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Cultivation of Toxoplasma in the Developing Chick Embryo.*

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Reports of the cultivation of toxoplasma in the developing chick embryo have appeared in the literature but these accounts are brief and give little experimental data. Levaditi, *et al.*,[1] and Wolfe, Cowen, and Paige[2] stated that inoculated fertile eggs hatched but that the chicks died within several days and their tissues were parasitized. Weinman[3] found that numerous small white nodules developed on the chorio-allantoic membrane after chorio-allantoic or yolk sac inoculation. In the above investigations recently isolated strains of toxoplasma were used for egg inoculation; deaths appeared to have been infrequent for they were not mentioned specifically. The data to be presented demonstrate that a strain of human origin, well adapted to mice, not only readily infected eggs but also produced such uniform embryo deaths as to make the embryonated egg a satisfactory titration medium.

The "R.H." strain of toxoplasma isolated by Sabin[4] from a fatal human case of encephalitis was used in these studies. It was

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1 Levaditi, C., Sanchis-Bayarri, V., Lepine, P., and Schoen, R., *Ann. de l'Inst. Pasteur*, 1929, **43**, 673.

2 Wolfe, A., Cowen, D., and Paige, B. H., *J. Exp. Med.*, 1940, **71**, 187.

3 Weinman, D., *Puerto Rico J. Pub. Health and Trop. Med.*, 1944, **20**, 125.

4 Sabin, A. B., *J. A. M. A.*, 1941, **116**, 801.

maintained in this laboratory by continuous intracerebral passages in mice and the 304th to 344th passage material was used for initiating the cultures in embryonated eggs. Fertile hen's eggs from pullorum-tested flocks were inoculated into the yolk sac. Incubation of the eggs both before and after inoculation was at 37°C. LD_{50} titers in mice and chick embryos were calculated according to the method of Reed and Muench.[5]

Injection of toxoplasma-infected, mouse brain suspensions into the yolk sac of embryonated eggs on at least 6 different occasions led to multiplication of the parasites with resulting death of the embryo in 4 to 10 days after inoculation. Examination of the dead embryos revealed the presence of numerous yellow-white plaques, 0.5 to 3 mm in diameter, on the chorio-allantoic and amniotic membranes (Fig. 1). The areas surrounding the lesions were thickened and histologically represented regions of dense cellular infiltration containing numerous toxoplasma (Fig. 2). Smears of the chorio-allantoic membrane and the yolk sac stained with Wright's stain revealed numerous toxoplasma both free and intracellular.

Three series of egg passages were studied in order to seek evidence of adaptation or change.

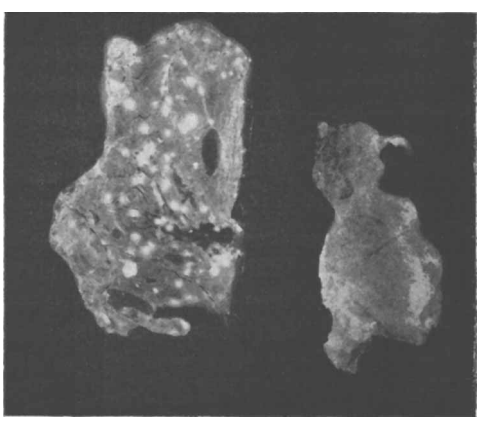

FIG. 1.
Toxoplasma in the developing chick embryo. Eggs 18 days old.
Left. Infected chorio-allantoic membrane.
Right. Normal chorio-allantoic membrane.

[5] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

One series was carried through 27 consecutive passages in 10- or 11-day-old fertile eggs (Table I). A second series was carried through 5 passages in 9- or 10-day incubated eggs, and three egg passages were done in which 6- or 7-day incubated eggs were utilized. No evidence was obtained that continuous or multiple egg passages altered the length of time between inoculation and death of the embryo. Most of the deaths occurred between the 5th and 6th days. Titrations performed in mice showed that LD_{50} titers for the various egg passages were about the same. Titrations of the various egg tissues revealed that while the chorio-allantoic membranes contained the greatest number of parasites the yolk sacs had only slightly less. Organisms were present in small number in the embryonic fluids (LD_{50} titers of $10^{-2.0}$ to $10^{-3.0}$) and the viscera of embryos (stained smears). Membranes harvested either from dead or living chick embryos at the time of the maximum number of embryo deaths possessed high titers (LD_{50} titers of $10^{-4.5}$ to $10^{-5.0}$) while the membranes of living eggs harvested two days before this maximum time gave lower titers ($10^{-3.5}$).

Although toxoplasma multiplied well in fertile hen's eggs between 6 and 11 days of age deaths in the younger embryos were spread over a larger number of days, *i.e.*, 4 to 8 days.

Several comparative titrations were performed in mice and chick embryos and the results are listed in Table II. In 3 of the tests higher LD_{50} titers were obtained in chick embryos. In these cases the amount injected into eggs was proportionately larger. However when similar inocula were used, *i.e.*, 0.5 ml per egg via the yolk sac and 0.5 ml per mouse intraperitoneally, the LD_{50} titers were identical. When the actual number of LD_{50} doses per gram of tissue were calculated the values obtained in three of the tests were slightly higher in mice than in chick embryos.

Other studies revealed that storage of infected membranes at 4°C yielded viable organisms for periods up to one month. Attempts to cultivate toxoplasma in the pooled embryonic fluids from 17-day fertile eggs were

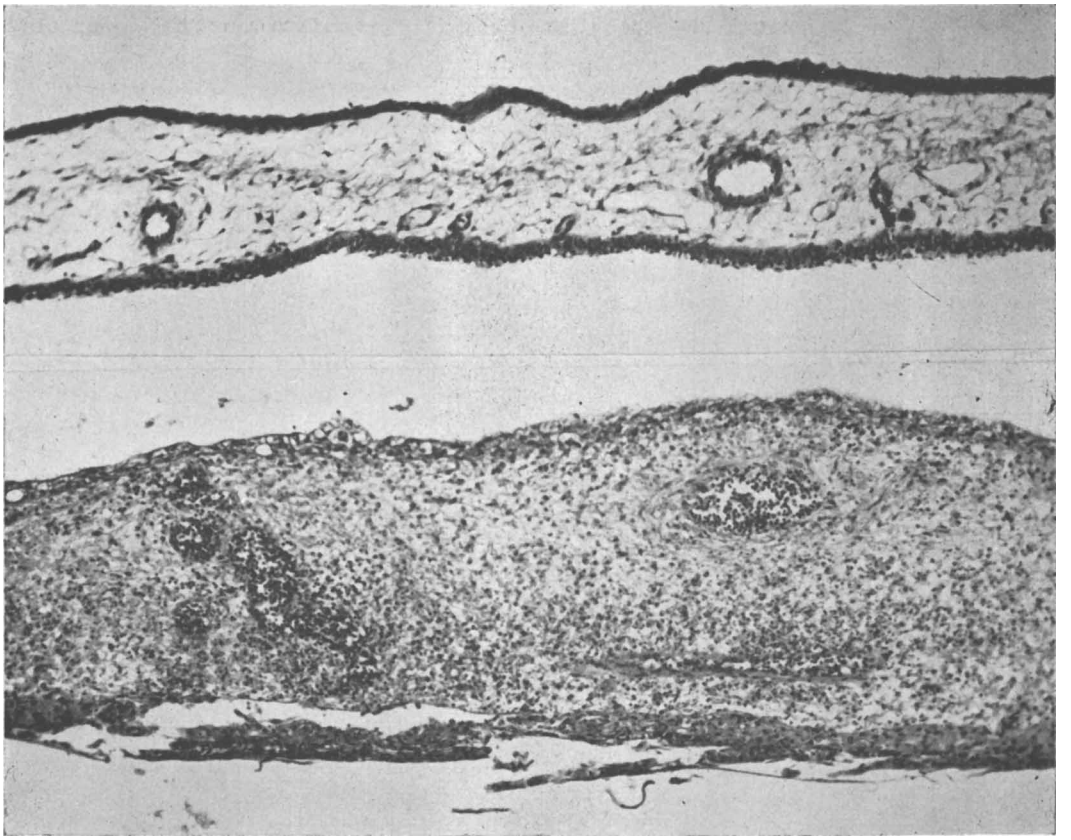

FIG. 2.
Toxoplasma in the developing chick embryo. Eggs 18 days old. H. & E. ×160.
Upper. Normal chorio-allantoic membrane.
Lower. Infected chorio-allantoic membrane.

TABLE I.
Toxoplasma Passages in Eggs Inoculated via the Yolk Sac.
0.5 ml per egg in 10- and 11-day-old embryonated hen's eggs.

Egg passage	Tissue suspension employed	Day of death	Intracerebral LD_{50} titers in mice		
			Yolk sac	Chorio-allantoic membrane	Membrane pool*
1	10% mouse brain	5,5,6,6,6,6,7,7,7,7	$10^{-3.5}$	$10^{-4.0}$	
4	5% Y.S.-C.A.*	6,6,6,6,6			$10^{-4.5}$
7	10% " "	5,6,6,6			$10^{-5.5}$
10	" " "	4,5,6,6,6,6,6,6,6			$10^{-5.0}$
12	" " "	4,4,5,5,5,5,5,5			$10^{-4.5}$
15†	" " "	6,6,6,10			$10^{-4.0}$
20	" " "	5,5,5,5,6,6,6,6			$10^{-5.0}$
27	" " "	6,6,6,6,6	$10^{-3.5}$	$10^{-4.5}$	

* Pool of yolk sac and chorio-allantoic membrane.
† Inoculated with membranes which had been stored 5 days at 4°C.

unsuccessful. More than 13 passages through eggs did not result in any obvious modification of pathogenicity for animals. Inoculation of infected embryonal tissues caused disease in mice, rats, chicks, rabbits, and a rhesus monkey. Preliminary results indicated

TABLE II.
Comparative Titrations of Toxoplasma Infected Tissues in Mice and Developing Chick Embryos. 10-day chick embryos inoculated via the yolk sac. Mice inoculated intracerebrally.

Passage	Inoculation: Tissue	Inoculation: Amt, ml	Titration in:	LD_{50} titer	LD_{50} doses per g of indicated tissue, × 1000
335	Mouse brain	.5	Embryos	$10^{-5.0}+$	200+
		.03	Mice	$10^{-4.5}$	1,000
336	" "	.5	Embryos	$10^{-5.0}$	200
		.03	Mice	$10^{-4.0}$	330
344	" "	.5	Embryos	$10^{-4.5}$	64
		.5*	Mice	$10^{-4.5}$	64
15	Y.S.-C.A.	.5	Embryos	$10^{-4.0}$	20
		.03	Mice	$10^{-3.0}$	33

* Mice in this case injected intraperitoneally.

that neutralization tests could be performed in the chick embryo.

The embryonated hen's egg may be an adjunct for the primary isolation of toxoplasma and although inoculation of eggs with the laboratory-adapted strain gave uniformly good results the probability is that for the initial recovery of strains the mouse is still the animal of choice.

Summary. A laboratory-adapted strain of toxoplasma was successfully propagated in the embryonated hen's egg. Uniform mortality was obtained and the majority of deaths occurred between 5 and 6 days after inoculation. The concentration of organisms in the chick embryo gave LD_{50} titers of $10^{-4.5}$ to $10^{-5.0}$ which approximated that usually attained in mice. Repeated passage through eggs resulted in no modification of pathogenicity for animals.

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Histochemical Differentiation Between Esterases.*

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Strikingly different properties of esterases (lipases) obtained from various species[1,2] have been reported by a number of workers. The differences include substrate specificity,[3] stereochemical specificity,[4] behavior towards activators and inhibitors, and pH optima.[5] Owing to multiple overlapping of differences and similarities between enzymes obtained from various sources, it is still undecided whether the various effects are due to the existence of several well defined individual enzymes or to the presence of unidentified accompanying substances.

With a histochemical method for the visualization of sites of lipase activity, similar differences between the esterases of various organs were found.

Experimental. Organs of 8 freshly killed white mice (5 males and 3 females) were fixed in chilled acetone, dehydrated, embedded

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1 Rona, P., and Bach, E., *Bioch. Z.*, 1920, **11**, 166.

2 Gyotoku, K., *Bioch. Z.*, 1928, 1939.

3 Falk, K. G., Noyes, H. M., and Sugiura, K., *J. Biol. Chem.*, 1924, **59**, 183.

4 Rona, P., and Ammon, R., *Bioch. Z.*, 1927, **181**, 49.

5 Davidsohn, H., *Bioch. Z.*, 1913, **49**, 249.