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Schizont, gamete, and oocyst production in the cat by human and porcine strains of *Toxoplasma gondii* from Japan

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Introduction

The recent discovery that *Toxoplasma gondii* is a coccidian which undergoes schizogony and gametogony in the intestinal epithelium of cats, and that the infectious forms shed in feces is the biological equivalent of the oocyst form of *Isospora* (Frenkel *et al.*, 1970; Hutchison *et al.*, 1970; Sheffield & Melton, 1970), has culminated an epochal period in the elucidation of the life-cycle and taxonomic status of this parasite. Remington (1970) has recently reviewed the series of investigations stimulated by the preliminary report of Hutchison in 1965, that concentrates of feces from cats fed chronically-infected mice could produce infections after incubation at room temperatures for several months.

The role of infectious oocysts and the oral route of infection in relation to human toxoplasmosis is yet unknown. Although this mechanism offers a reasonable explanation for many situations, several questions are immediately apparent which must be answered before the true epidemiological significance of this means of transmission can be assessed.

All of the reported investigations of the intestinal cycle of infection in cats have been carried out with only four strains of *Toxoplasma*: all are zoophilic strains, most of low pathogenicity and with an unusual tendency towards tissue cyst formation. The work of Hutchison and colleagues (1965-1969) was done with the Beverly strain which was originally isolated from a rabbit in England. A cyst-forming strain, M-7741, isolated from a sheep, was used by Jacobs & Melton, (1966, 1967), Sheffield & Melton (1969, 1970) and Frenkel *et al.* (1969, 1970) in the United States. An avirulent swine strain from Denmark, 119, which was used by Work & Hutchison (1969), and C-56, a cyst-producing chicken strain investigated by Sheffield & Melton (1970), complete the list of strains known to produce the oocyst forms, presumably as a result of schizogony and gametogony, in the cat. However, Frenkel (1969) reported that he did not have similar success with all strains; he could not produce oocysts with several strains of *T. gondii* used, although he had repeated successes using M-7741.

This raises the question as to whether the ability to undergo typical coccidian schizogony and gametogony is a characteristic of all forms of the species, including those producing human disease, or is peculiar to only certain strains; perhaps those with other special characteristics, such as relative non-pathogenicity and an increased propensity to form tissue cysts.

The present study was intended to determine (1) if this ability to undergo a developmental cycle in the feline gut is also characteristic of strains of *Toxoplasma gondii* from the Orient, an area from which strains have not previously been studied in this regard, and (2) if it occurs with a strain of human origin.

Materials and Methods

Two strains of *Toxoplasma gondii* isolated in Japan were provided through the courtesy of Dr. H. Matsubayashi and Dr. I. Nakayama of the Kitasato Institute, Tokyo. Strain S-273 was isolated from the brain of a pig. It is nonpathogenic for mice and produces many brain cysts; it is very similar to the Beverley strain in these characteristics. Strain KM was isolated from human cerebrospinal fluid, and is pathogenic for mice. Its behavior in mice is similar to the RH strain. The Beverley strain, also from Kitasato Institute, was used as a control infection since it was known to produce oocyst-like forms in cats.

Chronic infections in mice used to feed experimental cats were produced with the Beverley and S-273 strains by inoculating subcutaneously 0.1 ml of peritoneal fluid from a mouse with an acute infection. For the KM strain, peritoneal fluid in graded dilutions was similarly inoculated subcutaneously into mice which received drinking water containing 50 mg% sodium sulfadiazine for protection. Some mice did not survive the infection even with this prophylactic treatment. With all three strains, chronic infections were later produced by intraperitoneal (ip) inoculation of washed oocyst suspension from cat feces of previously successful experiments. Infection in all strains was verified by a positive serologic test three weeks or more postinoculation, and fresh brain-squash preparations were examined microscopically for presence of cysts in most cases.

Sera of mice were tested with the indirect fluorescent antibody test using commercially prepared fluorescein-conjugated antimouse globulin of rabbit origin.* The toxoplasmas used as antigen were produced from mouse ascitic fluid 3 days postinfection and were washed 4 times prior to fixation in 1% formalin-saline, to eliminate mouse proteins likely to produce a nonspecific reaction.

A total of 25 domestic cats which were donated by families from a U.S. military housing area was used in this series. They ranged in age from 5 days to mature adults, and were of both sexes. Immediately before and during the experiments they were housed in individual steam-sterilized cages and fed commercial canned cat food. Fecal examinations were performed daily for several days prior to exposure.

Food was withheld for one day prior to feeding toxoplasma-infected mice. In most cases the skinned carcass of one mouse, including the head, was fed to each cat. With a few cats, a second mouse was fed on the succeeding day. Brain homogenate of an infected mouse was given to young kittens via stomach tube. Trophozoite infections were attempted in two cats by feeding livers and spleens of infected mice, and in two cats by means of peritoneal fluid and liver/spleen homogenate administered by stomach tube.

Feces of the cats were collected daily and checked for presence of oocysts by microscopic examinations of concentrates made by zinc sulfate or sucrose (sp gr 1.15) flotation technique. Regardless of microscopic findings, fecal concentrates were incubated in 2.5% potassium dichromate solution at room temperature (23–27 C) for a minimum of 6–10 days, and then washed in tap water and injected ip into six mice. The mice were observed daily for evidence of infection, and if no acute infection developed, tail blood was taken after 21 days for testing in the indirect fluorescent antibody test (IFAT). Mice with positive serologic tests were checked for tissue cysts by microscopic examination of brain-squash specimens.

Portions of intestine of cats killed at varying number of days after exposure were fixed in 10% formalin, sectioned at 7–10μ, and stained with hematoxylin and eosin to demonstrate the schizogonic and gametogonic stages.

Results

Oocyst production with the Beverley strain

* Colorado Serum Co.

Table 1 Microscopic findings and confirmation by mouse-inoculation of fecal excretion of oocysts by cats fed mice infected with three strains of *Toxoplasma gondii*

Strain	Cat			Stage Fed	Fecal Oocysts			Other Coccidian	Inf./Exp.**
					Stool	Mouse Inoc.			
	no.	age	sex			Toxo	IFA		
Beverley	1	adult	♂	cysts	+	troph.	*	—	8/8
	2	5 days	♀	cysts	+	cysts	+	—	
	3	3 m.	♀	cysts	+	troph.	*	—	
	4	8 w.	♀	cysts	+	cysts	+	—	
	5	8 w.	♀	cysts	+	—	+	—	
	6	8 w.	♀	cysts	+	cysts	+	—	
	7	adult	♂	cysts	+	troph.	+	*I. felis*	
	8	adult	♀	cysts	+	cysts	+	—	
S-273	9	adult	♀	cysts	+	cysts	+	—	5/5
	10	3 m.	♂	cysts	+	cysts	+	—	
	11	3 m.	♀	cysts	+	cysts	+	—	
	12	3 m.	♀	cysts	+	cysts	+	*I. felis*	
	13	adult	♂	cysts	+	—	+	—	
KM	14	2 m.	♀	troph.	−	—	−	*I. felis*	4/12
	15	2 m.	♂	troph.	−	—	−	*I. felis*	
	16	adult	♀	troph.	−	—	−	—	
	17	adult	♂	troph.	−	—	−	—	
	18	3 m.	♀	cysts	−	—	−	—	
	19	3 m.	♂	cysts	−	—	−	—	
	20	5 days	♂	cysts†	−	—	−	—	
	21	5 days	♂	cysts†	−	—	−	—	
	22	3 m.	♂	cysts	+	cysts	+	—	
	23	2 m.	♀	cysts	+	cysts	+	*I. felis*	
	24	3 m.	♀	cysts	+	cysts	+	—	
	25	adult	♀	cysts	+	cysts	+	*I. felis*	

* not done † not seen in brain squash
** no. of cats infected/no. of cats exposed

was observed in all eight cats infected (Table 1). The typical 10–12μ oocyst described by other investigators (Fig. 1) was observed in the feces in each case. In three cases mice inoculated with these fecal specimens developed ascites containing typical proliferative forms, and in four, cysts were seen in the brain and they developed antibodies demonstrated by the IFA method.

With the S-273 strain the results were also uniformly positive, and oocysts were seen on examination of feces in all five cats infected. Mice inoculated with fecal concentrates of these cats became serologically positive in all cases, and in four of the five, cysts were found in the brain.

Much less consistent results were encountered with the human KM strain, however. In only four of the eight cats fed chronically infected mice were oocysts seen on fecal examination, and confirmed by presence of brain cysts and positive serology

in inoculated mice. No oocysts were obtained from four cats fed with mice with acute infections.

In all three strains the time of appearance of oocysts in feces was quite consistent, 3 to 5 days after feeding the infected mice (Table 2). However, the period of time

Table 2 First day of patency and total duration of oocyst excretion in feces of cats fed mice with tissue cysts of three strains of *Toxoplasma gondii*

Strain	Cat no.	lst day of patency	Days excreted
Beverley	3	4	4
	4	5	1
	5	7	1
	6	5	7
	7	3	14
	8	5	4
S-273	9	3	6
	12	3	10
	13	3	10
KM	23	4	9
	24	5	8
	25	3	12

during which they could be demonstrated by examination of fecal concentrates was variable for the Beverley strain with extremes of 1 and 14 days. Of the three cats examined for each of the other two strains, there was less variation, with extremes of 6 and 10 days for S-273 and 8 and 12 days for the KM strain.

No difference in susceptibility of cats was observed on the basis of sex; both male and female cats were infected with each strain. Prior natural infection with another species of coccidian likewise did not seem of influence susceptibility to *Toxoplasma*. Cats excreting a large ($30\times40\mu$) oocyst (Fig. 3), considered to be *Isospora felis*, could be infected, and produced oocysts with each of the three strains (Table 1).

Tissue stages in the epithelial cells of the small intestine which correspond closely with those described and illustrated by others (Frenkel *et al.*, 1970; Hutchison *et al.*, 1970) were observed in great numbers in a kitten experimentally infected with the Beverley strain, while none were seen in litter-mate controls. Similar forms were seen in cats fed S-273 and KM strains and killed after they began to excrete oocysts. Unmistakeable schizonts (Figs. 4, 5), macrogametes (Fig. 6), and microgametes (Figs. 6, 7) were identifiable. However, in many cases the stage of development of the parasite could not be identified by us with certainty.

Discussion

There is no obvious explanation for the observed lower rate of oocyst production among cats fed the KM strain as compared with those receiving either the Beverley or S-273 strain. It is possibly related to the number of tissue cysts in the infected mice. Although no quantitation was attempted, fewer cysts were seen in the brains of the KM mice which had received drug protection to induce chronic infection than were seen in the other strains. In fact, none were seen in mice fed to two cats (Table 1), and infection was determined only by a positive IFA reaction. Oocysts from cat No. 22 were found to produce a chronic infection much more easily. Mice thus infected, and harboring more cysts, were used to feed cats No. 24 and No. 25. The immune status of the cats could also have a bearing on these results. It is unfortunate that no prefeeding serological results are available for these cats, and it cannot be determined if the refractive cats had had a prior natural exposure.

The experiments reported here were not designed to provide absolute proof that the intracellular parasites seen were developmental stages in the cycle of *T. gondii*. The meticulously controlled and executed studies of Frenkel and colleagues and the agreement of other studies provide convincing proof of the identity of these forms. The similarity to the forms in histologic

sections from the Beverley strain infection was accepted as evidence that the intracellular forms in cats which were excreting oocysts after exposure to S-273 and KM strains represented developmental forms of the schizogonic and gametogonic cycle in these strains.

After this study was completed, we learned of the paper by Witte and Piekarski (1970) which reported the intestinal cycle and infectious oocyst production in cats fed tissue cysts of a human strain from Germany. This adds additional evidence to the present study that the ability to undergo schizogony and gametogony in the feline host is characteristic of *Toxoplasma gondii* strains from a wide geographic area and variety of hosts.

Summary

Two strains of *Toxoplasma gondii* of Japanese origin, S-273 from swine and KM from a human, were investigated for the capacity to undergo gametogony and schizogony in the intestinal epithelium of the domestic cat. Tissue cysts in chronically infected mice of each strain, and of the Beverley strain used as a control, were fed to a total of 25 cats.

All of five cats fed S-273 strain, and four of eight fed KM strain, began to excrete typical *Toxoplasma* oocysts from 3 to 5 days later and continued for a period of from 6 to 10 days. These were shown to be infectious to mice after sporulation.

With both strains, cats which were killed while shedding oocysts were demonstrated to have intracellular parasites similar to those observed in infections with the control (Beverley) strain. Unmistakeable schizonts, macrogametes, and microgametes, as well as many unidentified stages were observed in histologic sections of intestinal epithelium.

This indicates the ability to undergo typical *Isospora*-type development in the feline gut is characteristic of strains from a wide geographic area, and of those causing human infections.

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日本産 *Toxoplasma gondii* の人体株および豚株の猫体内での schizont, gamete, oocyst の発育について

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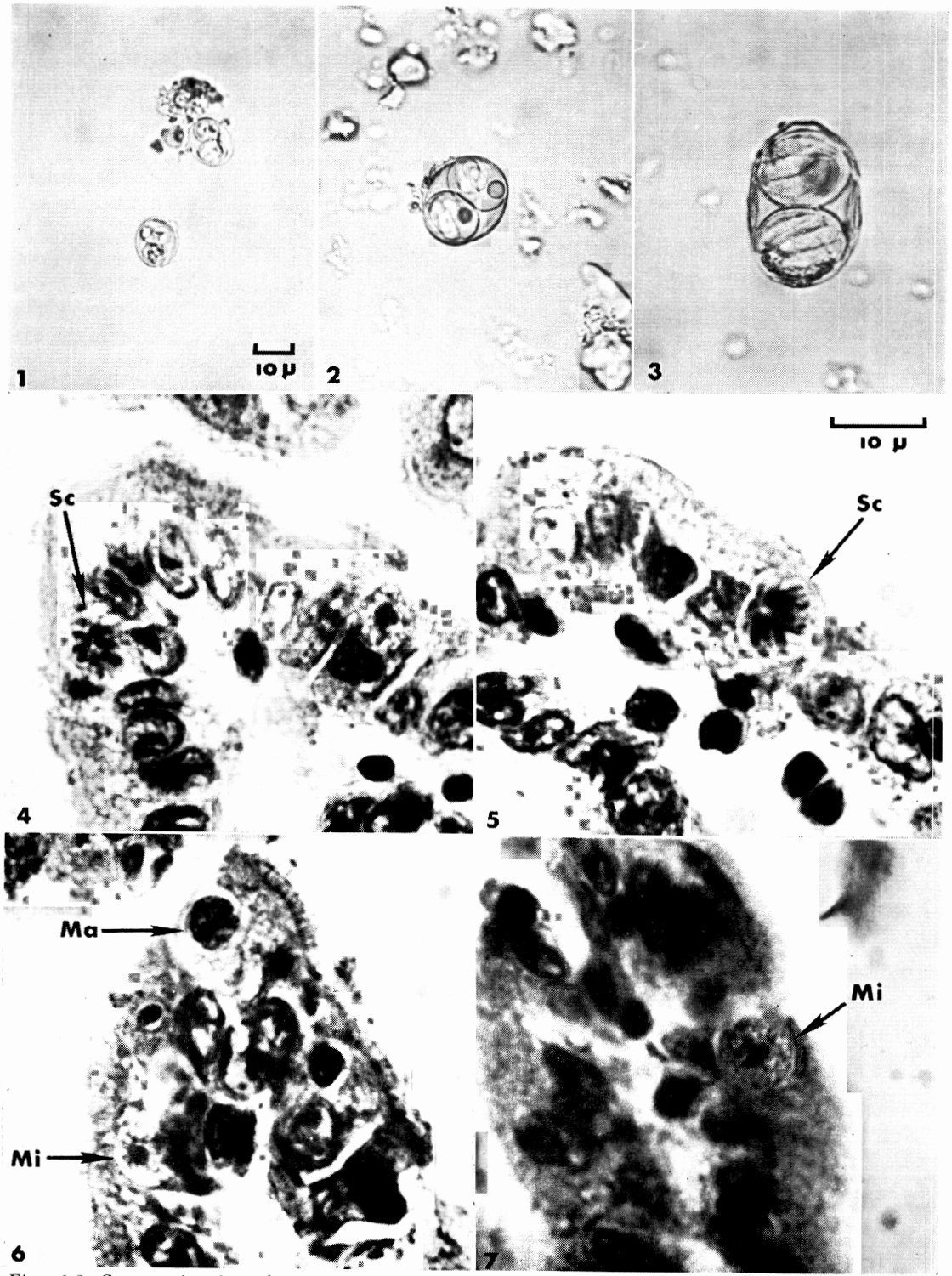
豚よりの S-273 株および，人体よりの KM 株の日本産 *Toxoplasma gondii* 2 株が猫の腸上皮細胞内で gametogony および schizongony の stage に発育するか否かについて検索を行つた．すなわち，それぞれの株に慢性的に感染しているマウスの組織内 cysts を，また対照群として Beverley 株に感染しているマウスの cysts を，合計 25 匹の猫に与えた．

S-273 株を与えた 5 匹の猫すべて，および KM 株を与えた 8 匹中の 4 匹が，投与後 3 日より 5 日の間に，典型的な *Toxoplasma* の oocyst を排出した．その後 6 日より10日後まで排出が続いた．更に，これら oocysts は，胞子形成後，マウスに感染することが判つた．

Oocysts を排出中の猫を剖検したところ，対照群の Beverley 株の感染においてみられたものとよく似た，細胞内原虫を両株について認めることができた．すなわち，schizont, macrogamete および microgamete などの stage 像が，多くの識別し難い stage 像と共に，はつきりと腸上皮細胞の組織切片に認められた．

この結果，猫の腸内において，典型的な *Isospora* type の発育が行われ得るということは，それが，広範囲の株の特長であるということ，および人体感染をおこす株の特長であるということを示している．〔特別掲載〕



Figs. 1-3 Comparative sizes of oocysts found in Japanese cats. Sporulated oocysts (×400) of *Toxoplasma gondii*, *Isospora rivolta*, and *Isospora felis* respectively.

Figs. 4-7 Intracellular forms (×1000) in intestinal epithelium of a kitten 7 days after feeding brain cysts of KM strain of *Toxoplasma*; schizont (Sc), macrogametocyte (Ma), and microgametocyte (Mi).