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Malarial Infections in the Duck Embryo.

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Infection of duck embryos with *Plasmodium cathemerium*, *P. lophuræ*, and *P. elongatum* has been reported.¹ In the present work only the first two species were used in attempts to produce standardized, highly-reproducible infections. After intraembryonic inoculation of infected duck blood the 11- to 16-day duck embryos were incubated at 37°C or 40°C. Inoculation doses ranged from about 12 to 50 million parasitized cells per embryo. (Intravenous injection of such numbers of either species of *Plasmodium* into ducklings produces infections with no prepatent period. A high immediate mortality (50%) within 2-3 days follows intraembryonic injection of as little as 0.05 ml of any material.) A total of 72 embryos was inoculated after various preliminary experiments.

A prepatent period of variable length occurred in the embryo. This depends probably on dosage, site of inoculation, temperature of incubation, and especially the species of parasite. It was consistently longer with *P. lophuræ*. This difference in rate of reproduction of the two species is true for ducklings as well as for the duck embryo. If permitted to run its course, *P. cathemerium* regularly killed the embryo in from 6-11 days with evidence that a high degree of parasitemia occurred (up to 50% of the erythrocytes). With inocula of 20 to 25 million parasitized cells per embryo and incubation at 37°C death occurred regularly on the 10th or 11th days. Both species can develop in the embryo at either 37°C or 40°C. The earliest embryonic deaths with *P. cathemerium* occurred at the higher temperature. The *P. lophuræ* infection developed more slowly and even 14 days

after inoculation did not show the intense parasitization of the embryo regularly seen in the duckling. For the purposes of this work *P. lophuræ* therefore was not a satisfactory parasite.

An embryo of 19 days incubation still shows an occasional primitive erythrocyte of yolk sac origin. Two embryos of this age exposed to similar temperatures of incubation, size of inoculum and level of parasitization (18 and 27% respectively) were examined in detail. The one, infected with *P. cathemerium*, revealed only an 11% infection of the primitive erythrocytes while in the other, infected with *P. lophuræ*, 76% of the rare primitive red cells were parasitized. This differential rate of infection of erythrocytes probably is confirmatory evidence of the influence of the age of the erythrocyte on its susceptibility to penetration by plasmodia.² In one embryo elongated gametocytes of *P. cathemerium* were seen, some with jagged edges, such as those described as occurring in ducks infected with *P. relictum*.³

As in the duckling, infection of the embryo with either species led to splenomegaly and malarial pigmentation. Separating the spleens of those embryos uninfected, dying or killed early in the infection from those dead of malaria or killed late in the patent period (only *P. cathemerium* data were sufficient) we found that it is unlikely that a spleen of gross size more than 19 mg is from an uninfected embryo even when the embryo is 18 or more days old. Spleen weight-body weight ratios ranged from 1.2 to 2.4 mg per g in the heavily infected embryos (the mean of 9 determinations was 1.8 mg/g). In the other group the mean ratio of 13 specimens, some of which had been incubated more than 24 days, was only 0.7 mg/g with only one specimen as large

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1 Wolfson, F., *Am. J. Hyg.*, 1940, **31**, Sec. C, 26.

2 Hewitt, R., *Bird Malaria*, Baltimore, 1940.

3 Wolfson, F., *Am. J. Hyg.*, 1939, **30**, Sec. C, 123.

as 1.2 mg/g. Fatty changes, deposits of bile pigment and erythropoiesis in the liver were additional features of the pathological picture encountered only in infected embryos.

Having established that reproducible malarial infections of the duck embryo could be obtained with inoculations of erythrocytic stages of *P. cathemerium*, we undertook a preliminary study of the possibility of producing infections using the ground organs of infected mosquitoes (*Culex pipiens*).[†] Though no infections have been produced as yet some embryos can survive at least 14 days after such inoculation when incubated at 40°C. No attempt was made to produce sterile inocula

[†] The strain of *Culex pipiens* was kindly supplied by Dr. F. Wolfson.

beyond the use of completely sterile equipment.

Summary. Intraembryonic inoculation of duck embryos with the 3T strain of *P. cathemerium* causes reproducible fatal infections with a high level of parasitemia 6-11 days after inoculation. Splenomegaly occurs in these infected embryos. Intraembryonic inoculation of *P. lophurae* produces much more variable results. Infection requires a considerably longer period to develop. This may depend upon the maturity of the erythrocyte in the embryo. Both species of plasmodia can develop in the embryo at either 37°C or 40°C. Duck embryos can survive the intraembryonic inoculation of ground organs of malaria-infected mosquitoes if they are incubated at 40°C.

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Relative Susceptibility of Staphylococci to the Bacteriostatic Action of Antibiotics.

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During the last few years many antibiotics have been studied experimentally and several, notably penicillin, tyrothricin, streptothricin, and streptomycin, have been employed successfully in the treatment of human infections or are being investigated at the present time regarding their clinical usefulness. It is well known that the spectra of activity vary from compound to compound. Certain antibiotics, however, are more effective than others against some species of microorganisms. Herrell and Heilman¹ found penicillin superior to gramicidin in bactericidal activity toward staphylococcus, pneumococcus, and hemolytic streptococcus. The question presents itself as to whether or not various strains belonging to one and the same species differ in their susceptibility to the action of antibiotics. Crowe

and Ward² state that certain strains of staphylococci are insensitive to tyrothricin or crude penicillin. On the other hand, Rammelkamp and Maxon³ reported that 29 different strains of staphylococci varied only slightly in their resistance to penicillin. The results of an investigation into the relative activity of penicillin, tyrothricin, and streptothricin on various strains of staphylococci are embodied in the ensuing report.

Material and Methods. Strains of *Staphylococcus aureus*, isolated from patients at this hospital, were used in this investigation. In addition, several strains (Nos. 3, 20, 209) were obtained through the kindness of Dr. Jean V. Cooke, Washington University, St.

¹ Herrell, W. E., and Heilman, D., *Am. J. Med. Sci.*, 1943, **206**, 221.

² Crowe, S. J., and Ward, A. T., *N. Y. State J. Med.*, 1945, **45**, 61.

³ Rammelkamp, C. H., and Maxon, T., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 386.