and one had *T. cati* eggs appearing in the stool 2 days after completion of the experiment. No *T. cati* eggs were found during the experimental period. Therefore, the infective form of *Toxoplasma gondii* was presumably present in the flotation material and unassociated with nematode eggs.

In 16 cats with which transmission was achieved with or without *Toxocara cati* infections, infection was not produced by feces collected before day 4 nor after day 11 with two exceptions as noted above. This indicates that the parasite must remain at least 3 days in the cat before its transmissible form can be passed in the feces. The parasite must then remain outside the host for a minimum of 3 days before becoming infective to mice, since we have not been able to get transmission before day 3 of incubation. The transmissible forms are no longer present in the feces approximately 12 days after infection. Some parasites, collected and incubated, retained their infectivity for more than 100 days.

In previous reports (*1, 2, 4, 6*) the role of the *T. cati* egg has been discussed as a protective habitat for the toxoplasma organism during exposure to unfavorable conditions outside the host. Our results do not necessarily rule out the possibility of *Toxoplasma gondii* being transmitted through nematode eggs but show that transmission can occur in their absence. A new resistant form of *T. gondii* evidently develops in the cat. Further studies are necessary to identify this form and describe its development.

Note added in proof: Hutchison *et al.* (*7*) have reported fecal transmission of *T. gondii*. After being fed cysts of *T. gondii*, 2 of 21 *Toxocara*-free cats passed feces which, after concentration and incubation, produced toxoplasmosis in mice.

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References

1. L. Jacobs, *Adv. Parasitol.* **4**, 1 (1968).
2. W. M. Hutchison, *Nature* **206**, 961 (1965).
3. J. P. Dubey, *J. Protozool.* **14** (Suppl.) 42 (1967).
4. ———, *Vet. Bull.* **38**, 495 (1968).
5. Transmission of *Toxoplasma gondii* to mice through feces of a cat free of *Toxocara cati* was also observed by J. K. Frenkel and colleagues (personal communication).
6. W. M. Hutchison, *Trans. Roy. Soc. Trop. Med. Hyg.* **61**, 80 (1967).
7. ———, J. F. Dunachie, K. Work, *Acta Pathol. Microbiol. Scand.* **74**, 462 (1968).

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Toxoplasma gondii: Fecal Forms Separated from Eggs of the Nematode Toxocara cati

Abstract. *Cats excreted the protozoan* Toxoplasma gondii *in feces, generally between 5 to 12 days after ingesting mice with chronic toxoplasmosis.* Toxoplasma gondii *and eggs of the nematode* Toxocara cati *occurring together could be separated by washing them through sieves that retained the eggs. This finding negates the postulated role of* Toxocara cati *in the transmission of toxoplasmosis.*

Ever since Hutchison described transmission of *Toxoplasma gondii* through the cat ascarid *Toxocara cati* (*1*) we have tried to repeat his observations and to identify the forms of *Toxoplasma* within the nematode and its eggs. We found that several *Toxoplasma* strains, including one derived from the Beverley strain with which Hutchison worked, were not transmitted in the feces of cats. However, *Toxoplasma* strain M-7741, originally isolated from sheep, was recovered 41 times from the feces of ten cats that had been fed mice infected with *Toxoplasma* 2 to 18 days earlier. Between 12 and 15 days after the infectious meal, transmission became irregular. Mice fed fecal floats from cats prior to being fed *Toxoplasma* did not develop toxoplasmosis.

The feces, which generally contained *Toxocara* eggs also, were collected daily, and were subjected to flotation at specific gravity of 1.180 in a solution of zinc sulfate; the top layer was aspirated, diluted, and sedimented in tap water. Such sediments were maintained at 21° to 24°C in water without a preservative, and their infectivity was titrated by feeding to groups of two to six mice. The fecal preparations frequently caused death in 6 days, with *Toxoplasma* visible only in the lesions of advanced enteritis. Surviving mice developed antibody to *Toxoplasma*, as measured by the Sabin-Feldman dye test, and *Toxoplasma* cysts were found in the brain.

We noted that immature eggs and infertile eggs "transmitted" toxoplasmosis although devoid of the hatching enzymes normally secreted by the developed larva (*1*). Hence we titrated a fecal suspension with a known number of *Toxocara* eggs to determine their ability to transmit toxoplasmosis and found that a suspension containing one or two intact eggs produced toxoplasmosis in five out of six mice. The fact that an end point was not reached where expected suggested that *Toxoplasma* might not be "packaged" with *Toxocara* eggs.

Efforts to separate infectious fecal forms of *Toxoplasma* from *Toxocara* eggs were successful. By passing feces containing *Toxocara* eggs (60 to 80 μ) through a 44-μ U.S. standard sieve and flushing the retained portion with water, we obtained identical or ten times more *Toxoplasma* in the filtrate than in the retained portion. After straining feces through an 88-μ sieve, to remove assorted debris and fungal mycelia, we completely separated *Toxoplasma* from the accompanying *Toxocara* eggs by flushing one sample with running tap water for 5 minutes through a 44-μ sieve. The integrity of the sieve was indicated by the fact that it completely retained 40,000 eggs. The filtrate, 1.6 ml, examined drop by drop, contained between 20 and 200 *Toxoplasma*. In addition, 7000 larval *Toxocara* were completely separated from associated *Toxoplasma* by migration in agar. At the site of deposit 13,000 larvae remained with 10 to 100 *Toxoplasma*.

Feeding a *Toxocara*-free cat for three successive days with mice infected with *Toxoplasma* resulted in shedding of fecal forms of *Toxoplasma* at day 5 through day 14 thereafter (Table 1). Results were the same for two other cats. One of these cats shed *Toxoplasma* 2, 3, 5, 6, and 7 days after a contaminated meal.

The characteristics of one lot of fecal forms of *Toxoplasma* were compared. They were infectious to mice when administered orally, intraperitoneally, and subcutaneously. They were generally not infectious on the day that they were passed in the cat feces, but developed infectivity within 1 week. They could be stored at room temperature for at least 3 months. The fecal forms of *Toxoplasma* passed through 44- and 37-μ wire mesh sieves and Pyrex C fritted-glass filters (pore size, 40 to 60 μ) but were retained by a Pyrex M fritted-glass filter (pore size, 10 to 15 μ). Infectious fecal forms of *Toxoplasma* resisted 5 percent sodium hypochlorite for at least ½ hour and 1 percent formalin solution overnight.

Table 1. Infectivity to mice of fecal forms of *Toxoplasma*. The cat was free of *Toxocara* eggs before and after the experiment. It was fed three mice with chronic toxoplasmosis on days 0, 1, and 2. Abbreviations: T, *Toxoplasma* in sections; t, typical lesions only. Each letter refers to one mouse.

Day	Mice dead/ inoculated	Serologic test	Histopathology	Organ passage
5	1:3	+	t	+
6	3:3		tTT	+
7	2:3	+	tTT	+
8	3:3	+	ttT	+
9	3:3	+	TT	+
10	3:3	+	TTT	
11	3:3	+	TT	
12	2:3	+	TT	
13	2:3	+	TT	
14	0:3	+		

However, if placed in formalin on the day that they were shed, they did not develop infectivity, although the accompanying *Toxocara* eggs embryonated, in agreement with a previous report (*3*). Fecal forms of *Toxoplasma* were not infectious after drying on glass for 24 hours.

That we could separate *Toxoplasma* from *Toxocara* eggs by washing is contrary to Hutchison's experience. However, our findings indicate that fecal forms of *Toxoplasma* cling to remaining fecal debris and fungal masses and that considerable washing may be necessary to remove them. After we removed mycelia and large debris in an 88-μ filter, it was easier to separate *Toxoplasma* from *Toxocara* eggs in a 44-μ filter.

We recovered fecal forms of *Toxoplasma* from cats freed of *Toxocara* by piperazine. Jacobs obtained *Toxoplasma* from six fecal specimens devoid of visible *Toxocara* eggs (*2*). Dubey also observed transmission of *Toxoplasma* in 2 of 109 fecal samples free of *Toxocara* eggs (*3, 4*). But these were isolated findings in a mass of data where *Toxoplasma* was associated with *Toxocara* eggs. Furthermore, resistance to tap water, formalin, and sodium hypochlorite was incompatible with characteristics of known forms of *Toxoplasma* and suggested that the infectious form was inside the nematode eggs (*5*). We transmitted a compatible strain of *Toxoplasma* (M-7741) in association with *Toxocara* in only seven of 18 attempts and in only three of eight attempts in *Toxocara*-free cats; these results indicate that Hutchison's failure to transmit *Toxoplasma* in five of five *Toxocara*-free cats (*1*) is statistically not too improbable. At least two of Hutchison's cats were used twice. In this connection, we have observed transmission only once in each of seven cats fed repeatedly. Transmission occurred five of nine times on first attempt, twice in nine second attempts, and in none of two on third attempt. The presence of low antibody titers did not seem to interfere with fecal transmission; of all 15 cats used, four of five transmitted without antibody in undiluted serum, and six of ten transmitted even though they had titers of 1:64, 1:32 (twice), and 1:16 (three times), at, or close to the time of transmission. The five cats that did not transmit had titers of 0, 1:4, 1:16, 1:128, and 1:256 (original serum dilutions). None of the cats became sick.

Reinvestigation not only failed to confirm Hutchison's hypothesis (*1*) but provided a simpler theory of fecal transmission, confirmed independently in the accompanying report by Sheffield and Melton (*6*). By eliminating the role of *Toxocara*, as shown conclusively in our washing experiments, we can now refocus our investigations on identifying and characterizing *Toxoplasma* in the gut rather than the nematode egg.

Removing the worm from consideration should simplify analysis of the epidemiology of toxoplasmosis and might more easily provide clues to the variable incidence of infection. Among U.S. military recruits, this ranges from below 5 percent in the Pacific mountain states to over 25 percent in some eastern and southern states (*7*), and up to 100 percent in the population over the age of 30 in the lowlands of Guatemala (*8*). Transplacental transmission appears to account for only a minute proportion of human infections (*9*). Carnivorism could be important in infecting cats, and in their potential for fecal dissemination of *Toxoplasma*. Ingestion of raw or undercooked meat can infect humans (*10*). The relative importance of this mode of infection in humans remains to be assessed.

Note added in proof: Hutchison has now reported fecal transmission of *Toxoplasma* by 2 of 21 worm-free cats (*11*).

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References and Notes

1. W. M. Hutchison, *Trans. Roy. Soc. Trop. Med. Hyg.* **61**, 80 (1967).
2. L. Jacobs, *Advan. Parasitol.* **5**, 1 (1967).
3. J. P. Dubey, thesis, University of Sheffield, England (1966).
4. ———, *J. Protozool.*, in press.
5. ———, *ibid.* **14**, Suppl. 42 (1967); *Vet. Bull.* **38**, 495 (1968).
6. H. G. Sheffield and M. L. Melton, *Science*, this issue.
7. H. A. Feldman, *Amer. J. Epidemiol.* **81**, 385 (1965).
8. C. L. Gibson and N. Coleman, *Amer. J. Trop. Med. Hyg.* **7**, 334 (1958).
9. J. K. Frenkel, in *Comparative Aspects of Reproductive Failure*, K. Benirschke, Ed. (Springer, New York, 1967), p. 296.
10. G. Desmonts *et al.*, *Rev. Fr. Etud. Clin. Biol.* **10**, 952 (1965); B. H. Kean, A. C. Kimball, W. Christensen, *J. Amer. Med. Ass.*, in press.
11. W. M. Hutchison, J. F. Dunachie, K. Work, *Acta Pathol. Microbiol. Scand.* **74**, 462 (1968).
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Mycoplasma Membrane Lipids: Variations in Fatty Acid Composition

Abstract. *The fatty acid composition of the membrane polar lipids of* Mycoplasma laidlawii *B can be dramatically altered. These variations result in characteristic morphological changes, and in most cases the cells remain viable. This organism should provide a useful system for clarifying the role of fatty acyl chains in biological membranes.*

Chemical analysis of biological membranes derived from a wide variety of organisms has shown that the fatty acid composition of any particular membrane tends to be fairly specific under a variety of conditions (*1*). Changes in age, temperature, and chemical environment can cause alterations in the composition of a particular membrane but, in general, these alterations in fatty acid composition are not great (*1*). However, the lipid composition of the membrane of *Mycoplasma laidlawii* B can be regulated to an appreciable extent by the components of the growth medium (*2*). We now report that, by addition of high concentrations of exogenous fatty acids to a growth medium low in lipid content, *M. laidlawii* B cells which show striking variations in the fatty acid composition of the membrane polar lipids can be obtained. These variations are accompanied by characteristic changes in cell morphology. In this organism the polar lipids, which are localized almost exclusively in the cell membrane (*3*), consist of glucose phosphatides and