WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

WHO/Mal/67.606
WHO/VDT/RES/67.124

ENGLISH ONLY
(avec résumé en français)

SEROLOGICAL STUDIES ON MALARIA[1]

PART II

An evaluation of complement-fixation and immunofluorescent tests with a simian malaria parasite antigen, in a study of malarial antibody levels in a population in a malaria endemic area.

A. Voller,
Nuffield Institute of Comparative Medicine,
The Zoological Society of London, England

and

R. Schindler
Bernard Nocht Institute of Tropical Diseases,
Hamburg, Federal German Republic.

INTRODUCTION

In spite of the mass of recent immunological investigations on malaria, relatively few attempts have been made to utilize the new techniques in field studies. Voller & Bray (1962) indicated the potential of fluorescent antibody techniques in measuring the malarial antibody levels in a population in an endemic area. McGregor et al. (1965) expanded this theme and were able to show that in some circumstances the fluorescent antibody results seemed to reflect the immunological status of the population with regard to malaria. Curtain et al. (1964) were able

[1] We are pleased to acknowledge the support received from the Medical Research Council, England, and the Deutsche Forschungsgemeinschaft. This study received the financial assistance of the World Health Organization.

to correlate the intensity of malaria infection in two groups of New Guinea villagers with the mean immunofluorescent titres. The above projects were carried out in endemic areas where there was little difficulty in obtaining the human parasite, Plasmodium falciparum, as antigen.

The use of a suitable simian malaria parasite facilitates antigen preparation and allows the tests to be performed away from malaria endemic areas. The known immunofluorescent cross-reactivity of P. cynomolgi bastianellii with human malarial sera has already been utilized in measurement of human malarial antibody in a small study by Kuvin & Voller (1963) and Marsden et al. (1965), and recently by Coudert et al. (1965). Similarly Desowitz & Saave (1965) were able to measure the malarial antibody levels of a New Guinea population using simian malaria parasite antigen in a haemagglutination reaction.

The purpose of the present study was to determine the value of the monkey malaria parasite P. cynomolgi bastianellii as antigen for comparative measurement of human malarial antibody by the fluorescent antibody and complement-fixation tests.

METHODS

During 1965 and 1966 an epidemiological and serological survey of yaws was carried out in Northern Nigeria by the Treponematoses Epidemiological Team of the World Health Organization with the co-operation of the Government of the Federal Republic of Nigeria.

Sera were collected in various parts of Northern Nigeria. They were transported in liquid nitrogen to Paris by the method described by Guthe (1965). The advantage of this method of transport and storage of serum, particularly concerning the preservation of antibodies in an unaltered form, are fully discussed by Guthe (1966). Aliquots were made available for fluorescent antibody tests carried out in London, and for the complement-fixation tests done in Hamburg.

Data concerning serum donors (age, sex, tribe, geographical area of the residence of the donor etc.) were provided by the Treponematoses Epidemiological Team of the World Health Organization.

The Regional Malaria Unit, Northern Nigeria provided us with results of blood-film examination of donors.

P. cynomolgi bastianellii was used as antigen. It was maintained by blood passage in Macaca mulatta and Erythrocebus patas.

The complement-fixation test and fluorescent antibody reaction were performed as described by Schindler and Voller (1967).

RESULTS

185 sera from Northern Nigeria were tested for malarial antibodies by complement-fixation and fluorescent antibody methods.

Table 1 gives the results of the tests in terms of age groups. Few children and only about 20% of the adults yielded positive complement-fixation results. In contrast almost all of the children and adults gave a positive fluorescent antibody reaction. A quantitative analysis of the fluorescent antibody results in terms of age groups is given in Fig. 1. Low titres were found in children and the maximum level was not reached until adolescence.

Because of the small numbers of individuals in the lower age groups the remaining analyses were confined to the adults.

The results of complement-fixation and immunofluorescent tests correlated with current parasitaemia are shown in Table 2 and Figs. 2 and 3. There was no relation between positivity of complement-fixation reaction, malaria fluorescent antibody titre and current parasitaemia.

DISCUSSION

The complement-fixation reaction has been proposed as a diagnostic aid for malaria numerous times since the beginning of this century and the early works are admirably reviewed by Tobie (1964). Of particular relevance to the present work is the report of Kingsbury (1927) of extensive cross-reactions between P. falciparum and P. vivax. The careful study by Eaton & Coggeshall (1939) established that the simian malaria parasite P. knowlesi could be used as antigen to detect malaria antibodies from patients with P. falciparum or P. vivax infections. This

finding received confirmation by the studies of Rein et al. (1949). The careful work of Mayer & Heidelberger (1946) indicated that when proper controls were set up with uninfected red cell material as antigen, then the apparent cross-reactions with heterologous malaria antigens were of lower incidence than the reaction with uninfected red cell material.

A probable explanation of the divergent results different authors obtained with malaria complement-fixation may be that the antigens contained different amounts of the non-specific component which is also responsible for the positivity occasionally encountered with sera from treponemal infections. Recently d'Antonio et al. (1966) have shown that there is a complement-fixation cross-reaction between P. knowlesi and P. vivax but not between these two species and P. falciparum when a purified antigen is used.

The blood film results show that over 30% of the adults in the present study had current malarial parasitaemia, P. falciparum being by far the most fequent, with P. malariae infections relatively rarely encountered. Such a high parasite rate derived from a single blood film examination would indicate that all the adults would be found to be infected at some time if a longitudinal study was performed. This assumption is supported by the fluorescent antibody results which yielded only one negative out of the 96 adult sera tested. Thus it can be concluded that there is no cross reaction between human antibody provoked by P. falciparum infection and P. cynomolgi bastianellii antigen in the complement-fixation reaction. The lack of any correlation between current malarial parasitaemia and the complement-fixation positivity in the present study is clearly shown in Table 2.

The above discussion suggests that a factor other than malaria is responsible for the positive complement-fixation results which we obtained. Such false positive reactions have been a feature of virtually all the previous studies on malaria complement-fixation, and have in several cases been related to syphilitic infection. Mayer & Heidelberger (1946) concluded that the higher the proportion of malaria parasites in the malarial complement-fixing antigen the more it cross-reacted with syphilitic sera. They also pointed out that it was not the same antigen which was functional in the complement-fixation test for a malarial and

syphilitic sera. Of special interest is their finding that their particulate antigen of P. vivax had low reactivity with syphilitic sera but still gave positive results with about a third of the malarial sera. Their thesis that the syphilitic and malarial sera were at least in some cases reacting with different components of the antigen received further support from their observation that the antigen derived from P. falciparum was reactive with three quarters of the sera from syphilitic cases and only with one quarter of the malarial sera. Mahoney et al. (1966) have recently applied more delicate modern methods to antigen fractionation and they found that the complement-fixing activity was mostly in a particulate fraction in contrast to the most active haemagglutinating antigen, which was soluble. Evidence to be presented at a later date indicates that yaws infection may be responsible for the positive malaria complement-fixation results which were obtained in the present study.

The mean titres of fluorescent malarial antibodies shown (Fig. 1) in relation to age groups indicates that young children had the lowest levels and that maximum levels were not reached until adolescence after which there was little further rise. Although the simian antigen P. cynomolgi bastianellii was used here, the present results are very similar to those obtained by Voller & Bray (1962) in Liberia and by McGregor et al. (1965) in the Gambia. Our work supports the view of Coudert et al. (1965) that this simian malaria parasite antigen appears to be satisfactory for fluorescent antibody tests on human sera from malaria endemic areas. All the adults with positive blood films gave a positive fluorescent antibody reaction (Fig. 2) but their titre distribution was similar to those without detectable parasitaemia. This lack of correlation with detectable parasitaemia and malarial antibody level is not surprising because the detectable rate in these studies was determined by a brief examination of thick blood films, and understates the true malarial infection rate (Bruce-Chwatt, 1963).

It may well be that simian malaria parasites other than the species we tested will serve as better antigens for the detection of human malarial antibody. The opinion has been voiced by Desowitz et al. (1966) that P. cynomolgi may be the simian antigen of choice for detection of P. vivax antibody and P. coatneyi for P. falciparum antibody, and their results with haemagglutination tests support this view. Collins et al. (1966) decided that P. fieldi was the most suitable simian antigen for immunofluorescent

estimation of P. malariae and P. falciparum antibody and Meuwissen (1966) found that this antigen gave a good reaction with sera from patients infected with P. ovale. There are clearly many questions to be answered concerning the practical suitability of current malaria serological tests and their biological basis, and it is hoped that the present immunological onslaught on malaria will answer these questions in the near future.

ACKNOWLEDGEMENTS

We are most grateful to Dr J. S. Dodge and his staff of the Regional Malaria Unit, Kaduna, Northern Nigeria who carried out the blood-film examinations and to the WHO Treponematoses Epidemiological Team who collected the sera and provided data on the donors.

TABLE 1. RESULTS OF COMPLEMENT-FIXATION AND FLUORESCENT ANTIBODY TESTS ON NIGERIAN SERA, WITH P. CYNOMOLGI BASTIANELLII ANTIGEN

Age group	C.F.T. positive	F.A.T. positive	Total sera tested
0 - 5 years	3	33	35
6 - 10 years	2	37	37
11 - 15 years	0	17	17
Above 15 years	19	95	96
Total	24	182	185

TABLE 2. THE RESULTS OF MALARIA COMPLEMENT-FIXATION TESTS ON ADULT NIGERIANS WITH OR WITHOUT CURRENT DETECTABLE MALARIA PARASITAEMIA

	Total tested	C.F.T. positive	C.F.T. negative	% positive malaria C.F.T.
Blood film positive	34	8	26	23
Blood film negative	58	11	47	19
Total	92	19	73	21

RESUME

En 1965 et 1966 une enquête sérologique et épidémiologique sur le pian au Nigéria du nord a fourni l'occasion d'appliquer à l'étude du paludisme sur le terrain les dernières techniques immunologiques.

En vue de comparer la méthode de fixation du complément à celle des anticorps fluorescents pour la détermination des anticorps du paludisme humain, on a tenté d'évaluer l'intérêt qu'il y aurait à utiliser comme antigène un parasite paludique simien, Plasmodium cynomolgi bastianellii.

Sur 185 échantillons de sérum prélevés au Nigéria du nord, 89 provenaient d'enfants et 96 d'adultes (plus de 15 ans). La recherche des anticorps a été faite par la technique de fixation du complément à Hambourg et par la technique des anticorps fluorescents à Londres.

Seuls quelques enfants et environ 20 % des adultes ont présenté une réaction positive à l'épreuve de fixation du complément. Par contre, 87 enfants et 95 adultes se sont révélés positifs à l'épreuve des anticorps fluorescents, le titre étant faible chez les enfants et n'atteignant une valeur maximale qu'à l'adolescence. Bien que le seul examen des lames de sang ait indiqué chez les adultes une proportion de positives supérieure à 30 %, on peut penser, en s'appuyant sur les résultats de l'épreuve des anticorps fluorescents, qu'une étude longitudinale aurait révélé l'existence d'une infection chez la totalité des sujets.

Il n'existe pas de relation entre la parasitémie ordinaire, le titre en anticorps du paludisme humain déterminé par fluorescence, et de positivité à l'épreuve de fixation du complément. Le dépistage du paludisme par fixation du complément a donné des résultats plus fréquemment positifs chez les malades réagissant positivement aux épreuves de recherche du tréponème que chez ceux qui n'y réagissent pas.

Il faudrait donc attribuer, semble-t-il, la positivité de l'épreuve de fixation du complément à un facteur autre que le paludisme. Des études précédentes sur cette épreuve dans le cas du paludisme ont déjà conduit à des résultats pseudo-positifs de de genre.

REFERENCES

d'Antonio, L. E., von Doenhoff, A. E. & Fife, E. H. (1966) Military Medicine, 9, Suppl. 1152

Bruce-Chwatt, L. J. (1963) W. Afr. med. J., 12, 141 and 199

Collins, W. E., et al. (1966) Amer. J. trop. Med. Hyg., 15, 11

Coudert, J., et al. (1965) Bull. Soc. Path. exot., 58, 630

Curtain C. C., et al. (1964) Nature (Lond.), 203, 1366

Desowitz, R. S. & Saave, J. J. (1965) Bull. Wld Hlth Org., 32, 149

Desowitz, R. S., Saave, J. J. & Stein, B. (1966) Military Medicine, 9, Suppl. 1157

Eaton, M. D. & Coggeshall, L. T. (1939) J. exp. med., 69, 379

Guthe, T. (1965) Bull. Wld Hlth Org., 33, 864

Guthe, T. (1966) Arch. Immunol. Therap. Exper., 14, 689

Kingsbury, A. N. (1927) Trans. roy. Soc. trop. Med. Hyg., 20, 359

Kuvin, S. F. & Voller, A. (1963) Brit. med. J., 2, 477

Mahoney, D. F., Redington, B. C. & Schoenbechler, M. J. (1966) Military Medicine, 9, Suppl. 1141

Marsden, P. D., et al. (1965) Brit. med. J., 1, 89

Mayer, M. M. & Heidelberger, M. (1946) J. Immunol., 54, 89

McGregor, I. A., et al. (1965) Trans. roy. Soc. trop. Med. Hyg., 59, 395

Meuwissen, J. H. E. Th., (1966) Trop. geogr. Med., 18, 250

Rein, C. R. et al., (1949) Amer. J. Hyg., 49, 374

Schindler, R. & Voller, A. (1967) Serological Studies on Malaria, Part I (mimeographed document WHO/Mal/67.605)

Tobie, J. E. (1964) Amer. J. trop. Med. Hyg., 13, Suppl. 1, 195

Voller, A. & Bray, R. S., (1962) Proc. Soc. exp. Biol. N.Y., 110, 907

FIG. 1

THE GEOMETRIC MEAN TITRES OBTAINED IN MALARIA FLUORESCENT ANTIBODY TESTS ON NIGERIAN SERA.

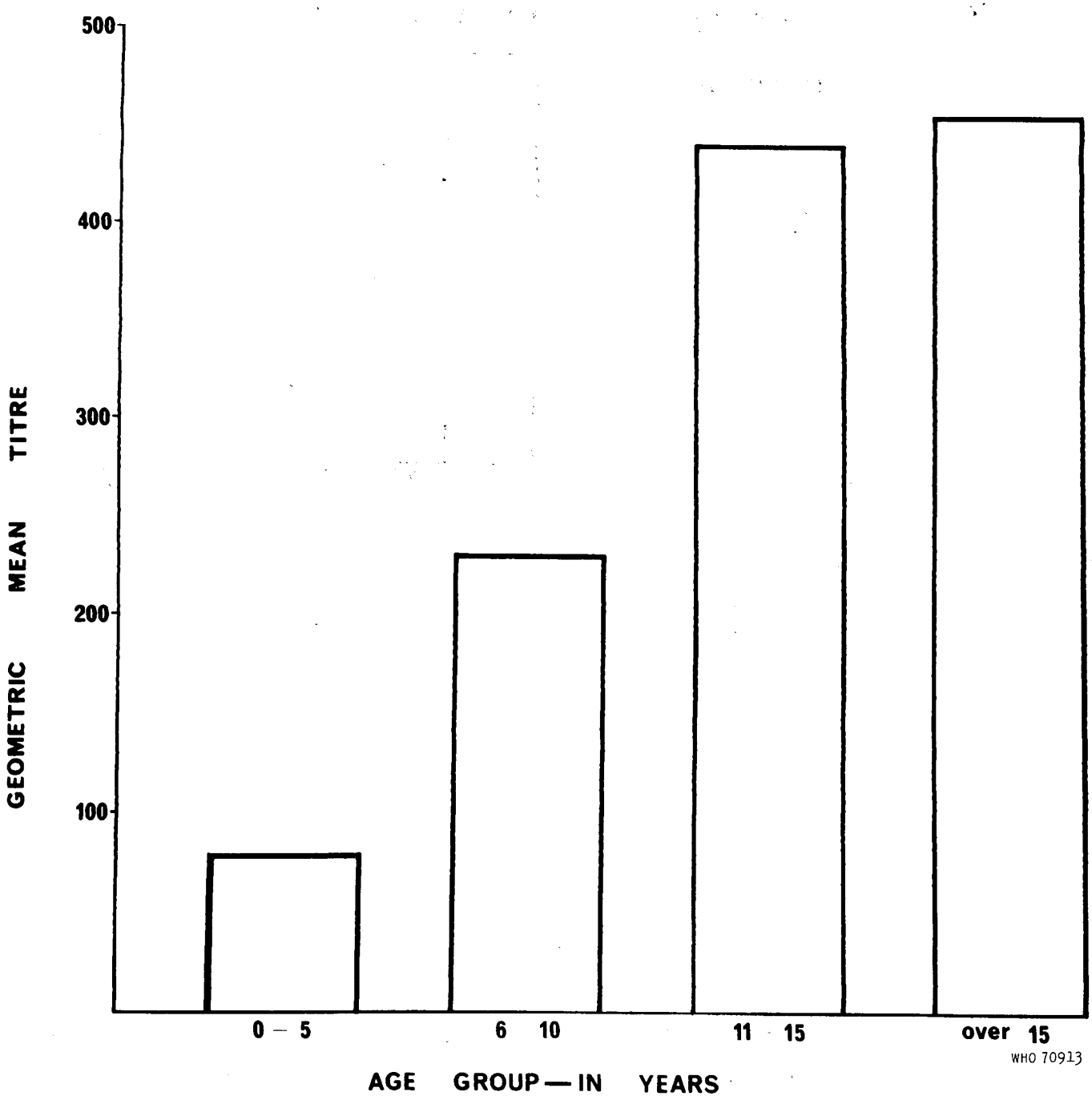
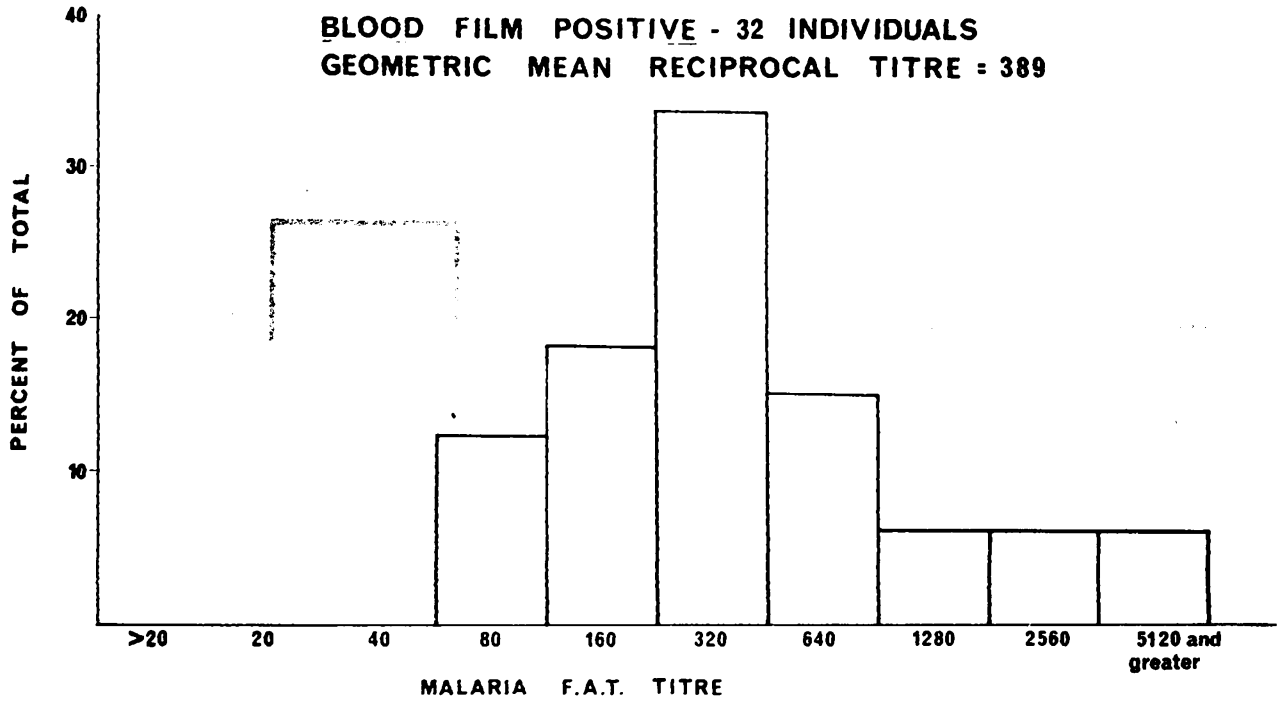


FIG. 2

THE DISTRIBUTION OF MALARIA FLUORESCENT ANTIBODY TITRES IN ADULT NIGERIANS IN RELATION TO MALARIA PARASITAEMIA.



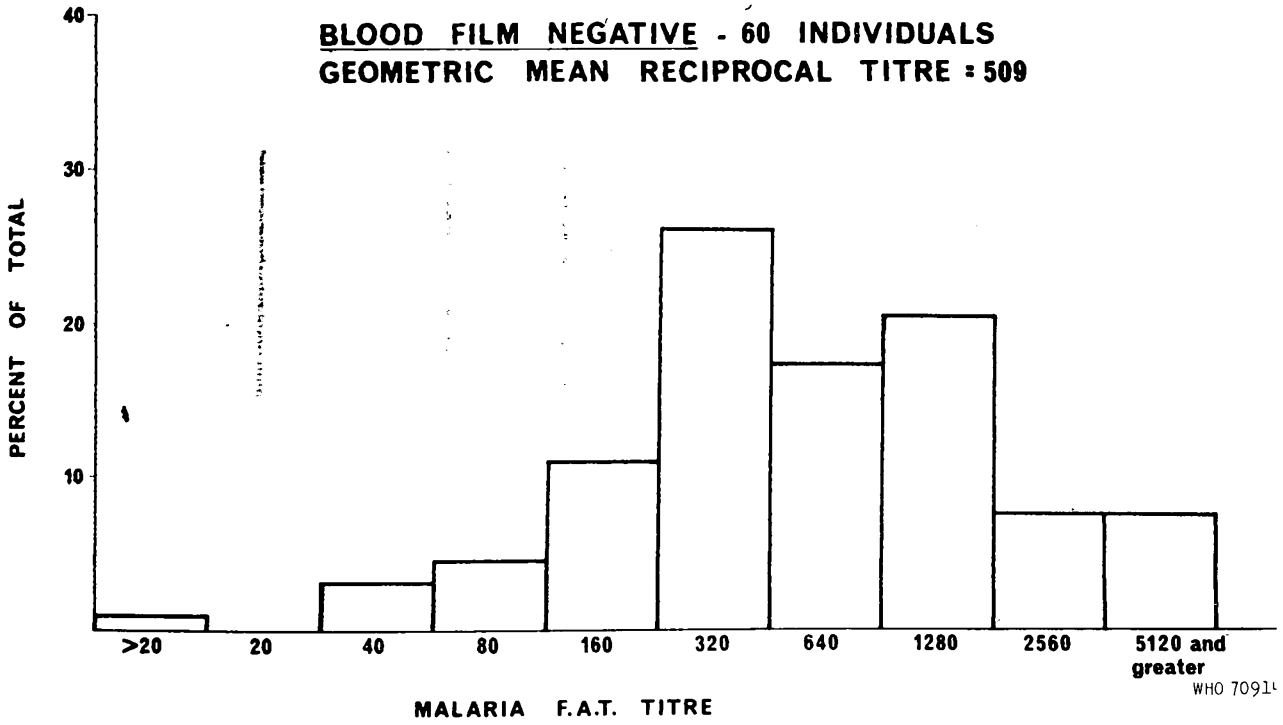
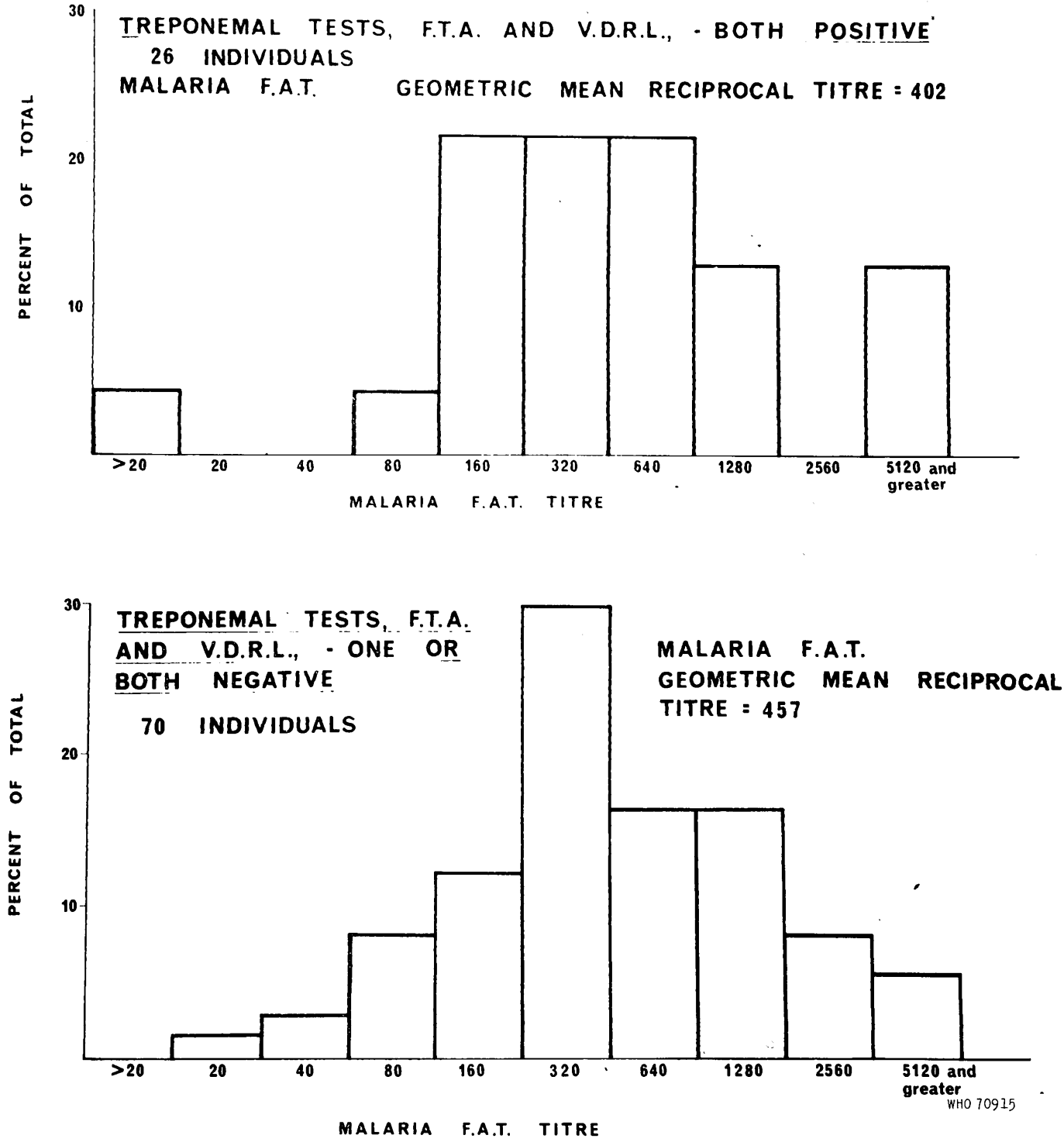


FIG. 3

THE DISTRIBUTION OF MALARIA FLUORESCENT ANTIBODY TITRES IN ADULT NIGERIANS IN RELATION TO RESULTS OF TREPONEMAL SEROLOGICAL TESTS.



The purpose of the WHO/Mal series of documents is threefold:

(a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;

(b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;

(c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.