Malaria Parasites: Fluorescent Antibody Technique for Tissue Stage Study

Abstract. *Tissue stages of avian and simian malarias were stained by the fluorescent antibody method. The fluorescent bodies proved to be parasites in tests for immunological specificity and on restaining with Giemsa. These results suggest a method for studying two important aspects of mammalian malarias—namely, the cycle in the mammalian host and the immunological relationships among malarias indicated by tissue stages.*

The fluorescent antibody technique has been used to stain malaria organisms (*1*). Antibodies produced against sporozoites were labeled with fluorescein isothiocyanate (FIC) and used to stain blood stages of the parasite. This cross-immunological reaction between two different forms of the organism indicated that tissue stages of malaria parasites might be studied by the fluorescent antibody method. Tissue forms of *Plasmodium gallinaceum, P cynomolgi* B strain, and *P. cynomolgi* PT strain (*2*) were stained by the fluorescent antibody technique.

Immune serum for staining *P. gallinaceum* was obtained from chicks that had recovered from an experimental infection produced by bites of infected *Aedes aegypti*. The chicks were subsequently challenged several times with blood forms of the parasite. *Plasmodium cynomolgi* B and *P. cynomolgi* PT were stained with serum pooled from two monkeys that had recovered from an experimental sporozoite-induced infection with B strain. These animals were challenged three times, and then were bled for immune serum. The serum was fractionated, labeled with FIC, and applied (see *1*). Except for the 34-percent saturation with ammonium sulfate in the fractionation of γ-globulin, the method was essentially that of Coons and Kaplan (*3*). The γ-globulin fraction was conjugated with FIC, and the unconjugated FIC was removed by dialysis against 0.01*M* phosphate-buffered saline (*p*H 7.1). The conjugate was absorbed with powdered monkey liver two or three times, spread over the tissue preparation, and left for 20 to 30 minutes. The tissue preparations on the slides were then washed with buffered saline and mounted in 10-percent buffered glycerol. The organisms were stained also by the indirect method (*4*). The slides were studied with a Leitz fluorescence microscope equipped with an HBO-200 mercury arc burner light source and a UG-1 primary filter.

Chicks were infected with sporozoites of *Plasmodium gallinaceum* introduced by bites of *Aedes aegypti*. After about 2 weeks the chicks were sacrificed and various tissues were removed. Direct smears, which were fixed for 10 seconds in methanol, were made from these tissues. Tissue for histological sections was fixed in Carnoy's fluid. Monkeys were infected by intravenous inoculation of salivary glands dissected from infected *Anopheles quadrimaculatus* and *A. freeborni*. Biopsies of the liver were made at intervals from 1 to 12 days after the inoculation of sporozoite. The tissue was fixed in Carnoy's fluid, sectioned, and stained by the fluorescent antibody methods described. Controls for the direct method were parasites exposed to labeled normal serum and to unlabeled immune serum. Controls for indirect staining were parasites exposed to labeled antiglobulin only.

Tissue forms of *P. gallinaceum* from lungs and brains of infected chicks stained well with conjugated antiserum to *P. gallinaceum*. Staining was inhibited by pretreatment with unlabeled immune serum. By the same procedures exoerythrocytic forms of the simian malarias were demonstrated in hepatic cells. These parasites were readily detected by their greenish-yellow fluorescence, and there were few confusing artifacts. Simian parasites 1 and 2 days of age contained a single nucleus and generally were seen next to the nucleus of the parasitized cell. The identity of the parasites was confirmed by inhibiting staining with unlabeled immune serum. Also, to confirm the identity of the organism, the fluorescent objects were located precisely and stained with Giemsa: The fluorescent antibody slides were placed in 70-percent ethanol to remove the glycerol, washed with buffered saline, and stained routinely with Giemsa. Early forms were not recognized by our laboratory staff until they had been first demonstrated by staining with fluorescent antibody. Figure 1 shows a 6-day-old exoerythrocytic parasite of *P. cynomolgi* B stained with fluorescent antibody. Figure 2 is a Giemsa stain of the same parasite.

Since publication of the report (*1*) showing that the fluorescent-antibody technique could be applied to the study of malaria, a number of papers on such application have appeared. (*5*). One major problem in the staining of malaria organisms by the fluorescent-antibody method is the difficulty of evaluating the results quantitatively. However, instrumentation for this purpose is being developed (*6*). Another difficulty is that of specificity. *Plasmodium cynomolgi* B stained as well as *P. vivax* did after treatment with antiserum to *P. vivax* (*4*). *Plasmodium berghei*, a rodent malaria, also stained with the serum, but less intensely. In our laboratory, blood forms of *P. gonderi, P. inui*, and *P. cynomolgi* PT have stained well with antiserum to *P. cynomolgi* B. Therefore, it appears that this method of staining malaria parasites may have limited value as a diagnostic procedure.

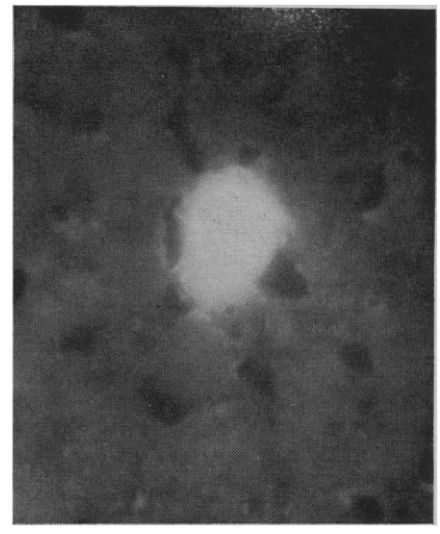

Fig. 1. Fluorescent antibody stain of a 6-day exoerythrocytic stage of *Plasmodium cynomolgi* B.

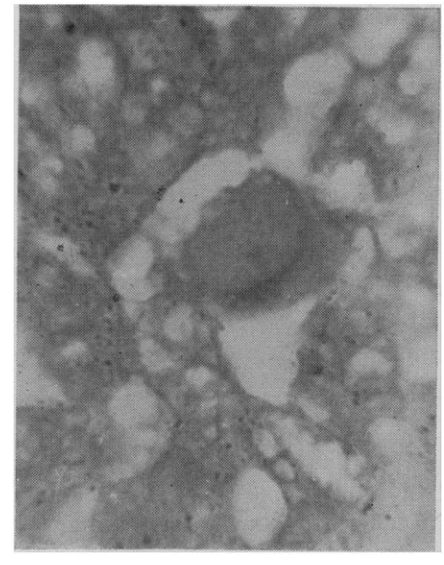

Fig. 2. Giemsa stain of a 6-day exoerythrocytic stage of *Plasmodium cynomolgi* B.

However, the work reported here (*7*) indicates that the fluorescent antibody method makes available a means for investigating immunological relationships among tissue stages of malaria parasites. These studies would be difficult to make by other immunological procedures. The fluorescent antibody method also provides a means for studying the cycle of malaria in mammalian hosts. In our laboratory we have shown, by this means, previously undescribed exoerythrocytic stages, 1 day old and older, of simian malaria in monkeys.

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Reference and Notes

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7. We thank J. B. Brooks for technical assistance in these studies.

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Assumptions in Tests for Meiotic Drive

Abstract. *Recent claims for the existence of meiotic drive in the ABO blood groups require the assumption of equal fertility (if there is no other selection operating) for the various mating types. There is evidence that does not support this assumption, and there are no convincing a priori grounds for making it.*

Matsunaga and Hiraizumi (*1*) presented data which they believe demonstrates prezygotic selection operating in the ABO blood groups in man, due probably to meiotic drive or sperm competition. Novitski (*2*) pointed out that their use of Hardy-Weinberg expectations to calculate the proportions of AA and AO fathers (and BB and BO fathers) is open to criticism. Hiraizumi and Matsunaga (*3*) agree with this criticism and present other data in support of their claim of prezygotic selection.

The methods used by them (*1*) include the assumption of equal fertility (in the absence of other selection processes) for the various mating types. However, this assumption, although essential to the methods, is not stated, and no justification for it is given. [Hiraizumi and Matsunaga (*3*) show that, in their data, the mean number of pregnancies from ♂O × ♀A matings does not differ from that from ♂O × ♀O matings; they are unable, however, at the 5 percent level of significance, to detect differences between 0 and 12 percent. Other matings, with the exception of ♂A × ♀O, are not compared in the report.] Since there are data on human blood groups which strongly suggest that this assumption is sometimes invalid (*4*), and since there are no overriding a priori grounds for making it, the validity of any conclusions that require the assumption remains uncertain.

It is worth emphasizing that, in theory, selection may occur anywhere in the life cycle of an organism. It may result from any of the well-recognized possibilities of gametic and zygotic selection (from fertilized egg to adult) affecting the proportion of gametes or zygotes, or it may result from deviations from equality in the mean number of potential conceptions (C_0) that a particular mating type, such as ♂A × ♀O, may have. The term C_0 is probably best viewed as the mean number of conceptions which would occur in the absence of any prior gametic selection. The means for number of conceptions from each mating type and the corresponding means for segregation proportions of the sperms and eggs, from each mating type, which actually participate in fertilization then determine the zygotic proportions at this stage. The new factor here is the weighting of the zygotic contributions from each mating type by the mean number of conceptions of that mating type.

It is perhaps of interest that in ten of the 18 comparisons of "family size equivalents" made by Matsunaga and Hiraizumi (*1*) a father heterozygous for the A or B allele is compared with an O father. From the discussion given here it follows that this comparison could as well be used to test for heterozygote advantage in fertility as for gametic selection. However, the fact that nine of these ten comparisons favor the AO or BO father is no better evidence for heterosis in fertility than for meiotic drive or sperm competition—these possible effects are all confounded. The difficulty referred to by Novitski (*2*) also remains.

There is still a possibility that gametic selection, in addition to the more convincingly demonstrated zygotic selection, occurs in the ABO blood groups, but the evidence presented by Matsunaga and Hiraizumi should not be cited as supporting it.

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I agree with Reed that the fertility of the mating types may differ, and indeed this was the reason why in our study (*1*) we selected only families having at least three children. After this selection had been made the two reciprocal mating types A × O showed very close agreement in mean number of pregnancies (P_{AO}, P_{OA}), mean number of children (C_{AO}, C_{OA}), and proportion of prenatal deaths (PP_{AO}, PP_{OA}). Since a large difference in fertility, which had existed before this selection had been made, disappeared, we believed that the effect of incompatibility (and of differences in fertility) had been minimized.

At the 5-percent level of significance, the value for P_{OA} ($= 4.913 \pm 0.211$) presented in our earlier report (*1*) could be larger than that for P_{AO} ($= 4.949 \pm 0.223$) by about 12 percent, but since only a fraction [perhaps less than 50 percent (*2*)] of this 12 percent would contribute to increase the number of O children, the excess actually observed seems to be greater than would be expected from fertility difference alone.

Exactly the same comparison can be made for the two reciprocal mating types B × O for families with at least three children. Here we obtain the same results as for A × O; that is, the values for *P*, *C*, and *PP* are nearly the same in the two reciprocal mating