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RHEUMATOID FACTOR IN NIGERIAN SERA

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SUMMARY

A high prevalence of rheumatoid factor has been found in the sera of apparently healthy inhabitants of two Western Nigerian villages. The prevalence of rheumatoid factor at high titre increased markedly with age. The rheumatoid factor found in the sera of these apparently healthy subjects showed a selective affinity for human γ-globulin rather than rabbit γ-globulin. Rheumatoid factor activity was shown to be restricted to the serum IgM fraction. In one of the two villages a significant correlation was found between the presence of rheumatoid factor at high titre and the presence of IgM malaria antibodies at high titre and the possible role of malaria in the induction of rheumatoid factor formation in these two communities is discussed.

INTRODUCTION

Only a small proportion of sera from healthy subjects living in countries of the temperate zones contain sufficient rheumatoid factor (RF) to give a positive result in standard tests (Lawrence, 1968; Valkenburg, Hijmans & Klein, 1968) except among the very old (Heimer, Levin & Rudd, 1963). There have, however, been a few reports of a high prevalence of RF among apparently healthy subjects living in tropical countries. Malawista, Boies & Seides (1959) recorded a high prevalence of positive F II tests (12·2%) in sera of apparently healthy Liberians and Houba & Allison (1966) noted a high prevalence of RF in members of the Luo tribe living in a malarious area of Kenya. In Uganda, Shaper *et al.* (1968) found a high prevalence of positive results in a slide latex test (21%) with sera from Rwandan immigrants to Uganda although the prevalence of a positive test among the indigenous Ganda (7·5%) was much lower. In New Guinea, Wells (1967) found a high prevalence of positive results in both the slide latex test and the sensitized sheep cell agglutination test with sera from apparently healthy individuals. However, in Jamaica, Lawrence *et al.* (1966) found that the prevalence of positive tests for RF in healthy subjects was no higher than

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that found in England, although very high titres in the bentonite flocculation test were found more frequently with Jamaican than with English sera. In a series of studies carried out at the Institute for Rheumatism Research, University Hospital, Leiden (Valkenburg *et al.*, 1968) sera from apparently healthy inhabitants of a number of countries in tropical and temperate zones have been tested for the prevalence of RF using standard techniques. These studies have shown that RF reacting with human γ-globulin, but not rabbit γ-globulin, is found more frequently in sera from New Guinea and Eastern Nigeria than in European sera. During the course of a population survey into the prevalence of rheumatic diseases in Western Nigeria a high prevalence of positive tests for RF was again found in the sera of apparently healthy Nigerians This paper reports the prevalence of RF in two Nigerian communities and records some of its characteristics

MATERIALS AND METHODS

Sera

Sera from 535 apparently healthy Nigerians (275 males and 260 females) were tested for the presence of RF. These sera were collected during the course of a population survey into the prevalence of rheumatic diseases carried out in two villages in Western Nigeria. One of the villages, Isheri, is situated in a zone of tropical forest and the other, Igbo-Ora, in open savanna. The pattern of disease occurring in the two villages shows many important differences and the relationship of these different disease patterns to the serological changes observed in samples collected in these two areas will be reported in more detail elsewhere (Muller *et al.*, to be published). Sera of blood donors and patients presenting at University College Hospital, Ibadan, Western Nigeria, were also studied.

The prevalence of RF in Nigerian sera has been compared with the results obtained on testing 741 sera from apparently healthy Dutch subjects collected during the course of a population survey into the prevalence of rheumatic diseases in three villages in the province of North Brabant, The Netherlands (Valkenburg *et al.*, to be published). The age distribution of the Dutch and Nigerian subjects from whom serum was collected is indicated in Table 1.

Tests for RF

All sera were tested for the presence of RF reacting with rabbit γ-globulin by the human erythrocyte agglutination test (HEAT) and for RF reacting with human γ-globulin by a latex fixation test (LFT) (Valkenburg, 1963). Sera giving agglutination in the HEAT at a titre of 1:32 or greater have been considered positive. The presence of a thermolabile inhibitor causes the occurrence of a number of prozones and false negative results in sera tested directly in the LFT (Schubart, Cohen & Calkins, 1959; Schubart, 1959; Bernhard, Cheng & Talmage, 1962; Klein *et al.*, 1966). All sera were therefore heated at 56°C for 30 min before testing and all the LFT results recorded in this paper refer to those obtained with inactivated sera unless otherwise stated. The prevalence of sera giving agglutination in the LFT at titres of 1:20 or more (the lowest dilution at which the sera were tested) and at titres of 1:640 or more have been recorded. The figure of 1:640 was taken as a convenient point of differentiation between high and low titre sera as a titre of 1:640 or more has previously been found to be a useful discriminant between 'normal' and 'abnormal' sera in epidemiological surveys and clinical practice.

Selected sera were also tested for RF reacting with human γ-globulin by the original LFT of Singer & Plotz (1956) and by the F II tanned sheep red cell test (Heller *et al.*, 1954) and for RF reacting with rabbit γ-globulin by the sensitized sheep red cell agglutination test (SCAT) (Ball, 1963) and the differential agglutination test (DAT) (Bywaters & Scott, 1960). Criteria of positivity in these tests largely follows those of the original authors and will be supplied on request.

Isolation of RF

Columns of Degalan V26 beads (Degussa Wolfgang, Hanau) coated with human γ-globulin were used to prepare purified preparations of RF (McCormick, Wilson & Greenwood, to be published). Degalan beads were thoroughly washed and then incubated for 60 min at 45°C in a 0·5% solution of human Cohn Fraction II γ-globulin that had previously been heat denatured by incubation at 63°C for 10 min. The beads were then left in the

TABLE 1. Age distribution of the 535 Nigerian subjects and the 741 Dutch subjects whose sera was tested for rheumatoid factor

	Nigerian subjects		
Age (years)	Igbo-Ora	Isheri	Dutch subjects
5–14	55	47	264
15–24	51	50	140
25–34	53	57	131
35–44	47	60	79
45–54	21	42	49
55 or >	8	44	78
Total	235	300	741

γ-globulin solution overnight at 4°C with constant stirring. A small column was poured and washed through with phosphate buffered saline (PBS) until the eluate was free of protein. An euglobulin preparation of test serum prepared by addition of 1% boric acid (Badin & Levesque, 1961) was made up in a small volume of PBS, applied to the column and eluted with PBS at pH 7·5. When the first protein peak had been completely eluted the PBS buffer was changed to a borate–citrate buffer at pH 4·5 and a second, smaller, protein peak was then obtained. It has been shown (McCormick *et al.*, to be published) that this peak contains almost all the RF activity of the original serum and that it is free of contaminating IgG.

Fractionation procedures

Several sera were fractionated by zone electrophoresis in Pevikon (Shandon, London). Gel filtration was carried out using Sephadex G-200 (Pharmacia, Uppsala) and ion exchange chromatography using DEAE cellulose (Whatman, Balston Ltd, England). The purity of the immunoglobulin fractions obtained was confirmed by immunoelectrophoresis using an antiwhole immunoglobulin antiserum (Burroughs Wellcome Ltd). Mercapto-ethanol

reduction was carried out according to the method described by Osler, Mulligan & Rodriguez (1966).

Immunoglobulin assay

Quantitative estimations of the immunoglobulin content of a subsample of the sera were determined by a modified Mancini method (Kalff, 1970) using monospecific rabbit anti-human antisera. Results are expressed as a ratio of those obtained with a pooled normal European standard.

Parasitological investigations

Most of the Nigerians investigated were examined clinically and parasitologically for infection with a number of specific parasites at the time that serum was collected. Details of the methods involved will be recorded elsewhere (Muller *et al.*, to be published). A subsample of the sera from Igbo-Ora were tested for the presence of schistosomal antibodies using a fluorescent technique (Bruijning, 1965) and a subsample of the sera from Igbo-Ora and Isheri were tested for malaria antibodies by a fluorescent technique (Voller, 1964) using *Plasmodium falciparum* in human blood films as the antigen. An antiwhole immunoglobulin conjugate and monospecific antiIgG and antiIgM conjugates were used in this latter test.

RESULTS

Prevalence of RF in Nigerian sera

Agglutination in the LFT at a titre of 1:20 or greater was given by 64% of the 535 Nigerian sera tested but by only 29% of the 741 Dutch sera studied. Agglutination at a titre of 1:640 or greater was given by 18% of sera from apparently healthy Nigerians but by only 4% of Dutch sera. No significant sex difference in the prevalence of RF was observed. The proportion of Nigerian sera giving agglutination in the LFT increased with age (Figs 1, 2), the age effect being particularly marked for sera giving agglutination at a titre of 1:640 or greater (Fig. 2). The prevalence of sera giving agglutination in the LFT at a titre of 1:20 or greater was similar in the two Nigerian communities investigated but sera from Igbo-Ora gave an agglutination at a titre of 1:640 or greater more frequently than sera from subjects of the same age living in Isheri (Fig. 2). This difference was most marked in younger subjects.

In contrast to the results obtained with the LFT similar proportions of Nigerian and Dutch sera gave agglutination in the HEAT at a titre of 1:32 or greater (Fig. 3). Four of the Nigerian sera positive in the LFT were positive in the HEAT and only one in the Brabant sample.

Reactivity with human and rabbit γ-globulin

In order to confirm the apparent affinity of the RF found in the sera of many apparently healthy Nigerians for human γ-globulin rather than rabbit γ-globulin, sera from 254 sick and healthy Nigerians were tested for the prevalence of RF by a number of standard techniques. Regardless of the nature of the carrier system used, sera from apparently healthy Nigerians frequently contained RF reacting with human γ-globulin but not with rabbit γ-globulin (Table 2). In contrast the RF found in the sera of Nigerian patients with

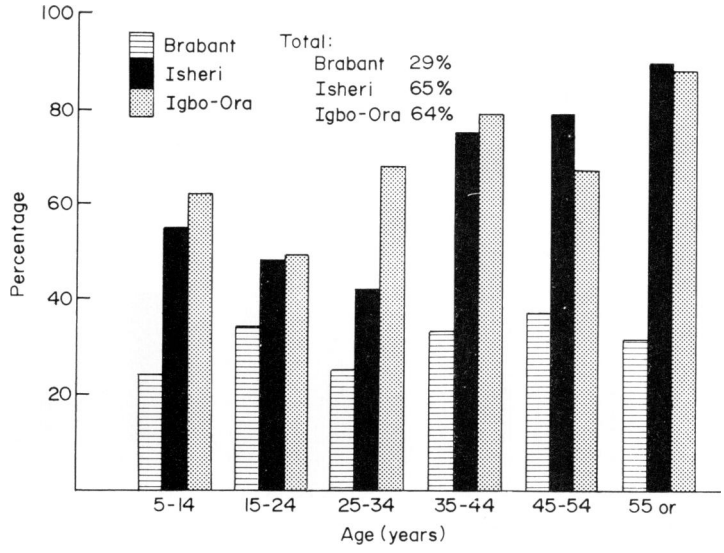

FIG. 1. Prevalence of a positive test in the LFT at a titre of 1:20 or greater in 741 Dutch sera, in 235 sera from the Nigerian village of Igbo-Ora and in 300 sera from the Nigerian village of Isheri.

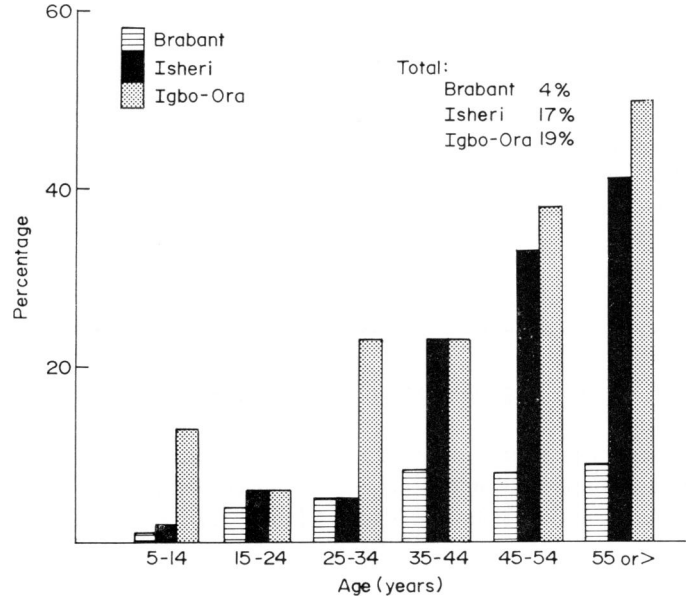

FIG. 2. Prevalence of a positive test in the LFT at a titre of 1:640 or greater in 741 Dutch sera, in 235 sera from the Nigerian village of Igbo-Ora and in 300 sera from the Nigerian village of Isheri.

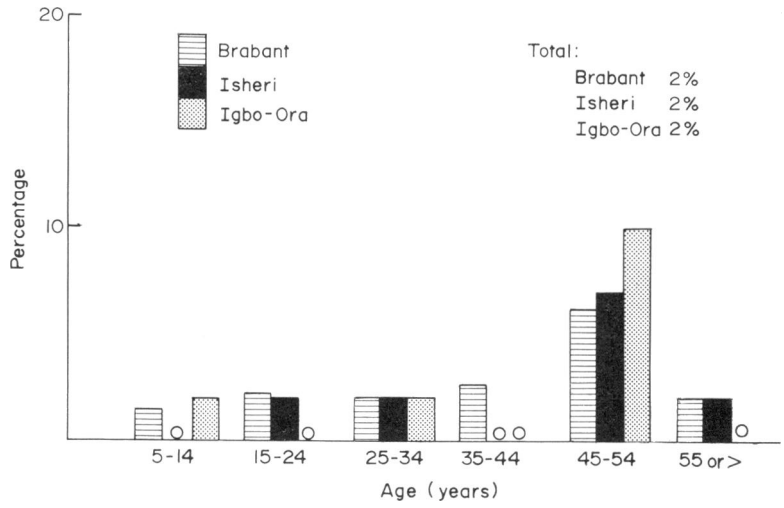

FIG. 3. Prevalence of a positive test in the HEAT at a titre of 1:32 or greater in 741 Dutch sera, in 235 sera from the Nigerian village of Igbo-Ora and in 300 sera from the Nigerian village of Isheri.

TABLE 2. Prevalence of a positive test for RF in different groups of Nigerian sera using a variety of test systems: results expressed as a percentage

Test system	Blood donors (n = 100)	Ward patients (n = 101)	Patients with rheumatoid arthritis (n = 53)
Human γ-globulin			
LFT (Singer and Plotz)	6	7	13
LFT (Valkenburg)	8	7	13
F II haemagglutination test	7	9	16
Rabbit γ-globulin			
HEAT	2	11	9
SCAT	1	5	7
DAT	0	1	2

rheumatoid arthritis and with general medical diseases frequently reacted with both human and rabbit γ-globulin.

A good correlation was found between the results obtained with the LFT and the F II tanned sheep red cell agglutination test. The HEAT was found to be slightly more sensitive than the SCAT in all the groups tested. The DAT was positive in only two of the 254 sera tested.

Reactivity with European and Nigerian IgG

Thirty Nigerian sera, selected to include a high proportion containing RF, were tested in the LFT using latex particles coated with IgG prepared by ion exchange chromatography

from pools of ten RF negative European and Nigerian sera. The solutions of European and Nigerian IgG used for coating the latex particles were made up to identical protein concentrations. In this experiment sera were tested without heat inactivation. Twenty-seven of the thirty sera agglutinated latex particles coated with European IgG to within one serial dilution of the results obtained with Nigerian IgG. However, one serum agglutinated Nigerian IgG coated particles to a titre of 1:320 but showed no agglutination of latex particles coated with European IgG and two further sera agglutinated latex particles coated with European IgG to titres of 1:640 and 1:160 respectively but failed to show any agglutination of latex particles coated with Nigerian IgG.

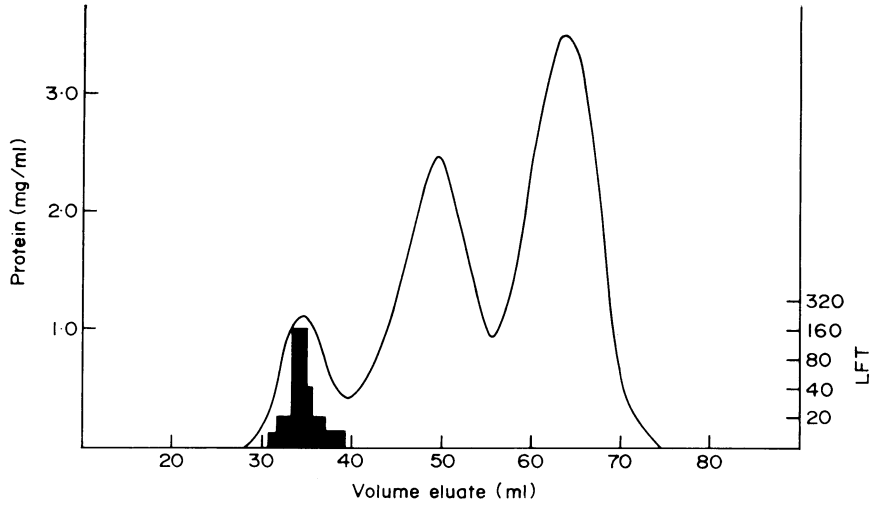

FIG. 4. Rheumatoid factor activity in the LFT of the serum of an apparently healthy Nigerian fractionated on Sephadex G-200.

Immunoglobulin class of the RF present in Nigerian sera

A number of experiments were undertaken to determine the immunoglobulin class of the RF frequently found in the sera of apparently healthy Nigerians. Agglutinating activity in the LFT was restricted to the γ-globulin fractions of four sera fractionated by zone electrophoresis in Pevikon and to the macroglobulin peak of four other sera separated on G-200 (Fig. 4). Rheumatoid factor activity of ten high titre sera was completely abolished by mercaptoethanol reduction suggesting that no free IgG RF was present. This view was supported by the fact that the IgG fractions of three pools of ten RF positive sera, prepared by ion exchange chromatography, did not show any activity in the LFT and by the fact that purified preparations of RF obtained from five sera by the use of Degalan V26 columns showed only the presence of IgM on immunoelectrophoresis.

RF and immunoglobulin levels

The immunoglobulin levels of twenty-eight sera from Igbo-Ora and twenty-seven sera from Isheri positive at a titre of 1:640 or greater in the LFT were measured and contrasted

with the levels found in the same number of RF negative sera from age and sex matched controls. The mean IgG of the RF positive sera was higher than that of the RF negative sera in both villages but the difference is only statistically significant for sera collected at Igbo-Ora (Table 3). The mean IgM level of the RF positive sera was higher than that of the RF negative controls at Isheri but not Igbo-Ora.

The contribution of RF to the very high levels of IgM found in many Nigerian sera was investigated by comparison of the IgM content of purified RF preparations with the IgM content of the serum from which they had been prepared. RF comprised only 0·5–3·5% (mean 2·2%) of the total serum IgM of the five Nigerian sera studied. Purified RF obtained from English patients with rheumatoid arthritis by this method comprised a higher proportion of the total serum IgM (McCormick *et al.*, to be published).

TABLE 3. Immunoglobulin levels of fifty-five Nigerian sera positive in the LFT at a titre of 1:640 or greater and of fifty-five sera from matched RF negative controls: mean levels and standard deviations given as a percentage of a pooled normal European serum standard

	Igbo-Ora		Isheri	
	RF positive (n = 28)	RF negative (n = 28)	RF positive (n = 27)	RF negative (n = 27)
IgG	247± 66	206± 60	260±118	225±94
	†St.W. P = 0·02–0·01		*n.s.	
	‡Wilc. P = 0·05–0·02			
IgM	226±135	225±211	257±175	170±91
	*n.s.		†St.W. P = 0·05–0·025	
			‡Wilc. P = 0·1–0·05	

* n.s. = not significant.

† St.W. = Student Welch test inducted for small sample size and unequal variances.

‡ Wilc. = Wilcoxon's test for two samples.

RF and parasitic infections

No significant correlation was found between the occurrence of RF at a titre of 1:640 or greater in the LFT and the presence of infection with schistosomiasis, onchocerciasis or filariasis at the time that the serum was collected. Schistosomal antibodies were found no more frequently in sera from Igbo-Ora positive in the LFT at a titre of 1:640 or greater than in sera from RF negative controls. Full details of these parasitological studies will be reported elsewhere (Muller *et al.*, to be published).

Malaria antibody levels were determined in the sera of eighty-eight blood donors from Western Nigeria selected without reference to the presence of RF. Mean titres of approximately 1:640 were obtained with antiwhole immunoglobulin and anti-IgG conjugates and a mean titre of approximately 1:160 was obtained with an anti-IgM conjugate. Malaria antibody levels were then measured in twenty sera from Igbo-Ora and ten from Isheri positive in the LFT at a titre of greater than 1:640 and in sera from RF negative aged and sex matched

controls from the same village. The proportion of RF positive and RF negative sera with malaria antibody levels above or below the mean values obtained in the preliminary study are indicated in Table 4. Higher malaria antibody levels were found more frequently in the RF positive sera than the RF negative sera with each conjugate but the difference obtained is only statistically significant for the anti-IgM conjugate and of borderline significance for the

TABLE 4. Malaria antibody titres obtained with three different conjugates in thirty Nigerian sera positive at a titre of 1:640 or greater in the LFT and in thirty sera from RF negative matched controls

	Anti-whole Immunoglobulin conjugate		Anti-IgG conjugate		Anti-IgM conjugate	
	>1.640	>1:640	>1:640	>1:640	>1:160	>1:160
RF positive	9	14	15	9	15	6
RF negative	4	21	10	15	7	18
	$P = 0{\cdot}1$–$0{\cdot}05$		$P = 0{\cdot}1$		$P < 0{\cdot}01$	

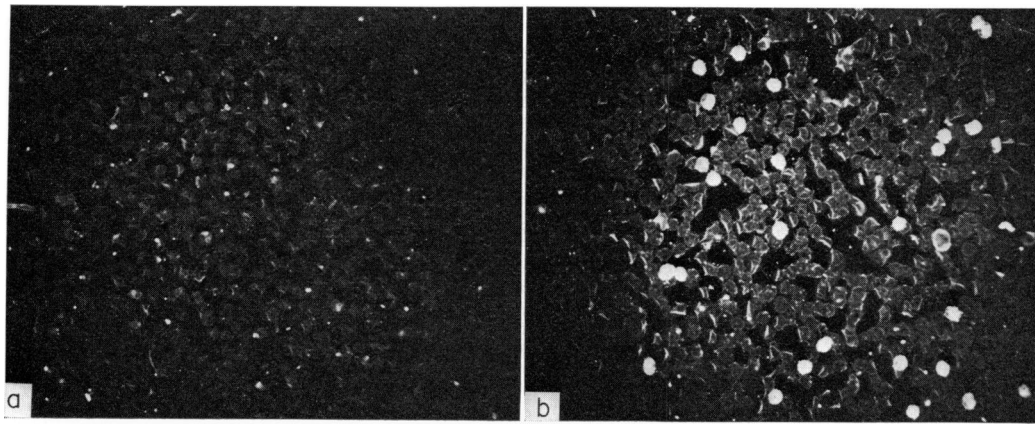

FIG. 5. Blood films from a patient infected with *P. falciparum* treated with (a) purified RF followed by an antiIgM conjugate and (b) IgG malaria antibody, followed by purified RF and then an antiIgM conjugate. Brilliant fluorescence of the malaria parasites is seen in (b) but not in (a).

anti-whole immunoglobulin conjugate. On separate analysis of the two villages it was found that the significant differences only occurred in Igbo-Ora and not in Isheri likely due to the small numbers of sera investigated.

The RF present in Nigerian Sera was tested for its ability to react with malaria antigen-IgG malaria antibody complexes. No fluorescence of malaria parasites was observed in blood films from a patient with *P. falciparum* malaria treated directly with pure RF preparations obtained from five Nigerian sera and counterstained with an anti-IgM conjugate.

However, if the blood films were treated with a purified IgG preparation containing malaria antibody in high titre before application of the purified RF preparations brilliant fluorescence of the malaria parasites was then observed (Fig. 5). RF preparations obtained from English patients with rheumatoid arthritis behaved in a similar way.

DISCUSSION

This study has demonstrated a much higher prevalence of RF in the sera of apparently healthy Nigerians than in the sera of healthy Dutchmen tested by identical laboratory techniques. It was found that the RF present in the sera of many apparently healthy Nigerians showed a selective affinity for homologous rather than heterologous γ-globulin whilst the RF found in the sera of Nigerian patients with rheumatoid arthritis and other general medical diseases usually showed reactivity with both human and rabbit γ-globulin. The RF found in a number of infectious diseases has previously been shown to have a similar selective affinity for human rather than rabbit γ-globulin (Peltier & Christian, 1959; Williams & Kunkel, 1962; Huntley *et al.*, 1966; Risemberg, Gomez & Rife, 1969).

Sera from sick and healthy Nigerians were tested for the presence of RF by a variety of standard techniques in order to confirm the apparent selective affinity of the RF found in normal Nigerian sera for homologous γ-globulin and to determine the most suitable test for routine use in this community. Difficulty was experienced with tests employing sheep erythrocytes due to the frequent occurrence in Nigerian sera of heterophile antibodies in high titre (Adeniyi-Jones, 1967; Greenwood, 1970). The presence of heterophile agglutinins rendered the DAT an unreliable test for RF in Nigerian sera and repeated absorptions with sheep red cells were sometimes necessary before the SCAT could be satisfactorily carried out. These difficulties were overcome by use of the HEAT, which employs human red cells coated with rabbit antibody rather than sheep erythrocytes, and this test was found to give comparable results to the SCAT in all the groups tested. The HEAT may be found to be a useful test for RF in other areas of the tropics where heterophile antibodies are prevalent.

Fractionation studies indicated that the RF activity found in the sera of many apparently healthy Nigerians was restricted to the serum IgM fraction and no free serum IgG RF was demonstrated. However, in another study (Greenwood & Torrigiani, unpublished) it has been found that when tested for the presence of antiglobulins to horse γ-globulin by an immunoabsorbent technique (Torrigiani & Roitt, 1967) many sera from apparently healthy Nigerians contain much higher levels of IgG than are found in the sera of healthy Europeans.

Both genetic and environmental factors must be considered as possible contributory factors to the high prevalence of RF found in apparently healthy subjects living in Western Nigeria. The vast majority of Nigerians studied in this investigation belonged to the Yoruba tribe suggesting that a genetic factor might be involved. However, in another preliminary study (Greenwood, unpublished) it has been found that sera from Nigerians living in the centre of Nigeria around the junction of the Niger and Benue rivers, who comprise a heterogeneous mixture of different tribal groups, also show a high prevalence of RF which shows a selective affinity for homologous γ-globulin. It thus seems unlikely that tribally restricted genetic factors are the only element involved in the high prevalence of RF in sera from Western Nigeria.

The importance of environmental factors in the development of the RF found in the sera of some apparently healthy individuals has been emphasized by the studies of Bennett

& Burch (1968) among the Pima Indians and of Adler *et al.* (1967) among immigrants to Israel. Active or past infection is probably one of the most important of these environmental factors as an increased prevalence of RF has been recorded in a number of viral, bacterial and parasitic diseases (reviewed by Bartfeld, 1969) including a number such as tuberculosis, leprosy, leishmaniasis, trypanosomiasis and visceral larva migrans which are prevalent in many tropical developing countries. It seems unlikely that any of these conditions was responsible for the high prevalence of RF in the sera studied in this investigation. None of the subjects from whom serum was obtained had any clinical features of leishmaniasis or trypanosomiasis, which are both rarely seen in the southern part of Nigeria, and only two had clinical features suggestive of leprosy. It is, however, likely that several had active pulmonary tuberculosis. Many of the subjects surveyed were found to be infected with onchocerciasis, schistosomiasis and filariasis but no epidemiological evidence was found to relate the occurrence of RF with the presence of these infections. Data on the presence of intestinal helminths was not obtained but it is likely that many of the subjects from whom serum was collected were infected with ascaris and hookworm.

Malaria is endemic in both villages from which sera were collected and most of the apparently healthy subjects studied had probably been infected with malaria on many occasions. Malaria may therefore have been one of the factors contributing to the high prevalence of RF in these communities. Our finding of an association between the occurrence of RF at high titre and the presence of malaria antibodies at high titre gives some support to this view. The occurrence of a strong association between the presence of RF and high titres of IgM malaria antibody may indicate the existence of a sub-group of the population with a particularly well-marked ability to make a 19S immune response. Studies in Kenya (Houba & Allison, 1966), New Guinea (Wells, 1967) and Uganda (Shaper *et al.*, 1968), have also suggested a relationship between malaria infection and the high prevalence of RF found in these areas. The experimental studies of Klein *et al.* (1970) have shown that a proportion of rhesus monkeys (*Macca mulatta*) infected with *P. cynomolgi* do develop rheumatoid-like factors during the period of malaria parasitaemia but that persistent RF formation is not seen after repeated challenge with malaria. However the relevance of these experimental findings to a consideration of the human situation is uncertain as it has been shown that inter-species variations occur in the ease with which RF formation can be experimentally induced.

Malaria infection could lead to the production of RF in several ways. It is possible that as a consequence of prolonged stimulation of immunoglobulin synthesis some IgM molecules are formed which, by change, have RF activity. If this process was occurring it would be anticipated that a strong correlation would be found between the presence of RF and the presence of high levels of total serum IgM. Although the mean IgM levels of RF positive sera obtained at Isheri was found to be higher than that of RF negative controls the association between the prevalence occurrence of RF and high IgM levels was not strong and it was not found among sera collected at Igbo-Ora. It has recently been shown that RF may show cross reactivity with other antigens (Hannestad, 1969) and it is possible that malaria infection might induce the formation of antibodies showing cross-reactivity with human γ-globulin. However, in an immunofluorescent test it was found that purified RF preparations obtained from Nigerian sera did not show any direct reactivity with malaria parasites. During malaria and other parasitic infections it is probable that large amounts of altered IgG antibody are formed as a result of combination of antibody with parasite antigens and it is possible that this altered IgG antibody is capable of inducing RF formation. We have

been able to demonstrate that the RF present in Nigerian sera reacts strongly with malaria antigen–IgG malaria antibody complexes but this reaction cannot be regarded as in any way specific and its demonstration cannot be taken to indicate that malaria antigen–IgG antibody complexes were responsible for the frequent occurrence of RF in Nigerian sera.

Although the findings of this investigation are compatible with the view suggested by the previous studies of Houba & Allison (1966), Wells (1967) and Shaper *et al.* (1968) that the high prevalence of RF found in apparently healthy inhabitants of a number of tropical countries is related to malaria infection our study does not provide any direct evidence to support this hypothesis and other factors must be considered. Our data do not suggest that any of the other parasitic or bacterial infections prevalent in Western Nigeria play a major role by themselves in the production of the RF frequently found in sera from this area. However, it is possible that the cumulative effects of infection with a variety of different organisms may ultimately lead to the formation of RF. This interpretation could explain the marked effect of increasing age on the prevalence of RF at high titre observed in Nigerian sera. A possible role for virus infections in the production of RF in Nigerian sera remains a further possibility to be considered. It is possible that the collection of data on the prevalence of RF in other parts of tropical Africa and its relationship to the local pattern of infectious disease may help to clarify some of these problems.

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