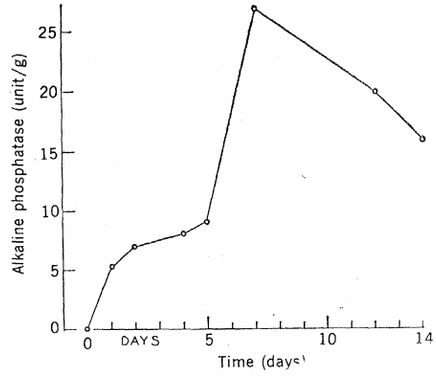

Fig. 2. Alkaline phosphatase (*7*) content (unit/g) of allografts of acid-decalcified rat tooth matrix in the subcutaneous tissues. Mean values of ten samples at each time interval are provided.

Autologous transplantation (*4*) of tooth to muscle leads to ossification that invades dentin and enamel and occurs in the pulp space. Urist *et al.* (*5*) discovered that the matrix of cell-free decalcified lyophilized bone, tooth, or cartilage can induce new cartilage and bone. The matrix was implanted as an allogeneic transplant in the muscle of rabbit and rat; deposits of new bone and cartilage were found 24 days after the operation. We now describe the early stages of the changes in responding cells induced by dentin matrix, which is highly cross-linked and exceptionally resistant to the deleterious action of strong acids.

The acid-soluble components of teeth were extracted at room temperature (28° to 30°C). Female Long-Evans rats, 25 to 30 days old, were the source of incisor teeth. The teeth were weighed and placed in 0.5*N* HCl (1 ml/mg) in a jar with a magnetic stirrer where they were propelled continuously around the vortex created by vigorous stirring. The rate of extraction of inorganic phosphorus (*6*) from the teeth was determined on portions of the acid. After 2 hours the tooth pulp was discarded, and the acid was replaced with an equal volume of 2 percent sodium bicarbonate to neutralize and wash out the acid. After 2 hours of stirring the teeth were implanted in the wet state subcutaneously on the abdominal muscles of allogeneic littermates of the donors of the teeth; the day of operation was counted as day 0. The grafts were excised subsequently on day 1 to day 365. The content (*7*) and localization (*8*) of alkaline phosphatase (E.C. 3.1.3.1) in the grafts were determined.

Before acid extraction the lower incisor teeth of our rats, age 30 days, weighed 29.9 ± 1.3 mg. The alkaline phosphatase activity was, in units: dental pulp, 292 ± 40; tooth shell, 10.5 ±