

References and Notes

1. The sample used was ESA reference standard methoxychlor consisting of 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane (89.5 percent) plus other isomers of methoxychlor and related compounds (10.5 percent) obtained through the courtesy of Dr. E. E. Fleck, U.S. Department of Agriculture.
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3. A. A. Nelson and G. Woodard, *A.M.A. Arch. Pathol.* **48**, 387 (1949).
4. B. Zondek and E. Bergmann, *Biochem. J.* **32**, 641 (1938).
5. The technical assistance of Donald Barber is gratefully acknowledged.

25 October 1960

Toxoplasma from the Eggs of the Domestic Fowl (*Gallus gallus*)

Abstract. Toxoplasmata of varying grades of virulence were isolated from eggs laid by clinically asymptomatic hens and from their internal organs. The strains so recovered were identified as *Toxoplasma gondii* by morphological, serological, cultural, and pathogenic criteria. These findings strongly suggest that toxoplasmosis may be contracted through the eating of raw or undercooked infected eggs.

Except for the report of Biering-Sorensen [cited by Siim (1)], who observed pseudocysts of *Toxoplasma* in the ovaries of naturally infected hens, indicating the possibility that eggs from such birds might be infected, we have been unable to trace any annotation referring to the isolation of the parasite from the eggs of the domestic fowl in the vast literature on toxoplasmosis, which now lists more than 2500 titles. Inasmuch as rapid clearing of tissues occurred in laying hens which received very heavy inocula, it seemed unlikely to Jones *et al.* (2) that the presence of *Toxoplasma* in the eggs of the hens would be anything other than an occasional finding. In view of the diver-

gent behavior of *Toxoplasma* in natural and experimental infections in chickens (2, 3), the problem of its localization in the eggs of naturally infected hens deserved a thorough study, which was undertaken by us during the course of our recent investigations into an enzootic of toxoplasmosis of fowls at one of the poultry farms in India.

Four of 42 eggs that have been screened to date were found to be infected, and extracellular and cyst forms of *T. gondii* were observed (Fig. 1) in scrape-smears of the chorioallantoic membrane. The identity of the parasite with *T. gondii* was confirmed by its transmission to mice and by serological tests. Surprisingly, none of the hens that laid these infected eggs had antibodies in their sera detectable by dye test, complement-fixation, precipitation, and indirect haemagglutination procedures. However, complement-fixation inhibition tests gave values ranging from 1:32 to 1:128.

Toxoplasmata were recovered from the diaphragmatic muscle, liver, brain, and ovaries of these asymptomatic hens when they were killed 2 weeks later. At necropsy, no gross lesions were seen in any of the internal organs but, interestingly, numerous cysts were demonstrated in crush-smears of the diaphragmatic muscle, liver, brain, and ovaries (Fig. 2) but not from spleen or lungs.

Immunologically, the isolates from laying hens were found to be identical with but pathogenetically different from the strains recovered from eggs. The egg-strains proved to be of low virulence for mice, and extracellular forms (Fig. 3) were detected in the peritoneal exudate of infected mice only by the third blind serial passage. The toxoplasmata recovered from the tissues of necropsied birds killed mice even in the first passage on or about the fifth day. Intracerebral inoculation of both strains into 1-week-old chicks

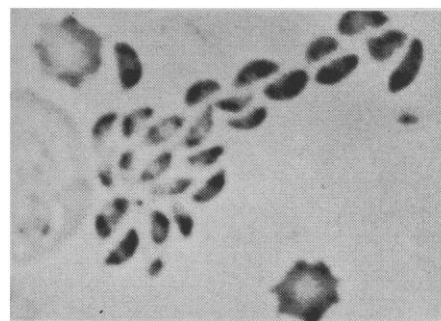


Fig. 3. Extracellular forms in the impression smear of peritoneal exudate of mice. Note the crenated erythrocytes (phase-contrast $\times 1500$). [May-Grunwald-Giemsa]

brought about their death with encephalitis in about 72 hours, while 10-week-old chicks that received heavy inocula by the subcutaneous route showed only mild transient parasitaemia and survived exposure. All the strains killed the embryos of 7-day-old embryonating eggs when they were inoculated directly into the yolk sac.

The susceptibility of embryonating hen's eggs to experimental infection with toxoplasma was established quite early (4), and today the chick embryo is considered to be the only available host in which parasites of low virulence can be maintained (5). But no natural infections have been previously described.

This is evidently the first report on the occurrence of toxoplasma in hen's eggs where a definite identification has been accomplished by morphological, serological, and animal recovery tests. Our data support the hypothesis that raw eggs could serve as sources of infections for human beings. This aspect of avian toxoplasmosis needs urgent and immediate attention; such an investigation is already in progress at this laboratory.

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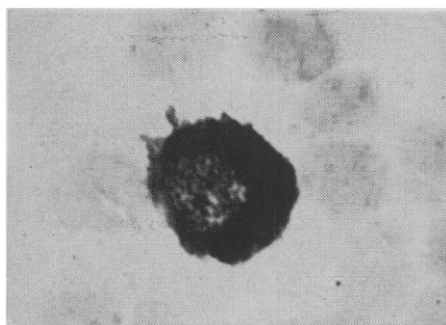


Fig. 1. Cyst stages of *Toxoplasma gondii* in the impression smear of chorioallantoic membrane. Note the absence of nuclei in the cyst wall (about $\times 1000$). [Giemsa]

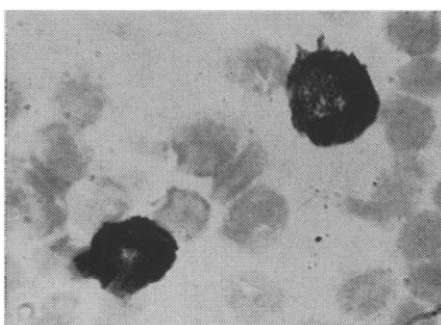


Fig. 2. Pseudocyst of *Toxoplasma* in the ovary of white Leghorn hen. Note the size that varies between 50 to 100 μ (about $\times 1000$). [Giemsa]