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Infectivity of *Hemophilus pertussis* for the Chick Embryo.

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For a long time the study of *Hemophilus pertussis* infections in the laboratory was hampered by the lack of a suitable animal of high susceptibility. White mice have been used frequently as the experimental animal, but in this species fatal infections are not regularly obtained through the usual routes of inoculation unless very large numbers of *H. pertussis* are administered. Recently Kendrick[1] has found that by the intracerebral route a much smaller dose of organisms will suffice to initiate fatal infection and this observation has been confirmed by other workers in studies on the chemotherapeutic activity of streptomycin[2] and the antigenic potency of vaccines derived from fluid cultures;[3] the LD_{50} in their experiments appeared to be a few hundred organisms.

In the course of studies which revealed the susceptibility of the developing chick embryo to a large variety of viruses and bacteria, Gallavan and Goodpasture[4] showed that *H. pertussis* would produce infection in 11- to 13-day embryos following introduction via the chorio-allantois or amnion. They did not attempt to study the quantitative aspects of the infection but did note that animals surviving 6 days or longer occasionally yielded positive heart's blood cultures. With this in mind, we thought it might be worthwhile to examine the susceptibility of the chick embryo to infection via the yolk-sac or allantoic cavity since these routes have proved so valuable in the investigation of other pathogenic agents.

Materials and Methods. The experiments were carried out with *H. pertussis* strain 934 obtained through the kindness of Dr. P. Kendrick of the Michigan State Department of Health. Cultures were propagated on sheep blood potato-infusion agar plates; typical hemolysis and other phase I characteristics were maintained throughout. After incubation for 48 or 72 hours at 37°C, the growth was removed and serial dilutions prepared in N-Z casein digest broth. To determine the number of viable organisms in the inoculum 0.1 ml of suitably diluted suspension was distributed over the surface of plates of the Bordet-Gengou medium with the aid of a glass spreader. Colony counts were made after 72 or 96 hours' incubation at 37°C.

Incubated fertile eggs were obtained from a local hatchery; no effort was made to secure eggs from a single breed of hen. For the yolk-sac route 0.5 ml of a given dilution was employed as inoculum; 0.1 ml was injected in the allantoic cavity. The eggs were subsequently incubated at 34-35°C and candled daily to determine the viability of the embryo. When the eggs were opened, cultures and smears stained by Wright's method were made from the yolk and allantoic fluid. Cultures of amniotic fluid, tracheal fluid, and heart's blood were also made from time to time.

Experimental Data. When fertile eggs of 7 to 11 days' preliminary incubation were infected via the yolk-sac with a small number (20 or less by plate count) of viable *H. pertussis*, death of the embryo usually resulted 6 to 9 days after inoculation. At autopsy, smears and cultures of the yolk showed numerous organisms. Occasionally small numbers of *H. pertussis* were recovered

[1] Kendrick, P., unpublished data, cited by Hegarty *et al.*[2]

[2] Hegarty, C. P., Thiele, E., and Verwey, W. F., *J. Bact.*, 1945, **50**, 651.

[3] Cohen, S. M., and Wheeler, M. W., *Am. J. Pub. Health*, 1946, **36**, 371.

[4] Gallavan, M., and Goodpasture, E. W., *Am. J. Path.*, 1937, **13**, 927.

TABLE I.
Infectivity of *H. pertussis* via the Yolk Sac in the Chick Embryo.

Inoculum				
Source	Dilution	No. of viable *H. pertussis*	Age of eggs when infected (days)	Results*
B-G culture	10^{-7}	144	7	D6, D7, D8
			11	D7, D9, 0
	10^{-8}	14	7	D6, D8, 0
			11	D6, D7, 0
	10^{-9}	1.4	7	D8, 0, 0
			11	D8, 0, 0
Infected yolk	10^{-8}	15	8	D5, D8, D9, D9
	$1/3x10^{-8}$	5	8	D6, D7, D8, D8, D9
	$1/9x10^{-8}$	1.7	8	D5, D6, D6, S10, 0
Infected allantoic fluid	10^{-9}	2	8	D8, D9, D9
	$1/3x10^{-9}$	0.7	8	0
			12	0, 0

* Results:
D_6 signifies death after 6 days' infection with many *H. pertussis* in yolk.
0 signifies no evidence of infection.
S_{10} signifies survival for 10 days, with positive culture from yolk.

in cultures from the heart's blood and amniotic fluid; none were found in the allantoic fluid. The heavily-infected yolk of embryos succumbing to infection was diluted in broth and titrated by the same route in other embryos. As few as 5 organisms deriving from the yolk were found to be capable of initiating a fatal infection. Table I summarizes experiments which exemplify these results.

H. pertussis from cultures on Bordet-Gengou medium or from heavily-infected yolk were also introduced via the allantoic cavity of 10- or 11-day embryos. A considerably larger number of organisms, usually at least several thousand, was necessary for the initiation of a fatal infection and deaths were infrequent. In those embryos succumbing, however, many organisms were seen in smears of the allantoic fluid and cultures revealed them to be present also in the heart's blood and tracheal fluid. *H. pertussis* in the allantoic fluids proved to be of high virulence when tested by the yolk-sac route (Table I).

Conclusion. The chick embryo is highly susceptible to *H. pertussis* when infected via the yolk-sac. The results of inoculation via the allantoic cavity are much less satisfactory. The chick embryo would seem to promise considerable utility in the quantitative experimental evaluation of antibody preparations and chemotherapeutic compounds versus *H. pertussis*.

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Prothrombin Level and Effect of Vitamin K Substitutes in Thrombocytopenic Purpura in Rats.*

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The administration of antiplatelet serum to animals produces purpura, a prolonged bleeding time and a low capillary resistance. These abnormalities are generally attributed

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