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Cultivation of *Trypanosoma equiperdum* in Yolk-Sac of Developing Chick Embryo.

E. ALTURE-WERBER. (Introduced by Geoffrey Rake.)

From the Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.

Chabaud[1] cultivated *Trypanosoma rhodesiense* on the chorio-allantoic membrane of 7- to 10-day-old chicken embryos using citrated or defibrinated mouse blood containing about 50 trypanosomes per field. All of the eggs inoculated with the citrated blood became infected, but only 50% of those inoculated with the defibrinated blood. Trypanosomes could be found 96 hours after inoculation and the number of them increased until the 6th day, whereafter they diminished, and finally disappeared by the 9th day. The average life time of the embryo was 7 days, but some survived for 9, 10, and 11 days. Chabaud also was successful in cultivating *T. brucei* and *T. equinum* in the egg. With both, the embryos first showed infection in 86 to 90 hours and lived an average of 7 to 9 days. With *T. lewisi* he could not obtain any infection of the chick embryo although he incubated the infected eggs for 5 to 15 days. Longley, Clausen, and Tatum[2] inoculated *T. rhodesiense* into the allantoic cavity of 8- to 10-day-old chicken embryos. The embryos usually died 5 days after inoculation with a heavy infection in the blood. These authors were able to transfer this strain through 8 subcultures by using blood diluted with 0.9% saline during 41 days. They were also able to cultivate *T. equiperdum, T. brucei, T. evansi,* and *T. hippicum* in the same way. These trial experiments were carried on for 15 days. Oag[3] inoculated series of chick embryos, in age from 8 to 12 days, with *T. brucei, T. congolense* and *T. equiperdum.* Neither with *T. congolense* nor *T. equiperdum* could the protozoa be demonstrated after incubation. Seven eggs infected with *T. brucei* showed living trypanosomes 3 to 4 days after incubation. Blood of these positive eggs passed to another set of eggs gave no results. He assumed that his results were due to survival of the protozoa during the 3 to 4 days' incubation. No details of method are given.

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1 Chabaud, A., *Bull. Soc. path. exot.*, 1939, **32**, 489.

2 Longley, B. J., Clausen, N. M., and Tatum, A. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 365.

3 Oag, R. Knight, *J. Path. and Bact.*, 1940, **51**, 137.

In connection with studies on the effect of arsenical compounds in syphilis and trypanosomal infections the possibilities of cultivating *T. equiperdum* in the egg were investigated. A series of experiments was made using 6- to 10-day-old chick embryos. The infection was performed by injection of citrated mouse blood containing *T. equiperdum* diluted with infusion broth into the yolk sac of the egg. In the first experiment a dose of 0.5 cc diluted mouse blood, containing 1 to 2 trypanosomes per microscopic field (magnification 98×7.5) was used. In the following experiments a counted number of trypanosomes were inoculated. It was found that *T. equiperdum* inoculated in the egg could only be demonstrated subsequently if inocula containing 500,000 or more protozoa were used. Of the different dosages used 2 million trypanosomes per egg in ½ cc broth (or 4 per field) gave the most satisfactory results.

When 2 million *T. equiperdum* per egg were used, 14 out of 30 eggs died between the 7th and 11th day. Between the first and 10th day after infection 16 eggs were opened, of which 2 were negative both microscopically and by mouse test. The infected eggs opened 1, 2, and 3 days after inoculation did not reveal any trypanosomes microscopically in the embryo blood, or blood of the chorio-allantoic membrane vessels. (The mixture of allantoic fluid and blood is referred to as fluid in this paper.) Most mice injected with either the yolk or fluid of these early opened eggs became infected and died between 7 and 10 days. Among 4 eggs opened 4 and 5 days after inoculation 2 eggs were negative microscopically both in the yolk and in the fluid, while the other 2 showed trypanosomes in the fluid but none in the yolk. The 2 negative fluid specimens killed mice in 5 days and the 4 yolk specimens killed mice in 7 to 13 days with infection due to *T. equiperdum*. Eggs opened 7 days after inoculation had 12 to 21 parasites per field in their blood. The yolk of these eggs killed mice in 7 to 14 days. Four eggs opened 8 and 9 days after infection showed between 5 and 25 parasites per field in the fluid. Mice inoculated with the yolk of the last mentioned eggs died in 5 to 7 days. Occasionally trypanosomes were found in wet and stained yolk preparations of infected eggs. In all the experiments the consistency of the yolk made the microscopic demonstration of trypanosomes very difficult. Death of the chick embryo was usually associated with hemorrhages in the brain, liver, and pericardium. Once a large hemorrhage occurred on the chorio-allantoic membrane. Twice hemorrhages were found in living embryos. If embryos were examined shortly after death trypanosomes could be found by microscopic examination. However, a

few hours later the infection could be traced only by mouse passage and if too much time passed before inoculation in mice, no infection in mice could be obtained. Sometimes it took 15, 18, 29, and once 35 days to kill mice with *T. equiperdum* infection.

An inoculum of one million trypanosomes per egg also caused infection in the embryo. High numbers of trypanosomes were found in the blood of the embryo and in the fluid. The embryos died between the 8th and 11th day after infection. Twelve eggs infected with 500,000 trypanosomes gave the following results: nine embryos died between the 9th and 11th day with hemorrhages in the brain and in the pericardium, while 2 eggs opened on the 9th and 11th day did not show any infection microscopically. Unfortunately no mouse passages were performed in this experiment. One egg opened on the 12th day had numerous trypanosomes in its blood and fluid. The fluid count was 198 million per cc. Eggs infected with 150,000 trypanosomes and less showed no infection either microscopically or in mouse passage, although a great number of eggs died during the experiments.

Summary. *Trypanosoma equiperdum* can be cultivated in the developing chick egg by inoculation of the infected material into the yolk sac of the egg. An inoculum of 2 million trypanosomes per egg gave satisfactory results. In 93% of infected eggs microscopically visible infection occurred, or infection could be demonstrated by mouse passage. When trypanosomes could not be found microscopically in the egg, mice inoculated with the egg specimen usually became infected. Sometimes it took 14 to 35 days to have such mice die with *T. equiperdum* infection. Trypanosomes which did not infect the embryos seemed to have been destroyed or retained in the yolk sac. Death of infected embryos was accompanied with hemorrhages in the brain, liver, and heart. Inocula of 500,000 and one million trypanosomes caused infection in the egg. With inocula of 150,000 or less organisms satisfactory results were not obtained. Repeated passages in the egg do not change the virulence of *T. equiperdum*. To maintain strains in eggs it is necessary to inoculate a sufficient dose, *i. e.*, 2 million organisms, to use several eggs, and to transfer once a week. The advantages of the above method are: 1. The injection into the yolk sac is a simpler method than injection on the chorio-allantoic membrane. 2. By injection into the allantoic cavity the embryo is killed in (some) 5 days and frequent transfers are necessary. The injection into the yolk sac retards the process of infection in the embryo.