grant No. RG-7152, Division of General Medical Sciences, U.S. Public Health Service, and partly by the Puerto Rico Department of Health.

7. Larvae and pupae were taken by Michel Guion from a boat on 22 July 1960 at the Yacht Club, Boca de Cangrejos, Isla Verde, P.R.
8. As in previous tests 20 fourth instar larvae in 200 ml of solution in cardboard disposable containers were used (*4*). To obtain dilutions a previous scheme (*2*) was modified to involve only two tubes. Thus for Bayer 29493: tube 1, 1 gram 25 percent wettable powder plus 24 ml distilled water; therefore each milliliter contains 0.01 g. Bayer 29493. Tube 2, 1 ml of tube 1 plus 99 ml water; therefore each milliliter contains 0.0001 g. To obtain 2.5 ppm (0.00025 part per 100), add 5 ml of tube 2 to 195 ml water; and for 0.5 ppm (0.00005 part per 100) add 1 ml of tube 2 to 199 ml water.
9. Bayer 29493 was obtained from the Chemagro Corporation, P.O. Box 4913, Hawthorn Road, Kansas City 20, Mo. It is a 25 percent wettable powder, O-O- dimethyl O- [4-(methylthio)-*m*-tolyl] phosphorothioate, 25 percent; inert ingredients, 75 percent.
10. We thank Dr. W. E. Dove, Fairfield Chemical Division, P.O. Box 1616, Baltimore, Md., for piperonyl butoxide.
11. The value of a low ratio of synergist to toxicant has also been indicated in the case of Sevin and Sesoxane against resistant houseflies by M. E. Eldefrawi, R. Miskus, and W. M. Hoskins [*Science* **129**, 899 (1959)].
12. Larvae and pupae were collected by Arthur H. Boike, Jr., from rain barrels in Christiansted, St. Croix, V.I., 1 Oct. 1959.
13. R. W. Fay, *Proc. New Jersey Mosquito Exterm. Assoc. 46th Meeting, 1959*, p. 180.

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Uterotrophic Action of the Insecticide Methoxychlor

Abstract. Dusting of rats and mice, as well as oral treatment with the insecticide methoxychlor, produced uterine weight increase in these rodents. Ablation of the ovaries, adrenals, or pituitary did not alter this effect, thus indicating a direct trophic action of this agent on the uterus. It is apparent that the application of this insecticide to animals used for hormonal experiments introduces an additional variable.

During the past several months, marked uterine stimulation of unknown origin in immature female mice which had been obtained for use in hormone bioassay has been observed in this laboratory. After extensive examination and bioassay of diets, bedding, and several types of insecticide used in the animal production area, it was determined that one of the insecticide powders used for ectoparasite control contained the agent responsible for the uterotrophic effect. That this effect was not mediated through the ovaries was demonstrated by the marked uterine stimulation and concomitant vaginal opening induced in ovariectomized, immature mice and rats 3 days after these animals had received a single dusting with the insecticide. Each of the components of the insecticide was then tested by the dusting procedure and it was established that technical methoxychlor, an ingredient present at a level of 1.91 percent, produced the effects observed in the female genital tract.

In a series of quantitative studies that followed, graded doses of technical methoxychlor (*1*), in sesame oil solution, were administered orally to groups of ovariectomized mice (Table 1). The data indicate that technical methoxychlor stimulates uterine weight increase in the mouse. Similar data were obtained in the rat at higher dose levels. An unusual effect was noted in the vaginal cytology of methoxychlor-treated mice. Instead of the typical cornified vaginal smear induced by 3 days of estrogen treatment, the smear was characterized by large numbers of leukocytes. Continued treatment resulted in vaginal cornification. However, in the rat, treatment with the compound resulted in vaginal cornification although occasional leukocytes were seen. Because of the extremely weak activity of methoxychlor when compared with estrone on the basis of weight increase in the uterus of the ovariectomized mouse (0.02 percent or less), the possibility that the insecticide might act indirectly through the metabolic pathways of the adrenal cortex was investigated. However, uterotrophic effects produced by oral treatment with technical methoxychlor in adrenalectomized, ovariectomized rats (Table 1) did not differ from those observed previously in ovariectomized animals. Experiments carried out in female hypophysectomized rats further demonstrated that methoxychlor acts directly on the uterus rather than by way of anterior pituitary hormones (Table 1).

In order to compare the pituitary depressant with the uterotrophic potency of methoxychlor, its action on the anterior pituitary gonadotrophic response of the rat ovary was also tested in parabiotic rats. In castrated female and intact female rats placed in parabiosis at 28 days of age, a 30-mg total dose of technical methoxychlor, given daily by stomach tube for 9 days, resulted in a mean ovarian weight of 16 mg (six parabiotic pairs) as compared with a mean of 104 mg for untreated controls (five parabiotic pairs). Thus, in common with the estrogens and related compounds, this substance blocks the gonad-stimulating action of the anterior pituitary. This inhibitory effect may clarify earlier unexplained findings (*2*) of extraordinarily small tests in experimental rats (pair-fed) maintained on a diet containing 1-percent methoxychlor.

The known adrenocorticolytic effects of related insecticides such as DDD (*3*) suggested the possibility that methoxychlor might alter adrenocorticoid secretion. In two adult mongrel dogs treated with this technical material in daily oral doses of 200 mg/kg for 15 days, there was no unusual deviation from the normal range of values for adrenal venous 17-hydroxycorticoid secretion as measured by Porter-Silber chromogens. Moreover, no distinct morphological changes in the adrenal cortex were noted.

Highly purified *p,p'*-methoxychlor was also assayed for uterotrophic effects in the rat and mouse. This isomer was only half as active as the technical product in the rat and was much less potent in the mouse. Anisole, one of the starting materials in the synthesis of technical methoxychlor has been reported to have estrogenic activity (*4*). In view of this report, anisole was considered as a possible contaminant of the technical material. However, when anisole was administered orally to immature, ovariectomized mice in total doses of 15 mg (5 mg daily for 3 days), there was no effect on uterine weight.

These findings indicate that an unidentified isomer or contaminant of technical methoxychlor is responsible in part for the uterotrophic effect (*5*).

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Table 1. Uterotrophic effects of methoxychlor in the mouse and rat. Technical methoxychlor in sesame oil solution was administered daily for 3 days by stomach tube. Controls received sesame oil only. Body and uterus weight: mean and standard deviation. NIH general-purpose strain mice and Sprague-Dawley rats were used in these studies.

Animals (No.)	Total dose (mg)	Operative procedure	Final body wt. (g)	Uterine wt. (mg)
		Mouse		
9	0	Ovariectomy	9 ± 1	5.2 ± 0.6
10	0.5	Ovariectomy	10 ± 2	11.3 ± 3.3
9	1.0	Ovariectomy	9 ± 1	19.4 ± 3.7
10	5.0	Ovariectomy	10 ± 2	34.5 ± 6.7
		Rat		
10	0	Ovariectomy plus adrenalectomy	55 ± 4	24 ± 5
10	20.0	Ovariectomy plus adrenalectomy	54 ± 6	70 ± 5
10	0	Hypophysectomy	73 ± 4	17 ± 2
10	20.0	Hypophysectomy	70 ± 5	58 ± 4

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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5. The technical assistance of Donald Barber is gratefully acknowledged.

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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 divergent 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 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.

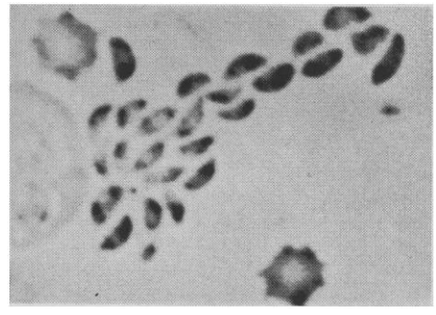

Fig. 3. Extracellular forms in the impression smear of peritoneal exudate of mice. Note the crenated erythrocytes (phase-contrast × 1500). [May-Grunwald-Giemsa]

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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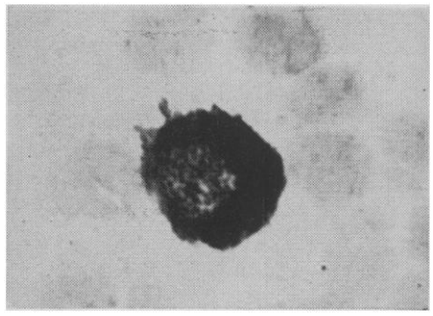

Fig. 1. Cyst stages of *Toxoplasma gondii* in the impression smear of chorioallantoic membrane. Note the absence of nuclei in the cyst wall (about × 1000). [Giemsa]

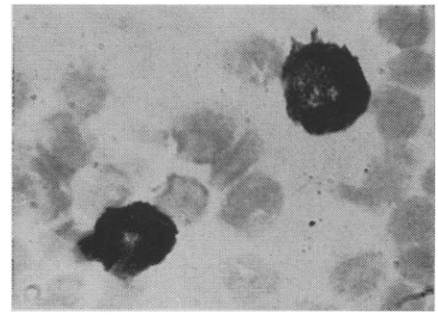

Fig. 2. Pseudocyst of *Toxoplasma* in the ovary of white Leghorn hen. Note the size that varies between 50 to 100 μ (about × 1000). [Giemsa]

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21 September 1960