# 17093 P. Infection of Mammalian Erythrocytes by the Avian Malaria Parasite, *Plasmodium lophurae*.*

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Reports concerning attempted infection of mammals with avian malaria parasites are limited, although recently Beckman obtained negative results following introduction of *Plasmodium cathemerium* parasites into man[1] and the guinea pig.[2] In a series of experiments unrelated to the immediate problem, the author noted that guinea pig erythrocytes injected into normal 10 day chick embryos survived for a period of 4 days. As a result of this finding, it was decided to investigate the possibility of securing infection of mammalian cells in chick embryos infected with *P. lophurae*, a parasite to which chick embryos have been found to be highly susceptible.[3]

White leghorn embryos of 10 days' incubation were given intravenous injections of $3 \times 10^7$ parasites, representing the 24th passage of *P. lophurae* in embryos. Two tenths cc each of twice washed erythrocytes of mouse, rat, rabbit, dog, sheep, and man were injected intravenously into embryos on the 2nd day after parasite introduction, at which time the infections averaged about 1100 per 10,000 avian erythrocytes. Blood films were usually made twice daily thereafter, although in certain embryos 4 or 5 smears were made in a 24 hour period. Giemsa's stain was used. Only cells agreeing in size, morphology, and staining reactions with the mammalian cell introduced were considered in determination of infection, since during the later stages of embryo infection artifacts arose which might have led to false identification.

Dog and rabbit erythrocytes were not well adapted to survival in chick embryos, the former killing the embryo in a period of 12 hours, the latter disintegrating rapidly. Sheep and rat red blood cells were well preserved even after 3 days but were uninfected. Guinea pig cells, although surviving better than those of the rabbit, disintegrated to the point that they were difficult to separate from abnormal avian erythrocytes. Therefore, although many infected cells suggesting those of the guinea pig were seen, the results were considered negative. Human erythrocytes retained their characteristic morphology throughout the period studied. Two infected cells were found, both with early trophozoites of *P. lophurae*. Infections were so low, however, that this type of cell was regarded as unsuitable for further study.

Erythrocytes of Swiss mice maintained their morphology and were readily parasitized. The proportion of mammalian to avian erythrocytes was usually 1:2. Infected mouse cells were found as early as 12 hours after introduction, although the percentage of infected erythrocytes remained low never exceeding 10 per 10,000. Very few doubly infected cells were observed. Early trophozoites were similar to corresponding stages in the avian erythrocytes. More mature trophozoites frequently elongated into the band forms characteristic of *P. malariae* but, like this parasite, they were sometimes seen as spherical masses in the center of the host cell (Fig. 1a). Pigment granules were small, golden-brown spheres. The cytoplasm was homogenous and stained similarly to *P. lophurae* parasites in chicken erythrocytes. Nuclear structures of trophozoites were identical with *P. lophurae* in chicken red blood cells. After two nuclear divisions parasites occupied a large proportion of the host cell and in later stages caused the cell to enlarge. Pre-segmenting forms with 7-10 nuclei were seen after 32 hours, and fully segmented parasites were seen 36 hours following introduction of erythrocytes (Fig. 1b) at

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1 Beckman, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 401.

2 Beckman, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 172.

3 McGhee, R. B., *J. Parasitol.*, 1949, in press.

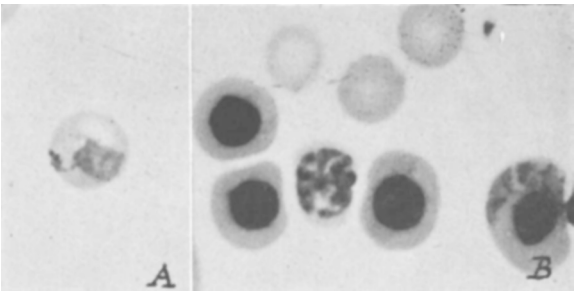


FIG. 1a. A medium trophozoite of *P. lophurae* in a mature mouse erythrocyte. ×1770.

FIG. 1b. A segmenting parasite of *P. lophurae* in a slightly enlarged mouse erythrocyte, from a smear made 36 hours following introduction of mammalian cells. ×1600.

which times avian cell infections averaged about 8000 per 10,000 red blood cells. Five to 11 merozoites, smaller than those in avian erythrocytes, were produced, generally in a rosette form. No gametocytes were found.

Cultures prepared according to the formula of Trager[4] using whole mouse blood in conjunction with infected chick embryo blood indicated a certain amount of invasion of mouse erythrocytes *in vitro*.

In view of the high degree of host specificity of avian malarias, it is surprising that cells of hosts so distantly related as to be placed in another phylum are invaded. Parasitism of mouse cells might, therefore, demonstrate that in certain cases the resistant principle lies in the serum constituents, rather than in the erythrocyte. *P. lophurae* is transmitted readily by *Anopheles quadrimaculatus* and this fact, along with the successful infection of mouse erythrocytes, might indicate a closer relationship between *P. lophurae* and mammalian malaria parasites than was previously supposed.

[4] Trager, W., *J. Parasitol.*, 1947, **33**, 345.

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## 17094. A Falling Sphere Method for Studying Clotting in Systems of Purified Blood Components.

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Although the conversion by thrombin of fibrinogen to fibrin is generally used as an indicator of reactions occurring earlier in the process of blood coagulation, methods for the study of this conversion, with very few exceptions, measure only a single end-point in fibrin formation.[1] It is apparent that a method which measures quantitative changes in the coagulation system up to some definite end-point might be of value in following the kinetics of the system. An involved technic has been reported[2] for measuring early alterations in clotting systems by recording changes in intensity of transmitted light as coagulation proceeds. Methods based upon changes in viscosity of a clotting solution have

[1] Quick, A. J., The Hemorrhagic Diseases and the Physiology of Hemostasis, Charles C. Thomas, Springfield, 1942.

[2] Nygaard, K. K., Hemorrhagic Disease. Photoelectric Study of Blood Coagulation, C. V. Mosby Company, St. Louis, 1941.