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ON THE GAMETOGONIC CYCLE OF *TOXOPLASMA GONDII*

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SUMMARY

A suspension of the crushed brains of albino mice, rich in cysts of *Toxoplasma gondii*, was administered through a gastric tube to four 25 days old kittens; on the 4th or 5th day thereafter, the kittens started to eliminate immature oocysts identical to those recently described for toxoplasma. The feces of one of the animals have been positive up to the 28th day.

The kittens have had positive dye tests and the parasite was re-isolated from their tissues. As for the mother cat, kept together with the infected litter and inoculated in the same manner 2 ½ months later, the dye test has been always negative and no toxoplasma oocysts were found in the stools.

The oocysts of toxoplasma became mature in about 4 days, when they contained 2 sporocysts, each with 4 sporozoites and a residual body. They were infective for albino mice, through the gastric route, 5, 7, 24 and 32 days after being shed.

INTRODUCTION

As probably did everyone else interested in trying to solve the problem of *Toxoplasma gondii* life cycle, we have been studying the infection in cats since HUTCHISON [3], in 1967, suggested transmission through the eggs of the nematode *Toxocara cati*. Our work led to: a study of the infection in cats with various strains of toxoplasma [7]; negative findings about the relationship of the nematode with the protozoon, thus confirming others' work [1, 5]; and, the reproduction of the recently described [2, 4, 6, 8, 9] gametogonic cycle. The characteristics of this cycle such as observed with one of our strains are here described.

MATERIAL AND METHODS

The strain of toxoplasma that went through the intestinal cycle in cats was registered as "AS-28" and was isolated in November 1969, from a wild brown mouse *(Mus musculus)* captured in our animal house. When killed the mouse had a positive dye test of 1:16,000. In laboratory albino mice infection by this strain has a tendency to become chronic, with the production of large numbers of cysts in the brains and high titer dye tests.

For the experiment we used a litter of 4 kittens aged 25 days, and their mother. They were kept together in an isolated pen, inside a large cage with bottom made of strips of wood, so that excreta could fall through on the cement floor of the pen. To collect feces, each animal was maintained separately in a smaller cage until he defecated.

Infection of the litter was obtained passing through a gastric tube a saline suspension (0.7 ml for each kitten) of the crushed brains of 2 mice inoculated 3 months before with strain "AS-28". Microscopical examination of the ingested material showed several toxoplasma cysts. The mother cat was not inoculated at the same time, but 2½ months later she was fed 1 ml of a similar material, rich in toxoplasma cysts, strain "AS-28".

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The feces were examined by Willis' floatation method before inoculation, almost daily during the first weeks after inoculation and less frequently when they became negative for toxoplasma oocysts. These were collected with a platinum loop from the surface of the feces suspension in saturated saline solution and kept in tap water with 1% formalin, at room temperature.

At necropsy, smears and sections of various portions of the small intestines and other organs were examined, in fresh preparations and stained by Giemsa's or eosin-hematoxylin, and tentatives were made to re-isolate the parasite from various tissues.

Albino mice fed upon, or inoculated with tissues or fecal material from infected cats were observed daily and killed when showing signs of illness, tissues and peritoneal washings being examined and inoculated into other mice. The survivors were killed one month after inoculation, their blood collected for the dye test, their brains ground, suspended in saline and examined for cysts. Passages into new mice were made only if the first were negative.

RESULTS

The whole feline family had negative fecal examinations prior to inoculation. On the 4th day thereafter, 3 of the inoculated kittens were passing immature oocysts identical to those recently described [2, 6, 8] for toxoplasma, and on the 5th day all kittens were positive (Fig. 1).

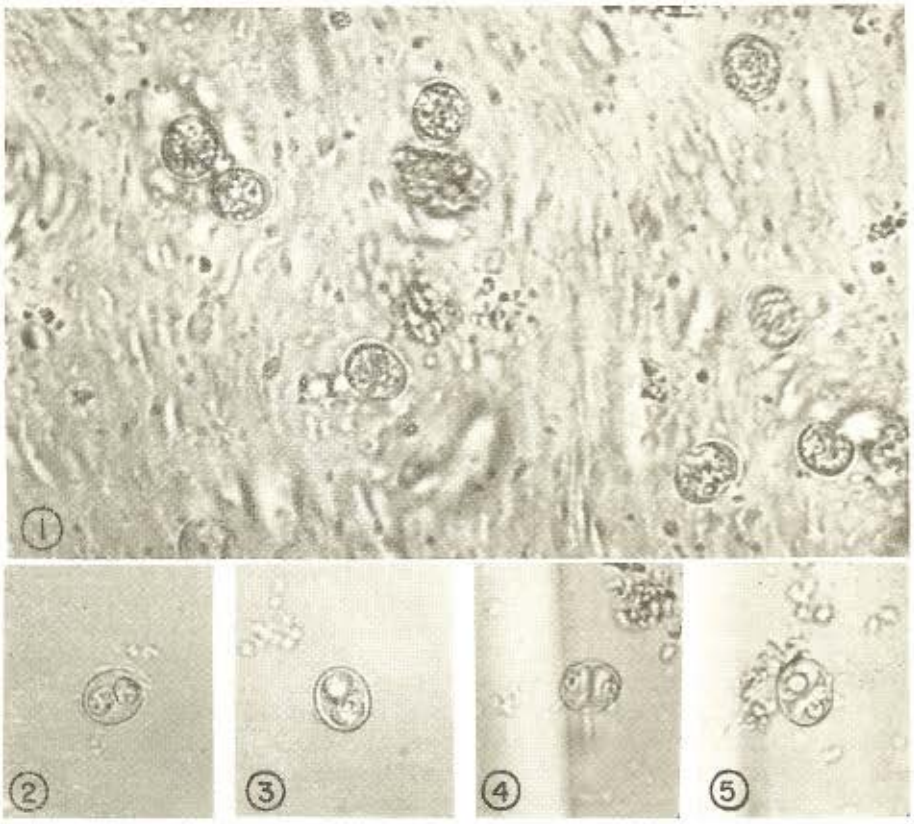

Fig. 1 — Immature oocysts in cat feces, Willis' floatation method. Figs. 2 and 3 — Oocysts with 2 sporocysts, 24 hours after conservation at room temperature, in 1% formalin. Figs. 4 and 5 — Mature oocysts, 4 days after conservation at room temperature. Fresh preparations, 800 X

One of the kittens was killed on the 7th day, a second died on the 13th day (apparently from causes other than toxoplasmic infection) and a third one died accidentally (after a heart puncture) on the 17th day; all 3 still had positive stools on the day of their death or on the day before. Fecal examination of the only surviving kitten has been positive up to the 28th day after inoculation, the number of oocysts varying much during this period.

From the 11th day on some oocysts of *Isospora felis* began to appear in the feces of the surviving kittens. The mother cat was later found also to harbour *I. felis* and was probably the source of infection for the young; she has been negative for toxoplasma oocysts, before and up to now, 15 days after her own inoculation.

The kitten killed on the 7th day had at this time a 1:4 positive dye test. The other 3 had a titer of 1:1024 on the 12th day and the only survivor showed again the same titer one month after. In the mother cat, the dye test — negative before — continues to be negative more than 2 months after the kittens inoculation and 15 days after she herself was fed infected material.

Toxoplasma has been re-isolated from the brains, lungs, mesenteric lymph glands (enlarged) and scrapings of the mucosa of the small intestines of all 3 dead kittens, through intraperitoneal inoculation in mice. In these animals the infection has been chronic and detected through the finding of cysts in the brains and high titer dye tests (up to 1:128 000) in mice killed one month or more after inoculation. Thus, after re-isolation from cats, strain "AS-28" still produced in mice an infection with the same characteristics found before.

After about 24 hours most oocysts kept at room temperature contained 2 sporocysts (Figs. 2 and 3) and they maturated between the 3d and 4th days, when each sporocysts had 4 sporozoites and a residual body (Figs. 4 and 5). Many oocysts, however, remained immature, especially those passed later in the infection.

Mature oocysts were fed to mice through a gastric tube, after 5, 7, 24 and 32 days at room temperature; in each of these groups of mice infection has been detected by the finding of toxoplasma proliferative or cystic stages in organs and/or the dye test.

In the duodenum of the kitten killed on the 7th day of infection large numbers of parasites were found within the epithelial cells of the villi; they probably represented the various stages of the toxoplasma cycle, since in this animal no oocysts of *I. felis* were found.

DISCUSSION

As already pointed out [6], the fact that cats are so often naturally infected with different coccidia makes the study of the whole cycle of toxoplasma in these animals quite difficult. It is probable that during our own studies on toxoplasmosis in cats [7], when the feces of the infected animals were regularly examined, we have misidentified the smaller coccidian oocysts.

In the experiments here described the oocysts found in the feces of the infected kittens were only of two types, one being the very large *Isospora felis* the other type was identical with the oocysts described for toxoplasma [2, 6, 8]. The fact that the assexual cycle characteristic of toxoplasma was reproduced in mice fed with fecal material from the kittens is a clear proof that the parasite was present in this material.

In our experiment the kittens only were susceptible to intestinal infection by toxoplasma. Although *I. felis* was spreading among the family, the mother cat apparently did not catch toxoplasma from her litter, nor did she present any evidence of infection after she was herself fed a large number of toxoplasma cysts. Her dye test has been, before that feed, negative, but based on observations described in another paper [7] we think that a negative test among these animals does not indicate absence of a previous infection. The great difference in titers between mice and cats inoculated with the same strain of the parasite is an indication that infection among the latter animals may be concomitant with a low titer dye test.

It is obvious that several aspects of the newly discovered cycle of *T. gondii* need to be clarified, one of these being the actual importance of cats in the spread of the in-

fection among humans and other animals, considering that cats appear to shed oocysts during so short a period in their lives and become resistant to re-infection.

RESUMO

Sôbre o ciclo gametogônico do Toxoplasma gondii

Os Autores reproduziram, em gatos, o ciclo gametogônico do *Toxoplasma gondii,* tal como foi recentemente descrito. A quatro gatinhos de 25 dias de idade fizeram ingerir, por meio de sonda gástrica, triturado de cérebro de camundongos albinos rico em formas císticas de toxoplasma, amostra "AS-28", recém-isolada pelos Autores de um camundongo cinzento silvestre *(Mus musculus).* No 4.° ou 5.° dia os gatinhos começaram a eliminar com as fezes pequenos oocistos característicos. Três dos animais inoculados foram sacrificados ou morreram acidentalmente no 7.°, 13.° e 17.° dias após a infecção, todos ainda eliminando diàriamente oocistos; no 4.° animal, conservado vivo, a eliminação prosseguiu até o 28.° dia, tendo sido os exames repetidamente negativos desde então.

A gata-mãe, não inoculada ao mesmo tempo, mas conservada junto com os filhotes, teve as fezes sempre negativas para oocistos de toxoplasma. Continuou negativa mesmo após ter sido inoculada do mesmo modo que os filhotes, dois meses e meio depois dêstes.

A reação do corante foi positiva a 1:4 no gatinho sacrificado no 7.° dia e a 1:1024 nos demais, do 13.° dia em diante. Na gata-mãe foi e continua negativa.

Os oocistos de toxoplasma, conservados à temperatura ambiente, amadureceram em cêrca de quatro dias, quando passaram a apresentar dois esporocistos, cada um com quatro esporozoitos e um corpo residual. Os oocistos infetaram camundongos por via digestiva, 5, 7, 24 e 32 dias depois de maduros, porém não houve nestes animais nenhuma evidência da existência de um ciclo esporogônico.

O toxoplasma foi recuperado dos tecidos dos gatinhos necropsiados, por passagem em camundongos.

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