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Detection of Malarial Antibodies in Man by Fluorescent Antibody Test Using *Plasmodium gallinaceum* as Antigen

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Abstract

The results obtained by the serological testing of 38 malaria cases are reported in this paper. The indirect fluorescent antibody test (FAT) was used. *Plasmodium gallinaceum* was used as antigen. Antibodies were detected in 84% of the patients. The results were more satisfactory in cases affected by falciparum malaria.

These results suggest that the FAT with *P. gallinaceum* as antigen can be regarded only as a complement to the clinical and laboratory diagnosis of malaria.

One of many valuable applications of the Fluorescent Antibody Test (FAT) is the detection and measurement of antibody levels during present or past infection with malaria parasites. Such a test is useful for clinical purposes (1, 5, 8) as well as for epidemiological investigations (2, 7, 20). Several papers have been published concerning the use of various *Plasmodium* species (3, 4, 13) in performing the test. Besides homologous parasite antigens (7, 14, 21), which are rather difficult to obtain, primate infecting *Plasmodia* (1, 4, 13, 17–19) have also been used as antigen for the FAT. KIELMANN and others (9–12) reported positive results when using *Plasmodium gallinaceum* as antigen. This offers many advantages. Whereas working on monkeys can be tedious, the handling of chickens to obtain the antigen is easier and the procedure is far less expensive.

This paper relates our own further experience with the serological investigations by FAT of human malaria using *P. gallinaceum* as antigen.

Materials and Methods

Sera of patients: These were obtained from 38 Europeans of both sexes who suffered from malaria after having returned from the tropics. All cases were confirmed by direct peripheral blood investigation, which was parasitologically positive.

The first serum sample was taken before standard therapy (chloroquine); further samples were taken during and after treatment.

Antigen: P. gallinaceum (the same strain used by KIELMANN et al. 9, 11) was taken as antigen. Chicken blood films were made between the 9th and 11th day after infection and were preserved at –20 °C.

Indirect immunofluorescence test after Coons: The basic technique used by AMBROISE-THOMAS (1) and KIELMANN et al. (11) was followed. Antigen was fixed with acetone for 10 minutes before the test was started. Small circles (⌀ 6 mm) were drawn over the blood films, on to which the serum dilutions were then

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Table 1. Distribution of fluorescence antibodies among different *Plasmodium* species **

Malaria	Number of cases	< 20 negative	Antibody titers (reciprocal) 20	40	80	160	320	640	Mean values
P. falciparum	13	2	4	2		1	3	1 *	150
P. vivax	12	3	3 (1 *)	4	1	1			40
P. ovale	1			1					40
P. malariae	1			1					40
P. falciparum + *P. vivax*	1		1						20
Unclassified	10	1	5	1		3			64
	38	6	13	9	1	5	3	1	

* Late-positive cases.
** *P. gallinaceum* used as antigen.

Table 2. Relation of fluorescence antibodies positives to parasitological diagnosis

Malaria	Number of cases	Antibody positive	%	negative	%
P. falciparum	13	11 (1 *)		2	
P. vivax	12	9 (1 *)		3	
P. ovale	1	1			
P. malariae	1	1			
P. falciparum + *P. vivax*	1	1			
Unclassified	10	9		1	
	38	32 (2 *)	84.21	6	15.79

* Late-positive cases.

dropped and incubated for 30 minutes at 37 °C. The initial serum dilution was 1/20. After washing with phosphate buffer pH 7.2 the antigen-antibody complex was covered with one drop of fluorescein-labelled antihuman globulin (Institut Pasteur, Paris) which had been diluted 1/40. Evans Blue was used both as a background contrast and as a dilution medium for the antiglobulin. Repeated incubation followed. After washing and drying, the slides were covered with the buffered glycerine solution. They were examined under blue light using a Wild fluorescence microscope, M 12. The evaluation and the specificity of the test has been described in an earlier publication (16).

Results

Tab. 1 and 2 show that in a serie of 38 cases with positive blood films, it was possible to detect malarial antibodies in 30 of them during the acute stage, before treatment was administered. In 2 cases the antibody titer became positive later, during specific treatment (chloroquine). 6 cases showed titers lower than 1/20 after repeated testing. Antibodies with a titer higher than 1/20 were detected in 84.21% of our patients. Positive reaction is shown in fig. 1.

The best results, as successful confirmation by FAT of the positive peripheral blood findings and as titer levels, were elicited in patients with falciparum malaria. Out of 13 such cases, only 2 remained consistently negative in subsequent tests. Interestingly enough, one of these was affected by severe cerebral malaria with renal failure; initially about 25% of the erythrocytes were parasited. After i.v. chloroquine the parasitaemia dropped rapidly to 2% and below; the patient, however, had to be dialysed twice before he finally recovered.

There were 12 patients infected with *P. vivax,* and these showed lower titers than those with *P. falciparum*. This evaluation is in agreement with the conclusion drawn by SADUN et al. (15), who used *P. falciparum* lysates as antigen for the FAT.

The few cases of unclassified, ovale and malariae malaria cannot be properly evaluated because of their small numbers.

Graph 1 shows the course of the antibody titers during and after specific treatment (all patients received chloroquine; some of those affected by *Plasmodia* other than *falciparum* received also primaquine). In a former publication (16) it has been established in concordance with KIELMANN et al. (11) 1/20 as the lowest positive titer. With regard to the course of the titers, it was noticed that in some cases of *P. falciparum* infection there was a rise in titer levels during treatment and only thereafter a decline. However, this rise does not appear to be significant. In cases of vivax malaria the antibodies curve was flatter and showed no relevant differences between treatment and post-treatment period.

We could follow up some of our patients up to 60 days. However, it ought to be emphasized that the follow-up of malarial patients offers considerable difficulties as they frequently do not report back for check-ups after the acute attack has been controlled. One patient, who had been affected by a malarial infection where exact identification of the *plasmodium* had not been achieved, could be followed up for 4 months. His titer was still positive 1/20 as at the beginning of the investigation.

Discussion

The results show, in concordance with KIELMANN et al. (9–12), that *P. gallinaceum* can be used as antigen for the detection of antibodies by FAT in cases of human malaria, of course homologous antigen will yield more exact results.

Positive serological findings (FAT) can support the clinical diagnosis, particularly in cases either without or with doubtful direct parasitological findings in the peripheral blood.

After therapy one can observe a decline of antibody titers.

The fact that the overall results were more satisfactory (judged as number of positive titers) in cases of *P. falciparum* infections could simply be explained by the usually higher parasitaemia in such cases. The "antigen load" could therefore have been higher, leading to a more pronounced antibody formation than in *P. vivax* infections.

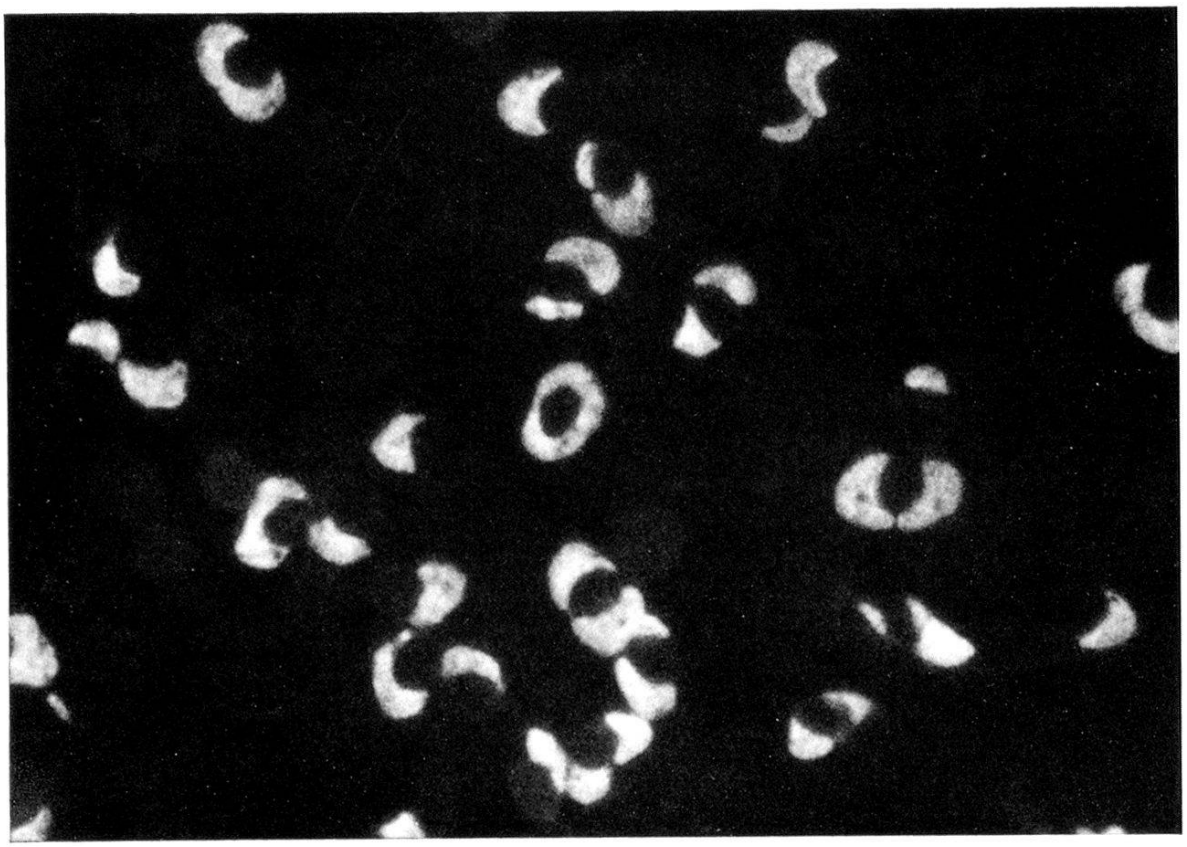
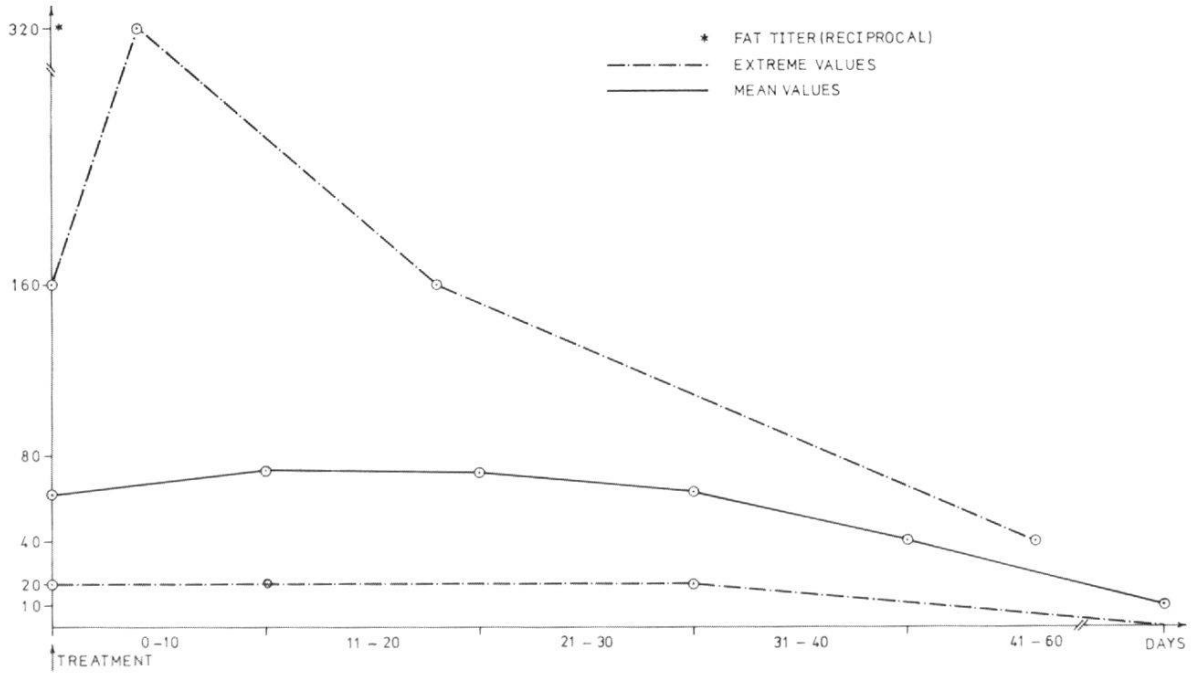


Fig. 1. Plasmodium gallinaceum as antigen in FAT. Chicken blood film after contact with serum from a patient with *P. falciparum* malaria, giving positive reaction.



Graph 1. Course of the antibody titers (*Plasmodium gallinaceum* used as antigen).

The single case with cerebral malaria, very high parasitaemia but a negative FAT is unexpected; however, it could perhaps be explained on the basis of a scarse ability to form antibodies by the person involved. Another patient, who came to our notice *after* what appears to have been an untreated acute malaria attack, showed in the blood films only very rare *P. falciparum* gametocytes and was at that time negative on FAT.

Nothing definite can be said about the nature and function of the antibodies revealed by the FAT.

The role of the antigen derived from the erythrocytic phase of the parasite seems to be relevant for the production of antibodies, as all the positive cases had a confirmed acute attack of malaria. On the other hand, it is very interesting that one single patient who had suffered from an acute attack of malaria in Mexico was negative both on direct blood film examination and on FAT when she came to us for a general check-up 5 months later, being at that time in normal health condition. Subsequently, however ($1^1/_2$ months later), she developed a relapse and was found to have a *P. vivax* infection. The former negative FA became positive during the attack with titer 1/20. This seems to lead towards the exclusion of a supposed role of the exoerythrocytic phase in the stimulation of antibody production. Of course one single observation like this has only a limited value, but could, if confirmed by further similar cases, support this theory significantly.

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